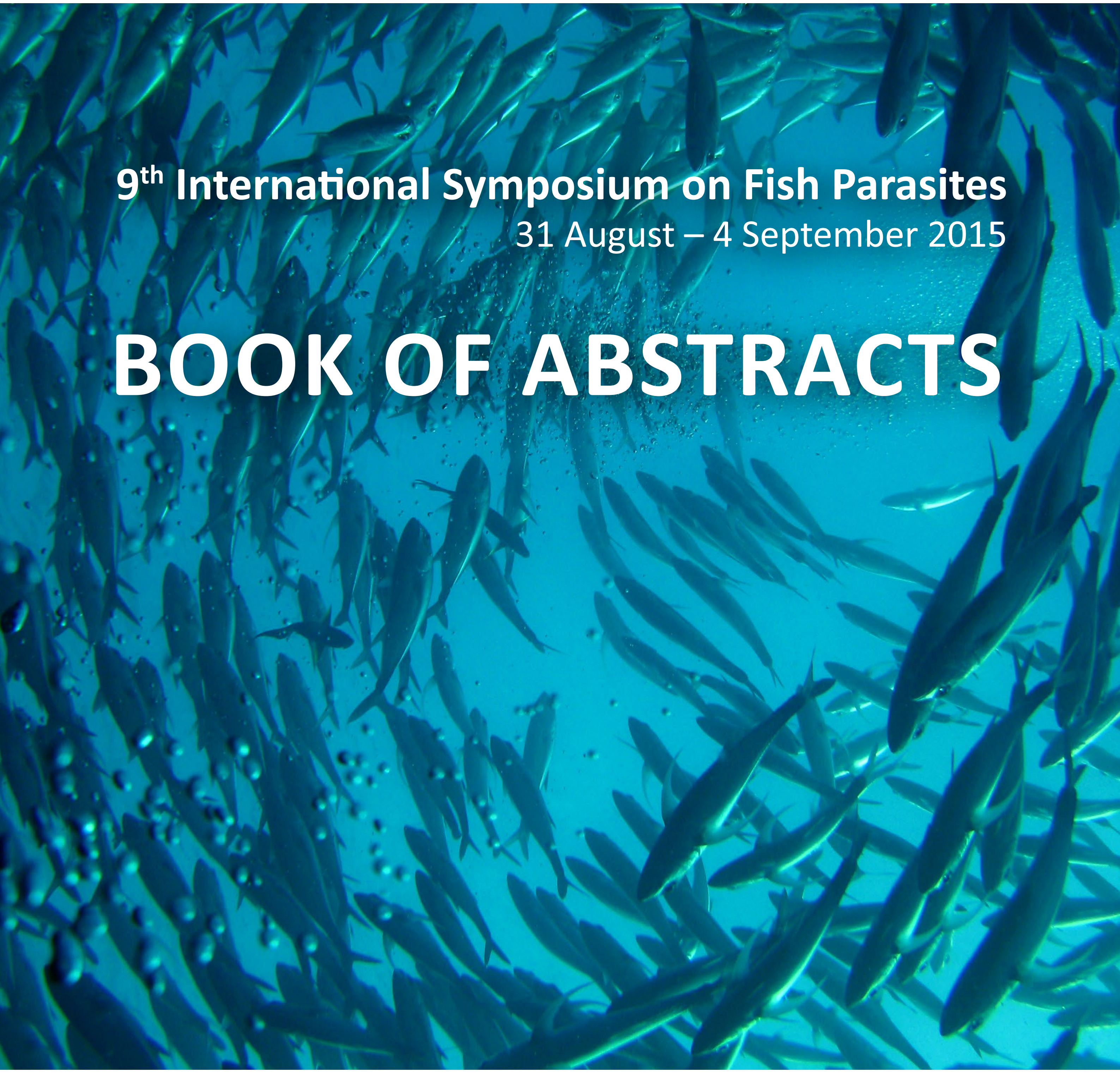
9th ISFP
VALENCIA 2015



9th International Symposium on Fish Parasites

31 August – 4 September 2015

BOOK OF ABSTRACTS

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

VNIVERSITAT DE VALÈNCIA

9th International Symposium on Fish Parasites
Valencia 2015

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F. Javier Aznar, Jerri L. Bartholomew, Maite Carrassón, Bahram S. Dezfuli, Aneta Kostadinova, Arne Levsen, Simonetta Mattiucci, Francisco E. Montero, Marcelo E. Oliva, Ana Pérez del Olmo, Oswaldo Palenzuela, Maria João Santos, Nicholas J. Smit, Ariadna Sitjà-Bobadilla, Bernd Sures, David W. Thieltges, Juan T. Timi, Hiroshi Yokoyama.

PLENARY LECTURES

INTERNATIONAL SYMPOSIA ON FISH PARASITES: A KEY FACTOR FOR THE ADVANCEMENT OF ICHTHYOPARASITOLOGY

Scholz T.

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Science is based, among other things, on a free exchange of ideas including meetings of researchers and their discussions about hypotheses, sharing experiences, planning collaborative research and also learning from each other. Personal meetings of students and young researchers with leading experts may represent crucial moments in their career development and can be decisive of their future professional destiny. It was actually also my case when I met, as an undergraduate student of parasitology, Clive Kennedy, Jimmy Chubb, John Mackiewicz, Glenn Hoffman, Tellervo Valtonen, Christina Sommerville and many other 'gurus' of fish parasitology at the historical first world meeting focused exclusively on ichthyoparasitology in former Czechoslovakia in August 1983. I was impressed not only by their often excellent lectures, but also their accessibility and kindness to 'waste' their valuable time by replying to often very basic questions of a no-name beginner in fish parasitology. This experience actually decided the course of my scientific career. Unfortunately, I was not allowed as a PhD student to attend the second fish parasitology meeting in Hungary in 1988 – we lived in socialism with a very strict hierarchy, in which students were not considered to be worth attending international conferences abroad. For safety reasons, I also abstained from probably the weirdest meeting in Petrozavodsk, Russia in 1991 organised precisely in the time when orthodox Russian communists wanted to take power by a coup d'etat. However, I feel that my experience as a long-term member of the international committee of fish parasitology meetings as well as my usually active participation at the following conferences: in Munich, Germany (1995; principal organiser Rudoph Hoffmann), Budweis, Czech Republic (1999; Frank Moravec and Iva Dyková), Bloemfontein, South Africa (2003; Jo Van As), Viterbo, Italy (2007; Simonetta Mattiucci) and Viña del Mar, Chile (Marcelo Oliva) enable me to present a brief overview of these informal, friendly, but scientifically very sound and socially important meetings. Therefore, this introductory talk is based mainly on my personal experience and souvenirs (of course, quite subjective and incomplete, yet 'mined' from my detailed minutes I made during most of these conferences) , but also on valuable comments generously provided by several key players in fish parasitology, the names of which will appear at the end of my talk. Even though such a historical overview may be nostalgic and not interesting for young, dynamic researchers and students, they are strongly encouraged to profit from the unique opportunity to informally discuss their results and research plans with leading scientists. I hope that forthcoming conferences on fish parasites will represent as important stimuli for many young colleagues as they did for me. I wish that they enjoy the privilege to study such amazing creatures as fish parasites!

MODELING THE EFFECTS OF CLIMATE CHANGE ON DISEASE SEVERITY: A CASE STUDY OF *CERATONOVA* (SYN *CERATOMYXA*) *SHASTA*

Bartholomew J.L.[1], Ray R.A.[2], Alexander J.D.[1], De Leenheer P.[3]

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Shifts in future temperature and precipitation patterns will have profound effects on host-parasite interactions and the dynamics of disease in freshwater systems. The aims of this talk are to present an overview of myxozoan disease dynamics in the context of climate change, and to illustrate how these might be predicted over the next several decades by developing a case study of disease dynamics of *Ceratonova* (syn *Ceratomyxa*) *shasta* in the Klamath River, California USA. Our case study introduces a model ensemble for predicting changes in disease dynamics under different climate scenarios (warm/dry, moderate/median, and cool/wet) from 2020 to 2060. The ensemble uses Global Circulation Models (GCMs) and basin scaled models for the Klamath River to generate predictions for future water temperature and river discharge. The environmental data are used as inputs for a predictive model and a degree-day model to simulate effects of climate change on polychaete host populations and on *C. shasta* spore viability, respectively. Outputs from these models are then used to parameterize an epidemiological model to predict changes in disease dynamics under each climate scenario. The epidemiological model outputs were measured against baselines established using real data for low, high and intermediate disease risk years. In general, the epidemiological model predicts that, except for infrequent high discharge years, *C. shasta* dynamics will be similar to the high disease risk baseline. This suggests that the recovery and management of Klamath River salmon will continue to be impacted by *C. shasta*.

PARASITES IN FISH MARICULTURE

Nowak B.

University of Tasmania, Launceston, Australia

Successful health management of farmed fish is essential for sustainable mariculture. The level of potential control of parasites is related to the type of aquaculture system. Cage culture offers little control over parasitic infections and may contribute to free living organisms becoming parasitic. Additionally, interactions between farmed fish and wild fish are unavoidable in cage culture. Parasites can cause significant losses and contribute to production costs through the costs of treatment and loss in growth of the fish. Sea lice are one of the most significant health problems in mariculture worldwide. All major producers of Atlantic salmon are significantly affected by different species of sealice. While there are commercial treatments available, development of resistance can reduce usefulness of some of those products. Sealice can also affect farming of other mariculture species, such as tuna. Amoebic gill disease (AGD) is a condition affecting some species of farm-reared marine fish caused by *Neoparamoeba perurans*. AGD was initially reported only in Australia and USA but by now it has a significant impact on salmon production in Australia, Scotland, Norway and Ireland. Main treatments used commercially include bath treatment with fresh water or with hydrogen peroxide. Tuna mariculture in Japan and Australia is affected by blood flukes from genus *Cardicola*. Currently praziquantel is used as a treatment but there is a growing concern that the blood flukes may develop resistance. Improved knowledge of the parasites and host-parasite-environment interactions has resulted in promising management strategies which can reduce impact of parasitic diseases in mariculture.

PARASITES AS BIOLOGICAL TAGS IN AQUATIC ECOSYSTEMS: PRESENT STATUS AND PERSPECTIVES

Timi J.T.

Instituto de Investigaciones Marinas y Costeras, Universidad Nacional de Mar del Plata-CONICET, Mar del Plata, Argentina

First studies on parasite tags for host stock delineation were published nearly a century ago, when they were viewed as curiosities and it took many years before they were accepted and worthy of serious consideration. Nowadays, this methodology is widely applied and increasingly considered as a valuable tool for fisheries management. An analysis of more than 300 publications showed that, since its origins, there have been (i) a positive evolution in the publication rates, in the geographic distribution of investigations and in the quantitative methodologies; (ii) regular reviews along its history and actualizations of the guidelines for the selection of suitable tags and (iii) an overwhelming majority of papers dealing with parasites of teleosts, with very few studies on elasmobranchs, invertebrates and mammals. The main perspectives for parasite tags are also analysed, including (i) their integration in holistic approaches to stock studies utilising multiple techniques on the same samples and combining results across spatial and temporal scales to produce conclusive and reliable evidence and (ii) the use of molecular approaches for parasite genetic tagging, given that parasite genotypes often show an increased genetic variance relative to their hosts that could potentially be used to discriminate parasite (and host) populations with higher accuracy than by using the host’s genotypes alone. The possible extension of the study of parasite tags from a fishery to a zoogeographical context is also analysed and exemplified, which could help to delineate marine regions to strategically plan and prioritize new conservation measures and protected areas.

CESTODE AND TREMATODE PHYLOGENETICS – WHATEVER NEXT?

Littlewood D.T.J.

Life Sciences Department, Natural History Museum, London, UK

Estimates of cestode and trematode phylogenies rely heavily on molecular data to provide resolution, and a robust framework for evolutionary interpretation and taxonomic stability. Remarkable progress has been made in the last 20 years with taxon-rich comprehensive phylogenies now available for scrutiny. However, only a limited number of molecular markers have been employed, usually components of the nuclear ribosomal RNA gene array (fragments of 18S, 28S or ITS(1/2)) and the mitochondrial genome (usually fragments of *cox1*). Progress towards multi-gene phylogenetic assessments have been slow, largely because few researchers have access to the taxa required for comprehensive assessments or the resources for such endeavours. A consortium of researchers focussed on surveying tapeworm diversity has provided material for new multi-gene phylogenies over the last few years and has highlighted the need for additional markers to provide greater stability for cestode classification and to better understand patterns of radiation and diversification. This has provided an important framework for community-led systematics with substantial progress made in diversity discovery and molecular systematic effort. Meanwhile, next generation sequencing, and the opportunities to apply this technology to material already collected, provides options for a move towards phylogenomics and renewed efforts in taking cestode and trematode systematics still further. Here I assess where we are with the phylogenies of flatworms with complex life cycles, the confidence we have in their phylogenies, the implications of accepting them and the need to test them further. The basic taxonomic and systematic approach provides tools for diagnosticians, ecologists, pathologists, evolutionary biologists and parasitologists alike. A strategic community-led approach should not be too costly if we pool resources and share goals, but probably requires a radical review of collections-based research and how we engage with it.

PARASITES AND BIOLOGICAL INVASIONS

Thieltges D.W.

NIOZ Royal Netherlands Institute for Sea Research, Texel, The Netherlands

The introduction of species to ecosystems often results in dramatic impacts on native biota. Parasites can contribute to these impacts but they can also play other important roles in biological invasions. For example, a loss of parasites during the invasion process can lead to a competitive advantage for invading hosts (parasite release). Once established, invasive hosts can acquire native parasites, amplify their populations and re-infect native hosts (spill-back effect). Parasites can also be co-introduced with their invading hosts, potentially infecting native species (spill-over effect). Finally, invasive species may not act as hosts for parasites but interfere with their transmission dynamics, thus reducing infection risk for native species (dilution effect). In this talk, I will present a conceptual overview of the different mechanisms of how parasites can play a role in biological invasions and I will exemplify various impacts on native biota. I will do so by focussing on marine hosts, ranging from invertebrates to fish, with additional examples from freshwater and terrestrial systems.

ENVIRONMENTAL PARASITOLOGY: UNDERSTANDING PARASITE ECOLOGY FROM AN ENVIRONMENTAL PERSPECTIVE

Sures B.

Department of Aquatic Ecology and Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen, Essen, Germany
Department of Zoology, University of Johannesburg, Johannesburg, South Africa

In recent years, there has been an increasing number of papers showing how parasitism and environmental conditions such as pollution or eutrophication can interact with each other in aquatic organisms. Apart from parasitological aspects these interactions are also important in terms of ecological and environmental research. The current presentation aims at identifying four promising directions for future research in the interdisciplinary field of environmental parasitology. 1. Parasites as sinks for pollutants within their hosts: Some parasites are able to reduce pollutant levels in the tissues of their hosts. This is an interesting implication since parasites might be beneficial to their hosts from this perspective. This would alter our common understanding of parasites which – by definition – have to be harmful to their hosts. 2. Parasites as a diagnostic tool to test bioavailability and toxicity of substances. Whereas free-living parasitic stages such as cercariae respond very quickly to the presence of pollutants in their environment and therefore can be considered as reaction indicators, endoparasites can only take up and accumulate pollutants if these were metabolized by the host in an initial step. 3. The composition of parasite communities reflects environmental conditions of their hosts. Accordingly, pollutants might be important factors in structuring parasite assemblages which themselves might be suitable to draw conclusions about the state of the environment. 4. Parasites and simultaneously occurring pollutants can affect the physiological homeostasis of infected hosts. This might have negative consequences for the health of the host, but is also important from an ecotoxicological point of view.

RECENT ADVANCE IN SALMON LOUSE RESEARCH – A BASIS FOR INNOVATION ON SEA LICE CONTROL

Nilsen F.

Sea Lice Research Centre, Department of Biology, University of Bergen, Norway

Infection with the salmon louse (*Lepeophtheirus salmonis*) is currently causing large economical loss in the farming of Atlantic salmon in Europe. In Norway the cost was estimated to be about 0.5 €/produced kilo in 2014. An important reason for the increased cost is related to reduced efficacy of most of the available medicines that are used for salmon louse control. The emerging resistance is challenging the current control strategy which aims at a low environmental impact related to the salmon lice. Hence, there is a strong demand for new control tools and to enhance the current state of knowledge for this important parasite in order to further facilitate salmon louse control. During the last several years special designed experimental facilities (e.g. single fish tanks) for salmon louse research has been established together with protocols for gene silencing using RNA interference (RNAi) in both larva and pre-adult and adult parasites. In addition, the salmon louse genome has been sequenced and annotated which allow easy access to candidate genes both for functional studies and for the identification and evaluation of new treatment targets. The presentation will give an overview of the established infrastructure and present some properties of the salmon louse genome together with some recent examples of functional studies and how our research can facilitate innovation for sea lice control.

WORKSHOPS

WORKSHOP: "EPIDEMIOLOGY, PROPHYLAXIS & CONTROL OF PARASITES IN AQUACULTURE"

Chairs: Francisco E. Montero[1] & Barbara Nowak[2]

[1]Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Valencia, Spain
[2]University of Tasmania, Launceston, Australia

Pathogens are one of the major problems for the development of the aquaculture. The industry and research often pays more attention to bacteria and virus as these pathogens are usually more frequent and severe. However, some parasite taxa include notable exceptions, as amoebas, ciliates, myxozoans, monogenean platyhelminths or caligid copepods, mostly known because they affect to well-known fish cultures (as salmon, trout, amberjacks, sea-breams, etc). Moreover, new parasite pathogens emerge every year, apparently related to the intensification of aquaculture, the development of new fish cultures, or the global change. Is the industry ready for this changing scenario? The degree knowledge on the biology, pathogenic mechanisms, epidemiology and control methods of each parasite depends on factors, as the existence of similar previously known parasitosis, or the economical relevance and time of experience the culture. In this regard, the geographical situation of the fish culture clearly has been determinant, but, has this regionalised situation changed in this globalised world?

The 9th International Symposium on Fish Parasites provides a unique opportunity to bring together international researchers with different expertise, coming from different geographical regions worldwide in order to obtain the broadest possible view of the occurrence and impact of parasites in aquaculture around the world.

WORKSHOP: “THE BIODIVERSITY OF TREMATODES OF FISHES”

Chairs: Thomas H. Cribb[1] & Isabel Blasco-Costa[2]

[1]School of Biological Sciences, The University of Queensland, Brisbane, Australia
[2]Department of Invertebrates, Natural History Museum of Geneva, Geneva, Switzerland

THE MEDITERRANEAN: DISCOVERY RATES FOR A WELL-STUDIED DIGENEAN FAUNA

Pérez-del-Olmo A.[1], Kostadinova A.[2], Gibson D.I.[3]

[1]Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Valencia, Spain
[2]Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
[3]Department of Life Sciences, Natural History Museum, London, UK

Knowledge of digenean diversity in Mediterranean fishes is based on many contributions since the 1800s (e.g. by Rudolphi, Looss, Stossich, Bartoli, Bray and Gibson). We updated data from the NHM Host-Parasite Database, and listed 294 digenean species allocated to 145 genera in 29 families from 177 fish species (25% of the known fish-fauna) belonging to 122 genera and 70 families. The most diverse (25–44 species) digenean families (Opecoelidae, Hemiuridae, Didymozoidae) represent more than a third of the total richness (35% of species; 24% of genera). The mean number of species per host is close to recent global estimates for digenean richness in teleosts (1.66 *vs* 2.04), indicating a high diversity in the Mediterranean. Families harbouring the greatest diversity (i.e. 60–100%) of digeneans species (Clupeidae, Gadidae, Labridae, Mugilidae, Sparidae, Scombridae, Triglidae) are also the best studied. Digeneans were also recorded from all hosts of a number (21; i.e. 30%) of species-poor host families. However, five speciose host families (Gobiidae, Myctophidae, Rajidae, Scorpaenidae, Serranidae) appear understudied (only 5–30% with records) and no digenean reports exist for 100/169 fish families present in the Mediterranean. Thus, although Mediterranean fishes appear well studied, further efforts are needed. Nevertheless, descriptions of new taxa (three genera and 27 species) since 2000 indicate that focused efforts have resulted in a high discovery rate (1.9 species per year). Many of these new (often cryptic) taxa were the result of combined morphological and molecular methods, which promise more reliable estimates of digenean diversity in this region.

KNOWLEDGE OF MARINE FISH TREMATODES OF THE ATLANTIC OCEAN

Bray R.A.

Department of Life Sciences, The Natural History Museum, London, UK

The Atlantic Ocean and its adjacent basins (e.g. Mediterranean Sea, Gulf of Mexico, Caribbean Sea) are well represented in the early history of the description of the global marine digenean fauna. Workers such as Rudolphi, Dujardin, Stossich described many Mediterranean forms whilst van Beneden, Olsson, Odhner, Nicoll, Linton, Stafford and Manter, provided variously satisfactory descriptions of the basic fauna of the wider Atlantic Ocean. Life-cycles have been elucidated by such workers as and Stunkard and Køie. It would seem, therefore, that with this long history, the marine fauna of the Atlantic is quite well known and it would be possible to have some idea of the development of the fauna in this, the youngest of the Oceans. The knowledge is, however, not evenly spread, with little being known of the south-eastern Atlantic or the deep-sea, particularly south of the Equator. In addition, many of the earlier descriptions are not now recognisable. Nevertheless, our understanding of the diversity of the fauna will be described and discussed and a comparison with the fauna of the older oceans made, including consideration of such developments as the appearance of the Panama Peninsula and Lessepsian migration into the Mediterranean Sea. The importance of testing morphological results with molecular techniques will be stressed and recently discovered cryptic species considered.

KNOWLEDGE OF TREMATODES OF SHORE FISHES OF THE TROPICAL INDO-WEST PACIFIC

Cribb T.H.[1], Yong R. Q-Y[1], Diaz P.E.[1], Martin S.B.[1], Huston D.C.[1], Bray R.A.[2], Cutmore S.C.[1]

[1]School of Biological Sciences, The University of Queensland, Brisbane, Australia
[2]Department of Life Sciences, The Natural History Museum, London, UK

The tropical Indo-west Pacific (TIWP) ranges from the Red Sea and East Africa in the west to Hawaii and Easter Island in the East. This huge marine biogeographic region encompasses the “Coral Triangle” (the waters of Indonesia, Malaysia, the Philippines, Papua New Guinea, Timor Leste and Solomon Islands), the most diverse marine area in the world. In total, the TIWP has at least 4,000 shore fish species of which at least 2,000 occur in the Coral Triangle. Study of this fauna began relatively late; serious reporting began only in the 20th Century and there remains much to be done. In this review we consider how well the fauna of adult digenean trematodes is known in terms of the likely total number of species, how well the patterns of host-specificity are understood, and the development of understanding of patterns of biogeography in the region. The overwhelming paradigm is of concentrated knowledge of isolated taxonomic groups, host groups and geographical areas relating to sustained work by a relatively small group of experts. Important components of the fauna in all these categories remain poorly studied. The lack of contemporary work in the Coral Triangle is especially striking. We conclude by discussing potential approaches to expediting progress.

PROGRESS TOWARD THE DESCRIPTION OF THE TREMATODES OF FRESHWATER FISHES OF THE WORLD

Scholz T.[1], Cribb T.H.[2], Ostrowski de Núñez M.C.[3], Smit N.[4]

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Given that fresh water constitutes less than 1% of global water resources, it is remarkable that freshwater fishes comprise about 40% of all fish species. Of course, these fishes harbour a huge range of trematodes, both as sexual adults and as metacercariae. Here, continent by continent, we review knowledge of this fauna. Knowledge is highly uneven and clearly incomplete for almost all regions, sometimes dramatically so. A recent critical examination of published data on 148 fish species of 21 families has revealed the occurrence of 56 species of trematodes parasitising freshwater fishes in Europe as adults and 63 species that use fish as intermediate hosts (metacercariae). For Australia there are records of just 31 trematode species from 34 of about 300 fish species. For South America there are about 200 species known from the astonishing estimated total of 6,000–8,000 fish species. For Africa there are fewer than 100 species reported. Although the biggest problem remains the completion of the “first pass” of alpha taxonomy, there are in addition great problems relating to biogeography and elucidation of life cycles. For the latter, molecular data, i.e. matching DNA sequences of larval stages and corresponding adults, may represent a powerful tool that should be used in future studies. Another challenging problem represents the existence of cryptic species and, in particular, considerable decrease of experts in taxonomy and life cycles of trematodes.

BIODIVERSITY OF LARVAL DIGENEANS IN FRESHWATER ECOSYSTEMS IN EUROPE

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Analysis of two novel datasets containing 8,202 and 2,380 host-parasite-locality records for digenean parasites of fishes and molluscs, respectively, was used to assess the biodiversity of larval digeneans in the freshwater environment in Europe. The "fish" dataset comprised a total of 67 species belonging to 33 genera and 9 families that utilise freshwater fishes as intermediate hosts. The distribution of taxonomic diversity of larval digeneans was distinctly uneven, with a large number of genera (21) represented by a single species and a single species-rich genus (*Diplostomum*, 17 spp.). The "mollusc" dataset covers a large number of lymnaeid (11 spp.; 193 host-parasite records, HPR), planorbid (18 spp; 132 HPR), bithyniid (15 spp.; 82 HPR) and bivalve (13 spp.; 32 HPR) digenean intermediate hosts. Four snail hosts had an extremely rich fauna: *Lymnaea stagnalis* (41 spp.); *Planorbis planorbis* (39 spp.); *Radix ovata* (36); and *R. peregra* (33 spp.). A total of 171 species belonging to 89 genera and 35 families was recorded, the majority (113 spp.) comprising parasites that use tetrapods as definitive hosts. Larval stages of 52 fish digeneans (43% of all species recorded in fish) are reported in freshwater molluscs, thus indicating a relatively good knowledge of the life-cycles of fish digeneans in Europe. Although the digeneans in molluscs exhibited a higher taxonomic diversity than those using fish as intermediate hosts, the same uneven diversity distribution pattern was observed, with a few species-rich genera, including most notably, *Diplostomum* (9 spp.). We discuss the taxonomic and ecological implications of these data.

THE WORLD REGISTER OF MARINE SPECIES: A TOOL FOR FISH PARASITOLOGISTS

Boxshall G.A.

Department of Life Sciences, The Natural History Museum, London, UK
Chair, Scientific Steering Committee, World Register of Marine Species

The World Register of Marine Species (www.marinespecies.org) aims to provide an authoritative, global list of names of marine species and it currently holds nearly 230,000 valid species names. The species list, together with over 190,000 synonyms, is the product of over 200 taxonomic editors – the taxonomic experts who provide the key intellectual content. Taxonomic editors have responsibility for particular taxa, ranging from a family to a phylum, to which they have password controlled access. They update WoRMS – capturing the 2000 or so new marine species described each year as well as synonyms, etc. WoRMS seamlessly delivers marine names data derived from other Global Species Directories, such as FishBase, AlgaeBase and World of Copepoda. As a fish parasitologist working on copepods, I find WoRMS an amazingly useful tool. Most importantly, it lists recorded hosts (with bibliographic details) and links to the updated taxonomy of the host. The link is reciprocal so you can also check what species of parasitic copepod have been reported from particular hosts, and where (as geographical data are also included). It also stores images, links to pdfs, DOI numbers, other websites, etc. The aim of this paper is to introduce WoRMS and determine whether the fish parasitology community is interested in developing a thematic parasitological directory within WoRMS.

FRESHWATER PARASITES OF EUROPE DATABASE: DIGENEANS IN FISH

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We will share our experience during the creation of the first database of freshwater parasites in Europe within the framework of the BioFresh EC-funded project. The total database contains: (i) distributional species-level data (25,220 host-parasite-locality records; of these 18,543 georeferenced) for parasites in 68 species of freshwater molluscs and 172 species of fish (Acanthocephala, Cestoda, Digenea, Monogenea, Nematoda; 385 species, 22,261 host-parasite-locality records, 15,987 georeferenced); (ii) species lists for each higher taxon; and (iii) annotated bibliography (1,098 references, timeframe 1924–2012). The database is compiled by taxonomists from published records from freshwater (rivers, lakes, ponds) and coastal brackish localities in 35 European countries. Taxa names are cross-checked with Fauna Europaea and other taxonomy databases, data entered are quality checked, taxonomic adjustments are made (parasite names used in original records kept in comments for each host-parasite-locality record). The dataset on fish digeneans contains 8,202 host-parasite-locality records covering 122 species belonging to 49 genera and 19 families found in a total of 148 fish species of 21 families; of these, 59 species use fish as definitive hosts. Study effort is uneven in relation to the habitats with 50, 41, 7.4 and 2% of the records originating from lake, riverine, coastal brackish and pond ecosystems, respectively. The most intensely studied group is cyprinid fishes (55% of records) followed by percids (17%) and salmonids (8.5%). Digenean taxonomic diversity is distinctly uneven with just two relatively species-rich genera: *Phyllodistomum* (10 spp.) and *Asymphylodora* (8 spp.).

TOWARDS A STABLE TREMATODE PHYLOGENETIC AND TAXONOMIC FRAMEWORK

Littlewood D.T.J., Briscoe A., Waeschenbach A., Bray R.A.

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It is now over 10 years since a comprehensive molecular phylogeny for the Trematoda (with emphasis on the Digenea) and based on nuclear ribosomal gene data, was published. The phylogeny yielded a number of insights into the evolutionary history of the group, suggested a number of higher-level taxonomic changes and raised many new questions. Meanwhile, the phylogenetic framework has also found use in incorporating hitherto unsampled taxa and guiding taxonomy. Generally, concerted collecting for molecular systematics has been driven by a need for accurate diagnostics and stable taxonomy but usually at a relatively low taxonomic level. Little effort has been expended on developing the molecular toolkit or for wider biodiversity discovery of trematodes. Importantly, relatively poor support for deeper branches in the current digenean tree of life suggests a fresh, critical approach is required. Here we discuss problems associated with current estimates of trematode phylogenies and suggest possible solutions as to how greater diversity might be sampled and phylogenetic stability achieved cost-effectively and conveniently.

PROPOSAL FOR A STANDARDIZED STYLE FOR DESCRIBING TREMATODES

Overstreet R.M.

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No standardized style for descriptions of trematodes presently exists, at least on an international basis. Standardization should perhaps not be mandatory, but it seems to be the optimal approach to provide the most useful data for both present and future researchers. It can fit into any format, which in turn will follow journal requirements. One reason that researchers submitting descriptive taxonomic works do not follow a specific style is because they do not always appreciate why some reviewers disagree with their style or because the reviewer suggests a mixture of ways or styles of describing specific features. This presentation provides a most needed style that if followed will avoid most ambiguous clauses, be clear as to meaning, be concise, allow room for an abundance of modifiers, and permit authors to realize why a style makes sense. Moreover, because recent investigations have shown that many cryptic species exist and many other species follow convergent paths, critical descriptions of as many phenotypic characters as practical should be described to best support and assess contradictory molecular sequence data from multiple taxa. Descriptions should include molecular data or specimens from which later molecular analysis could be obtained. Also, the rationale and when specific fixatives, stains, dyes, methods, supplies, techniques are preferred will be presented. Additionally presented are definitions of shapes and structures as well as a discussion of which important structures require illustration.

MOLECULAR APPROACHES TO TREMATODE SYSTEMATICS – "BEST PRACTICE" AND THE IMPLICATIONS FOR FUTURE STUDY

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Morphological examination of putative trematode species has been the cornerstone to describing their taxonomic affiliation and congeneric relationships. However, since the early-1990s, there has been increased application of molecular technologies. In particular, data obtained by direct sequencing of genetic loci has overcome problems linked to phenotypic plasticity, provided comprehensive insights into genealogical relationships, and allowed a thorough exploration of geographic variation. Early reviews on the topic called attention to several theoretical and methodological issues, from the importance of sample size to the number of loci sequenced. How far have we progressed on these matters? With time, revolutionary advances in next-generation sequencing technologies and bioinformatics now allow unprecedented opportunities to explore a range of aspects linked to the fundamental biology of parasites, on a scale that was previously unimaginable. These advances provide the opportunity to take the next step beyond traditional molecular characterisation of species using one or two loci. Here, we discuss current methods, best practice and, perhaps most importantly, what that next step is and how it may i) allow the identification of more informative genes and subsequently better diagnostic markers for species delineation, ii) provide stronger resolution in inferring phylogenetic relationships between taxa, iii) enhance our understanding of morphological evolution, iv) improve knowledge about host-parasite distribution patterns and parasite life-cycles, and v) provide insights into population level variation that could aid epidemiological investigations *via* advanced identification techniques.

WHAT DO PARASITE ECOLOGISTS NEED FROM TREMATODE TAXONOMISTS?

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Research in parasite ecology requires accurate identification of studied species, and thus depends on taxonomic tools for identification. Traditional, as well as developing areas of research, such as geographical patterns of biodiversity and phylogeography, the influence of global climate change, the role of parasites in food webs, etc., are closely tied to research in systematics and taxonomy. This dependence applies to all parasite groups, however, the complex life-cycles of trematodes and their changing morphology and required hosts across the life-cycle pose greater difficulties for ecologists and require more concentrated efforts from taxonomists. A comprehensive, regularly updated, online database of trematode diversity would be a great step forward. Although the number of described and named trematode species is always increasing, often trematodes are only identified to genus or family level in community studies, especially those at larval stages. Furthermore, the number of fully resolved life-cycles remains very small. Parasite ecology would certainly benefit if taxonomists invested at least as much effort on figuring out life-cycles as on describing new species, thus revealing pathways of trophic transmission. The use of phylogenetic information in community ecology has grown dramatically in recent years, mainly stimulated by the idea that evolutionary relationships can provide insights into historical processes shaping community assembly. We need more resolved phylogenies in most groups of trematodes to identify ecological, evolutionary, and biogeographic drivers of parasite community composition. As parasite ecology expands and evolves, it will need taxonomic descriptions of both larval and adult trematodes, knowledge of their life-cycles and their phylogenetic relationships, all more readily accessible than at present.

WORKSHOP: "BIOBANKING: A NEW APPROACH FOR IMPROVING RESEARCH ON FISH PARASITE BIOREPOSITORIES"

Chairs: Ángel F. González, Santiago Pascual & Helena Rodríguez

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The general idea around the workshop on biobanking for parasite biorepositories is the presentation and future implementation of banking platforms for samples and associated data assuring the traceability of the processes involved and the high-quality of stored samples. A major trend in environmental research consists in addressing global phenomena around the study of large series of samples organized with well-defined and detailed criteria, with the specific information required in each case. The availability of traceable samples and associated information of high quality has been a plea that has been at the root of many of the most important advances in marine science. The collection of significant numbers of marine samples and quality information involves a major effort in planning, construction, and finally operation; it is very time and resource consuming constraining the accurate development of the marine research. Furthermore, the new paradigm beyond these platforms is providing a banking network for exchange of parasite material that allow a best-value for money approach and a significant improvement for high quality research. The main distinguishing feature of biobanks with respect to the classical concept of a collection of samples and associated data is its commitment to transferring samples and associated information in an open, transparent, and partnering way for the benefit and improvement of high-quality science. The biological biobanks scheme and infrastructure we developed is expected to provide a number of outcomes that will boost the development of a network of biobanks, first in marine and freshwater parasitology and later in other scientific fields.

FORUMS

INTERNATIONAL COLLABORATIVE FORUM: "EUROPEAN CENTRE OF ICHTHYOPARASITOLOGY, CZECH REPUBLIC: PROMOTION OF A RESEARCH AND TRAINING CENTRE"

Chair: Milan Gelnar[1], Tomáš Scholz[2], Pavel Jurajda[3]

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Fish parasitology is one of the very few areas of bio-ecological research in the Czech Republic that actually plays a leading role at the international level and has the highest international reputation. ECIP – European Centre of Ichthyoparasitology – represents a follow-up of previous research centres with several inovations with a primary focus on research complementarity and formation of young researchers. As well as a more intensive mutual collaboration of all three teams involved, namely Faculty of Science, Masaryk University in Brno, Institute of Parasitology, Biology Centre of the Czech Academy of Sciences of the Czech Republic in České Budějovice (Budweis) and Institute of Vertebrate Biology of the Czech Academy of Sciences in Brno. Altogether 49 mostly young researchers including PhD students from 13 countries and four continents (Algeria, Austria, Australia, Belgium, Bulgaria, Czech Republic, India, Mexico, Russia, Serbia, Slovak Republic, UK and Ukraine) are involved.

The ECIP project is based on four research modules: (1) Diversity of parasites and adaptation to parasitism; (2) Phylogenetics and molecular taxonomy; (3) Host-parasite relationships and molecular interactions; and (4) Evolutionary epidemiology and behavioral ecology of parasites. Each of them representing a different spectrum of methodological approaches applied to studies of seven major groups of parasites (protists, myxozoans, monogeneans, tapeworms, digeneans, nematodes and parasitic crustaceans) and their host fish. Multidisciplinarity and complementarity of individual teams headed by a well balanced mixture of internationally renowned senior researchers, which form the ECIP, are considered to be the strongest aspects of this complex research project supported by the Czech Science Foundation, project No. P505/12/G112. In addition to research, advanced teaching in methods of modern systematic, ecological and evolutionary parasitology represents another key activitiy of the ECIP staff.

ORAL PRESENTATIONS

O-001

PARASITES OF THE LUMPFISH, *CYCLOPTERUS LUMPUS*, AND IMPLICATIONS WITH THEIR USE AS CLEANER FISH IN CAGED REARED ATLANTIC SALMON

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The production of juvenile lumpfish intended for use as cleaner fish in salmon sea cages is significantly increasing and in 2014, 500.000 juveniles were successfully reared in Iceland for that purpose. A comprehensive knowledge on parasites infecting the cleaner fish is vital as they could cause problems in juvenile lumpfish production and furthermore pose a risk of transmission to the cage reared Atlantic salmon.

Wild lumpfish, some of which used as broodfish for production of cleaner fish, were screened for microparasites by examination of fresh preparations, histology and by molecular methods.

Five different microparasites were found: *Nucleospora cyclopteri*, *Goussia* sp. *Kudoa islandica*, *Parvicapsula asymmetrica* and *Cryptobia dahli*. Both *N. cyclopteri* and *K. islandica* were highly prevalent, the former being highly pathogenic and possibly vertically transmitted while the latter is apparently apathogenic but causes severe post mortem myoliquefaction. *Goussia* sp. infections were associated with mortality and caused severe intestinal pathology. *Cryptobia dahli* was prevalent but had no negative effect on the fish. P. asymmetrica infections were commonly mild and without any apparent pathology.

Parasites could cause health problems in the production of cleaner fish for commercial use. Furthermore, the transmission of pathogens from cleaner fish to cage reared salmon is possible. N. cyclopteri is a potential pathogen affecting the production of juvenile lumpfish while K. islandica is non-specific with regards to fish host and could possibly infect salmon and cause spoilage of the fish product. The Goussia species identified is pathogenic to lumpfish but its host specificity is unknown.

O-002

UTILISING SENTINEL CAGED SALMON & PLANKTON TRAWLS TO ASSESS A MODEL OF PARASITE DISTRIBUTIONS IN LOCH LINNHE, SCOTLAND

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The ectoparasitic crustacean *Lepeophtheirus salmonis* is considered to be a production pest of marine salmonid aquaculture, estimated to cost the industry in Scotland almost €40 million annually. One approach to managing *L. salmonis* infestation pressure on fish stock is to understand the transport of *L. salmonis* and thus interconnectivity between sites, so that sites can better co-ordinate practices to manage lice.

We aim to demonstrate the use of sentinel caged fish and plankton trawls to provide data to assess a feature of the modelled parasite distributions in Loch Linnhe, Scotland. We also use the data to determine the spatial and temporal abundance of lice.

A bio-physical model was utilised to predict the distribution of infective lice originating from farm sites. Model validation was carried out using plankton tows and nine sentinel cages positioned at different locations within the loch during May & October 2011 to 2013.

Sentinel cage data demonstrated that infestation pressure on sentinel fish varied by location and year with the highest values recorded in 2011 (Prevalence 86.91% and intensity 7.13) and 2013 (79.78% and 3.42). Nauplii were rarely sampled (21/528) whilst copepodids were in 160/528 trawls with the highest density found in May 2011 (0.09 cops/m^3). Relative lice count information was provided by producers allowing for Spearman Rank correlations demonstrated a correspondence across all year observations and simulations between modelled rank density and observed rank of: counts (ρ(52)=0.48***), prevalence, (ρ(52)=0.47***), abundance (ρ(52)=0.46***) and intensity ρ(52)=0.42**). Observed rank plankton copepodid density also corresponded with rank modelled density ρ(528)=0.19***.

O-003

OCCURRENCE OF SEVERE EMACIATION DISEASES CAUSED BY A NEW *ENTEROSPORA* IN CULTURED GROUPER FINGERLINGS IN SOUTH CHINA SEA

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Groupers are very important cultured fish species in South China Sea. Mass mortality of cultured grouper fingerling (including *Epinephelus fuscoguttatus, E. drummondhayi*, *E. coioides* and all other cultured grouper species) occurred along Hainan coast of South China Sea since 2011. The remarkable clinical signs include extreme emaciation, white faeces, anorexia, cachexia, lethargy, empty stomach, intestinal oedema, thin intestine wall and extremely atrophic liver. The disease generally occurred in juvenile fish of 3-12 cm (especially below 7 cm) in body length, during rainy season and the prevalence and mortality varied depending on the farm, but the maximum reached up to 100%. To investigate the possible pathogenic agents responsible for this disease, a thorough parasitological examination was conducted. A new microsporidium infecting the nucleus of intestinal epithelial cells was suspected to be responsible for this emaciation and white faeces syndrome of groupers. Histopathological analysis showed that this microsporidian induced severe pathological changes including accumulation of cell debris between the epithelium and lamina propria and severe detachment of intestinal mucosa, and degeneration of the epithelium associated with massive infiltration of inflammatory cells. The plasmodium contains several diplokarya and displays polysporous development, which occurs without an interfacial envelope. Based on the morphological, histopathological, ultrastructural and molecular evidences, a new *Enterospora* species is thought to be involved in the syndrome. This is the first intestinal microsporidian reported in fish from China. Further studies are needed, considering that grouper fingerlings bred in Hainan province are becoming an important source for cage-cultured groupers in South East Asia.

O-004

THREE SPECIES OF MONOGENEAN PARASITES EMERGING IN CULTURED MEAGRE (*ARGYROSOMUS REGIUS* ASSO, 1801) (TELEOST: SCIAENIDAE) FROM WESTERN TURKEY

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The first commercial production of meagre (*Argyrosomus regius*) was established in 2005 in Turkey. Farmers reported occurrence of scale losses and skin lesions already during the first season. Based on morphological characters, *Benedenia sciaenae* was identified from infected fish in that farm. Nowadays, in two different commercial companies, meagre fish started to show non-specific disease signs, such as lethargy, emaciation, gill anaemia and mortality. Thirty specimens of meagre from each farm were sampled for parasitological analysis. The microcotylid monogenean *Sciaenacotyle panceri* and *Calceostoma glandulosum* Johnston *et* Tiegs, 1922 were found on the gills of meagre from one cage farm. The prevalence of infection of these two monogeneans was 100 % and the mean intensity of infection was 1125 ± 73 and 8 ± 2, respectively. *Diplectanum sciaenae* was observed on the gill of meagres from the other farm. The prevalence and mean intensity of infection was 100 % and 1125 ± 75. Measurements were made using an ocular micrometer and drawings were made with using a camera lucida. No protozoan or other metazoan parasites were found.

O-005

CALIGUS ROGERCRESEYI SENSITIVITY AND TREATMENT EFFICACY OF ANTI-LOUSE CHEMICALS IN CHILE

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The sea louse *Caligus rogercresseyi* is a severe threat to the salmonid industry of Chile. Control of the parasites relies on chemical treatments, but reduced treatment efficacy due to resistance has been reported towards pyrethroids (deltamethrin) and emamectin benzoate (Bravo et al. 2008, Helgesen et al. 2014). An organophosphate, azamethiphos, was introduced in 2013 to improve treatment efficacy. The aims of the present study were to assess the sensitivity of *C. rogercresseyi* towards these three chemicals using bioassays, and to explore the correlation between the bioassay results and the treatment efficacies.

The study was performed in four fish farms in regions X and XI in Chile, from May 2013 to May 2014. Bioassays were carried out on adult farm-collected lice as described in Helgesen et al. (2014) (expressed as EC$_{50}$). Lice abundance was monitored weekly and treatment efficacy calculated. Sensitivity towards emamectin benzoate was low and similar in the four locations (EC$_{50}$ 125-327 ppb). The sensitivity towards deltamethrin varied; more resistance was found in the mid-west of region X (EC$_{50}$ 2.68-3.34 ppb). An early stage of resistance development towards azamethiphos was seen in the farms located in region X (EC$_{50}$ 0.88-2.36 ppb). Pyrethroids (deltamethrin and cypermethrin) and azamethiphos were the most commonly applied treatments. Efficacy of azamethiphos treatments ranged between 92% and 100%, in accordance with the sensitivity level showed by the bioassays. Pyrethroid treatment efficacy ranged between 38% and 79% in the farm with the highest EC$_{50}$-values and between 60% and 100% in the farms with the lowest EC$_{50}$-values.

O-006

LEPEOPHTHEIRUS SALMONIS (COPEPODA: CALIGIDAE) RESPONSES TO AZAMETHIPHOS AND CYPERMETHRIN

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Lepeophtheirus salmonis (sea lice) are the most economically important parasite of salmonid aquaculture in the Northern hemisphere. Veterinarians are limited to few therapeutic options for sea lice control and consequently, overuse of these products has lead to drug resistant populations. Two of these compounds, azamethiphos and cypermethrin, are still used globally despite the declining sensitivity of sea lice to their effects. To better understand the response dynamics of these treatments, sea lice were exposed to multiple doses of azamethiphos or cypermethrin using *in vitro* bioassays. Both larval and adult sea lice were exposed to either treatment or a seawater control for 24hrs. Lice survival was assessed before collection for transcriptional analysis using a 38K oligonucleotide microarray. Principal component analysis indicated differential expression due to drug exposure was minor compared to differences between sexes and stages of sea lice. Cypermethrin and Azamethiphos induce a similar gene expression response, with genes involved in stress response being upregulated compared to control. A distinct gene expression profile was also noted between those lice surviving treatment compared to those observed to be moribund post-24hrs treatment. Traditional drug response biomarkers showed differences between sexes and stages however neither treatment caused upregulation of these transcripts. These data show that constitutive expression differences between sexes and stages impact drug response in sea lice.

O-007

SINGLE MUTATION RESPONSIBLE FOR RESISTANCE TOWARDS ORGANOPHOSPHATE (AZAMETIPHOS) IN SALMON LICE (*LEPEOPHTHEIRUS SALMONIS*)

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Acetylcholinesterase (AChE) is the primary target for organophosphates (OP). OP has been used against salmon lice (*Lepeophtheirus salmonis*) in Norwegian salmonid aquaculture since late 1970s. Similar to other arthropods, frequent use of OP resulted in the development of resistance in *L. salmonis* against them in the 1990s*,* leading to a major economic loss afflicting the aquaculture industry. Understanding and unravelling the biochemical pathways underlying resistance in *Lepeophtheirus salmonis* against OP is, therefore, the need of the hour.

In the present study, full length cDNA sequences encoding two AChEs in *Lepeophtheirus salmonis* were identified and fully characterized. Interestingly, in contrast to most other arthropods, *Lepeophtheirus salmonis* has two different forms of *ace1* gene (*ace1a* and *ace1b*) with no orthologous of *ace2* gene.

The screening of whole cDNA sequences of *ace1a* and *ace1b*, in sensitive and azamethiphos-resistant sea lice samples, led to the identification of a missense mutation *Phe362Tyr* in *ace1a*.

Significantly higher frequency of the mutant allele (*362Tyr*) in the resistant strains (5 strains) compared to sensitive strains (2 strains) indicated the association of *Phe362Tyr* with resistance towards azamethiphos. The 3D modelling, short term survival experiments and enzymatic assays further supported the imperative role of *Phe362Tyr* in reduced sensitivity of *L. salmonis* towards azamethiphos.

In collaboration with PatoGen Analyse AS, a rapid diagnostic assay was developed for high throughput screening of *Phe362Tyr*. The epidemiological data from various fish farming sites in Norway (n = 48) collected from 2012 to 2014 further validated that *Phe362Tyr* alone is responsible for resistance in *L. salmonis* towards azamethiphos in Norway.

O-008

THERAPEUTIC AND BIOLOGICAL CONTROL MEASURES FOR CRUSTACEAN PARASITES (*CALIGUS* SP.) ON SEABASS (*LATES CALCARIFER*) IN MALAYSIA

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Studies of the biology of sea lice have been conducted from various perspectives in the world. However, this information is limited in Malaysia; most of the published literature has centered on the economically importance and on a wide range of species. This research has focused on identification, prevalence, studying developmental stages under different environmental conditions (temperature, salinity, nitrite, and alkalinity), behavior, including the application of genetic methods. Herbal extract (neem oil *Azadirachta indica*) has been used to control *Caligus* sp on Seabass. A total of 1074 specimens of caligids were isolated throughout the study. The results showed that there have five different species, including *C. minimus, C. epidemicus, C. rotundigenitalis, C.absen* and *C.robotus.* Their presences were observed within a narrow salinity range (25–28ppt) warm temperature (28.2-29.6 ^{0}C) Nitrite (0.25mg/l) and high alkalinity (60ppm). *Caligus* sp showed site preference for attachment which was at the body surface and fins of the fish. With different alkalinity concentrations, fecundity of C.*minimus* varied from 55.9±4.3 to 66.7±3.9 eggs and hatching rate is from 0 to 85.27±3.81 %, the highest is at 70 ppm treatment. The population of *C. minimus* strongly fluctuates among treatments; the highest dynamic is at 70 ppm of alkalinity. The study also determined LC50 of Caligus, Seabass with neem oil is 2ppt and 20ppt, respectively and neem oil can be used to control Caligus infection on Seabass at 5ppt.

O-009

AKIRIN-BASED CHIMERIC PROTEIN: POTENTIAL CANDIDATE VACCINE AGAINST SEA LICE PARASITES

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Sea lice ectoparasites are the most widely distributed marine pathogens in salmon industry. Vaccination could be an environmentally friendly alternative for sea lice control but is still in an early stage. P0 protein is a structural component of ribosomes in all organisms and essential for cell viability. Recent results in tick suggest that it might be a potential antigen candidate. Furthermore, the nuclear factor subolesin/akirin/my32 protein is highly conserved from insects to mammals, and plays an essential role in the innate immune system. We characterized the my32 gene/protein from two important sea lice species, *Caligus rogercresseyi* and *Lepeophtheirus salmonis*, based on cDNA sequence isolation, phylogenetic relationships, three dimensional structure prediction and expression analysis. We also demonstrated a 56 % of protection against sea lice in a vaccine trial in *Salmo salar* with my32 from *C. rogercresseyi.* In addition, vaccination with purified recombinant my32 from *L. salmonis* elicited a specific antibody response in mice and fish. These results suggest that the combination of these good candidate antigens could be a promising target against sea lice infestations. In this sense we design two chimeric proteins based on my32 protein and a 35 amino acid peptide from ribosomal P0 protein. Immunization in tilapias (*Oreochromis niloticus*) with these chimeric antigens elicits specific antibodies anti-my32 and anti-P0 which provide evidence of the dual effect of a chimeric vaccine. Based on immune response we demonstrate that the antigens in this study appear to be potential candidates for continued development of a vaccine against sea lice infestations.

O-010

WHAT HAPPENS TO REEFS WITHOUT CLEANER FISH?

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The known benefits of fish cleaning in reef communities are increasing. An ongoing experiment, involving the removal of cleaner wrasse *Labroides dimidiatus* from seven reefs and left undisturbed on nine reefs (64-285 m^2) for 15 years, has shown 1) decreases in the abundance and diversity of large visiting and small resident fishes, and abundance of recruited residents, juvenile visitors, and juvenile conspecifics; 2) a decrease in growth and increase in parasitic copepods in individuals of a resident species; and 3) a skew in the size frequency distribution of individuals towards more small fish in two resident species. However, the mechanisms involved in these community-wide and individual effects remain unclear. Each cleaner wrasse eats ~1200 parasitic gnathiid isopods a day. An increase in these blood-feeders could explain the observed effects on fish. Therefore, we tested whether their infection rate differed with cleaner presence. As clients, we used a resident (damselfish, *Pomacentrus amboinensis*) and a visitor (wrasse, *Hemigymnus melapterus*). On the experimental reefs, fish were placed in traps (~12h, day/night) which gnathiids could enter but not escape. Few gnathiids (0-3) infected damselfish, with more at night; and they were not affected by cleaner presence. In contrast, more gnathiids infected wrasse; with more on cleaner-absent (0-179) than on cleaner-present (0-14) reefs in the day; at night there was no cleaner effect (0-25, 0-24, respectively). This is the first demonstration that cleaner wrasse affect gnathiid infection rates. We suggest gnathiids are a causative agent for some of the observed changes in fishes.

35 YEARS OF ISOLATION AND HEATING CAUSES PARASITE INDUCED SHIFT OF THE SELECTION REGIME ON IMMUNE GENES (MHC GENES) IN PERCH (*PERCA FLUVIATILIS*)

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Genes that play key roles in host immunity such as the major histocompatibility complex (MHC) in vertebrates are expected to be major targets of selection. Environmental conditions affect host–parasite interactions and thus also selection on MHC. We analyzed MHC class IIß variability over 35 years in a population of perch (*Perca fluviatilis*) that was split into two populations. One population was subjected to heating from cooling water of a nuclear power plant and was isolated in an artificial lake, while the other population was not subjected to any change (control). The isolated population experienced a change of the allelic composition and a decrease in allelic richness of MHC genes compared to the control. The two most common MHC alleles showed cyclic patterns indicating ongoing parasite–host coevolution in both populations, but the alleles differed between the two populations. No such patterns were observed at alleles from nine microsatellite loci, and no genetic differentiation was found between populations. Differences in parasitism of the current perch populations suggest that a change of the parasite communities had occurred over the isolation period

We conclude that perch in the enclosed and heated artificial lake have experienced strong selection on immune genes induced by a shift in the parasite community as a result of the exceptionally high temperature in the artificial lake. The dynamics of MHC alleles over time however changed in variable ways indicating that the biological effects of a change in environmental conditions are to a large extent unpredictable.

LONG-TERM INFECTION DYNAMICS OF *OLIGOGONOTYLUS MANTERI* IN ITS INTERMEDIATE AND DEFINITIVE HOSTS FROM CELESTUN, YUCATAN, MEXICO

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Olygogonotylus manteri, is a digenean using the mayan cichlid fish *Cichlasoma urophthalmus* as definitive and second intermediate host, and the hydrobiid snail *Pyrgophorus coronatus* as first intermediate host. This trematode species has been one of the most frequent (mean prevalence 92± 10.1) and abundant (mean 27± 29) parasite species in the mayan cichlid along 10 years of monthly samples at Celestun, Yucatan, Mexico. It has been also a prevalent species in the snail host samplings throughout the last 6 years (1.0±1.2). The aim of this study was to identify temporal patterns of trasmission of *O. manteri* among hosts, and the potential effect of environmental conditions over these patterns through time. Fourier time series analysis was use to identify infection peaks through time and cross correlation among enviromental variables and infection parameters. This statistical technique was useful to identify the key time lags in the trasmission of *O. manteri*.

O-013

EFFECTS OF ENVIRONMENTAL STRESS ON HOST-PARASITE INTERACTIONS IN THE STICKLEBACK-*SCHISTOCEPHALUS* SYSTEM

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Global environmental change has considerable potential to influence the interactions between hosts and parasites, with consequences for parasite development, transmission and life cycle completion rates. Here we present results from recent experimental studies examining how environmental stressors – increased water temperature, reduced dissolved oxygen (DO) and endocrine disrupting chemicals (EDCs) – affect host-parasite interactions in three-spined sticklebacks experimentally infected with Schistocephalus solidus. The effects of temperature were dramatic. Over a 16-week post-infection period, a 5C temperature increase generated a fourfold increase in plerocercoid growth rate in experimentally infected fish, with all parasites reared in fish held at 20C developing infective worms, compared to none held at 15C. Furthermore, infected fish exhibited behavioural preferences for warmer temperatures, potentially generating a feedback loop accelerating parasite growth. An environmentally relevant EDC (17b-estradiol, E2) also affected the growth of plerocercoids in sticklebacks, with parasites growing most quickly in male hosts, suggesting sex differences in the consequences of EDCs for host-parasite interactions. The effects of Schistocephalus on the expression of host genes associated with the hypoxic response were also examined under normoxic and reduced DO conditions. Expression patterns were influenced by infection status, with the physiological responses to hypoxia of infected fish being impaired, with likely consequences for the survival of infected fish in hypoxic environments. Our results from this ideally suited model experimental system demonstrate that anthropogenic global changes have considerable potential to influence the outcome of host-parasite interactions in fish, with significant consequences for disease phenotypes and for life cycle completion rates under perturbed environments

O-014

POTENTIAL EFFECT OF CLIMATE IN THE TEMPORAL VARIATION OF *MEXICONEMA CICHLASOMAE* IN THE MAYAN CICHLID *CICHLASOMA UROPHTHALMUS* IN CELESTUN, YUCATAN, MEXICO

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Little is known about the temporal variability of tropical fish parasites. This is currently an issue in the tropics due to the potential effect of Global Climate Change (GCC) on parasite dynamics. Indeed, there is a lack of information on key environmental factors affecting parasite abundance through time. Rainfall has been suggested as a key factor because the host-parasite system experience variability due to annual patterns of precipitation. A further complication in the tropics is the influence of disturbances occurring at different time scales (e.g. El Niño Southern Oscillation (ENSO) 3-5 years), for which it is necessary to carry out long-term observations. The aim of our study was to determine the influence of environmental factors in the infection parameters of *Mexiconema cichlasomae* in its intermediate *Argulus yucatanus* and definitive hosts *Cichlasoma urophthalmus* from a tropical lagoon over a period of 10 years. Environmental factors and infection parameters of *M. cichlasomae* were analyzed using wavelets to determine potential recurrent patterns. Infection parameters of *M. cichlasomae* showed peaks every 6, 12, 24 and 60 months. The peaks of maximum variability were related with fluctuations of salinity, rainfall and temperature, suggesting the influence of ENSO. The present work showed a synergism among different environmental factors acting at a long temporal scale which in turn affect this host-parasite system. The temporal variability of infection parameters of *M. cichlasomae* expand over periods longer than a year suggest the need for long-term data sets in studying temporal variability in infection levels in the context of GCC

O-015

GEOGRAPHICAL DISTRIBUTION PATTERNS OF MONOGENEAN PARASITES OF BUTTERFLYFISHES (CHAETODONTIDAE) IN THE SOUTH PACIFIC

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Butterflyfishes are the iconic species of coral reefs. Many species are sympatric in the reefs. They host many species of parasites among which monogeneans of the subfamily Ancyrocephalinae. These direct life cycle parasites live on the gills and are generally regarded as very specific. We have studied the monogenean communities of over twenty species of Chaetodontidae in the South Pacific region (Australia, Palau, New Caledonia, Wallis, French Polynesia). Our results show a relative conservatism of the distribution pattern of the monogenean species found throughout the host community. Thus, some host species are never or almost never parasitized while others are consistently abundantly parasitized. Besides the fact that such results are in contradiction with the traditionally observed distribution patterns of species diversity in this region, i.e. decreasing gradient from west to east species, our results raise some questions about monogenean specificity. We will comment these results taking into account host phylogeny trying to identify why some species are never parasitized despite the fact that they live in sympatry with heavily infected ones.

O-016

BIOGEOGRAPHY OF PARASITISM IN FRESHWATER FISH: SPATIAL PATTERNS IN HOT SPOTS OF INFECTION

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Contrary to species occurrence, little is known about the determinants of spatial patterns of intraspecific variation in abundance, particularly for parasitic organisms. In this study, we provide a multi-faceted overview of spatial patterns in parasite abundance and examine several potential underlying processes. We first tested for a latitudinal gradient in local abundance of the regionally most common parasite species and whether these species achieve higher abundances at the same localities (shared hot spots of infection). Secondly, we tested whether intraspecific similarity in local abundance between sites follows a spatial distance decay pattern or is better explained by variation in extrinsic biotic and abiotic factors between localities related to local parasite transmission success. We examined the infection landscape of a model fish host system (common and upland bullies, genus *Gobiomorphus*: Eleotridae) across its entire distributional range. Our results showed that the most widely distributed parasites of bullies also achieve the highest mean local abundances, following the abundance – occupancy relationship. Variation in local abundance of any focal parasite species was independent of latitude, the abundance of co-occurring species and spatial distance or disparity in biotic attributes between localities. For only one parasite species, similarity of abundance between sites covaried with the extent of abiotic differences between sites. The lack of association between hot spots of infection for co-occurring species reinforces the geographic mosaic scenario in which hosts and parasites coevolve by suggesting non-deterministic, species-specific variation in parasite abundance across space.

O-017

ECOLOGY *VERSUS* PHYLOGENY AS DETERMINANTS OF HOST SPECIFICITY IN TRYPANORHYNCH TAPEWORMS OF SHARKS AND RAYS

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This study explores non-metric multidimensional scaling (nMDS) as a tool for investigating parasites as indicators of the biology of their hosts. A particularly novel and attractive feature of nMDS is its ability to allow assemblage-level parasite data to be simultaneously applied to questions of host biology. The method was examined using trypanorhynch tapeworms, which are known to be transmitted among their hosts by way of the food web (i.e, via predation), can unambiguously be identified in the intermediate and final hosts, and thus trypanorhynchs have obvious potential as indicators of host feeding biology. Analyses conducted in this study were focused on the trypanorhynch assemblages in the elasmobranch as definitive hosts. The relationship between trypanorhynch assemblage and investigated factors for all sharks were complicated but we noted that depth, together with diet and environmental realm, were the major influence factors. Based on analyses, we found several species of sharks had different character from their description that could be caused by change of shark behaviour or trypanorhynch host path. The relationship between trypanorhynch assemblage and investigated factors for carcharhiniform species was more robust than the relationship of trypanorhynch assemblage and all sharks. Among the factors investigated in the carcharhiniform analysis, the relationship between environmental realm and trypanorhynch assemblage was most remarkable. The low host specificity in this group of tapeworms results in a correlation between the worm assemblage and the host ecology, masquerading the long host-parasite co-evolution and elasmobranch phylogeny.

O-018

PREY AND PARASITES AS INDICATORS FOR THE ROLE OF *ETMOPTERUS SPINAX* IN LOCAL FOOD WEBS

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The role of small benthic deep-sea sharks may be crucial for characterising and understanding deep-sea ecosystems which is essential for management and protection measures considering prevailing anthropogenic impacts on these systems. By combining an examination of stomach contents yielding a snapshot of the most recent trophic niche and the structure of parasite communities reflecting a long-term feeding niche, the main goal of this study was gaining more comprehensive information on the role of the small-sized deep-water velvet belly lantern shark *Etmopterus spinax* in the local food webs of the Galicia Bank and the canyon and valley system of the Avilés Canyon (both NE Atlantic). Component parasite communities in *E. spinax* were relatively rich, whereas the infracommunities were rather depauperate, with similar low diversity at both localities. Analyses revealed significant differences in the composition and structure of both prey assemblages and parasite communities between the two areas, thus indicating differential effects of the two deep-sea ecosystems on both the recent and long-term trophic niches of *E. spinax*. These results underline the importance of the use of multivariate analyses for the assessment of geographical variation in shark populations based on diet data and parasites. To the best of our knowledge, this study provides the first comparative parasite infracommunity data for a deep-sea shark species.

O-019

A PARASITOLOGICAL SURVEY OF *GALEUS MELASTOMUS* RAFINESQUE, 1810 FROM DEEP WATERS OF THE NW MEDITERRANEAN SEA IN RELATION TO DIET AND HEALTH CONDITION OF THE HOST

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Despite their importance and vulnerability, deep-dwelling elasmobranchs are still poorly understood. In this context, this study aims to perform a parasitological survey of the blackmouth catshark *Galeus melastomus* Rafinesque, 1810 in deep waters of the north-western Mediterranean Sea describing variability on the parasite community between host maturity stages and its relation to diet and health condition of the host. A total of 120 specimens of *G. melastomus* were captured in 2010-2011 at 400-1,400 m depth in three different locations of the Balearic basin using a semi-balloon otter-trawl. Parasitological, dietary, enzymatic stress indicators and histological data were obtained according to standardized protocols. Main relationships among fish parasite load, size, condition indices, enzymatic activity of muscular acetylcholinesterase, lactate dehydrogenase and citrate synthase and intensity of hepatic melano-macrophages were tested. A total of 16 different parasite taxa were found: one coccidian, one monogenean, one digenean, five cestodes, seven nematodes and one copepod. The most important parasites in terms of prevalence and abundance showed a significant relationship with host size, probably reflecting hosts ontogenic diet shifts: the digenean *Otodistomum* sp., the cestodes *Scolex pleuronectis* and *Grillotia* cf. *adenoplusia* and the copepod *Eudactylina* cf. *vilelai* were linked to adult sharks, while the cestode *Ditrachybothridium macrocephalum* characterized juvenile hosts. Overall results considering associations between parasite infection parameters and health condition of their hosts yield the conclusion that, in the present study, the intensity of parasite infections does not negatively affect fish health.

The study was supported by the MICINN project ANTROMARE (CTM2009-12214-C02-02)

O-020

MOLECULES FOR ECOLOGY: DIVERSITY AND DISTRIBUTION OF *LEPIDAPEDON* SPP. IN DEEP-SEA FISHES OF THE WESTERN MEDITERRANEAN

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Totals of 652 fish belonging to four species: *Lepidion lepidion* (n = 262), *Mora moro* (n = 77), *Phycis blennoides* (n = 217) and *Trachyrincus scabrus* (n = 96) were collected using bottom trawling at depths of 400–1,800 m in the western Mediterranean between the Balearic Islands and the Catalonian coast of Spain. Specimens of *Lepidapedon* spp. were recovered from all host species with prevalence ranging between 27% (*M. moro*) and 81% (*P. blennoides*). Partial fragments of the mitochondrial *nad*1 gene were amplified for representative samples from each host. Combined molecular and morphological analyses confirmed the presence of *Lepidapedon desclersae* in the four hosts and *Lepidapedon guevarai* in *L. lepidion*, *T. scabrus* and *P. blennoides*. Totals of 17 and 6 unique *nad*1 haplotypes were identified for *L. desclersae* and *L. guevarai*, respectively. The most abundant haplotype of *L. desclersae* was recovered in *L. lepidion*, *M. moro* and *T. scabrus* predominantly from off Balearic Islands at depths of 1,000–1,200 m, whereas the most abundant haplotype of *L. guevarai* was found in *P. blennoides* from off Barcelona and at depths of 400–1,000 m. Analysis of the entire dataset revealed significant effects of host and depth on infection parameters of the two species, *L. desclersae* being most prevalent and abundant in *L. lepidion* and at depths of 1,400–1,800 m and *L. guevarai* predominating in *P. blennoides* and at depths of 400–1,000 m.

The study was supported by the MICINN project ANTROMARE (CTM2009-12214-C02-02) and the project ECIP (P505/12/G112).

O-021

TAXONOMY, HOST-SPECIFITY AND BIOGEOGRAPHY OF THE GENUS *HAMACREADIUM* LINTON, 1910 (TREMATODA: OPECOELIDAE), FOUR NEW SPECIES AND EVIDENCE FOR A CRYPTIC SPECIES COMPLEX

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The opecoelid trematode *Hamacreadium mutabile* Linton, 1910 is the only reported species of its genus from Australian waters. The current taxonomy suggested *H. mutabile* has a cosmopolitan distribution and an exceptionally low host-specificity, whereas most other nominal *Hamacreadium* species are represented by few published records. We compared sequences of *H. mutabile* from its type-host and type-locality, the grey snapper, *Lutjanus griseus*, Florida, with 52 sequences generated from specimens collected from a range of lutjanids from the Indo-West Pacific. Together with morphological examination of 697 specimens, these sequence data provide evidence for a cryptic complex of *Hamacreadium* species from the Indo-West Pacific, none of which represent *H. mutabile*. Following an extensive review of all past records of *Hamacreadium* species and with considerations for host-usage and biogeography, we present a redefinition of the genus and revise its composition. We conclude, contrary to numerous previous reports, that *H. mutabile* does not occur beyond the West Atlantic, and that the definitive host-range of species of *Hamacreadium* is overwhelmingly restricted to shallow-water piscivorous lutjanids. We argue that only three of 41 nominal species can be recognised as belonging to the genus and add two new combinations and four new species. Finally, we emphasise replication and the importance of considering morphology, sequences, host-usage and biogeography in parallel when proposing new trematode taxa or revising problematic ones such as *Hamacreadium.*

O-022

MORPHOLOGICAL DIVERSITY *VERSUS* MOLECULAR SIMILARITY: THREE NEW BLOOD FLUKES (APOROCOTYLIDAE) FROM BALI AND AN APPARENT CASE OF RAPID DIVERSIFICATION

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Investigations into the fish blood fluke (Digenea: Aporocotylidae) fauna of the tropical Indo-west Pacific region revealed a number of new species in fishes of the order Tetraodontiformes, including a complex of species in pufferfishes of the genus *Arothron*. Three new species were discovered from waters off Bali, Indonesia, one of which infected the narrow-lined puffer *Arothron manilensis* and the spiny blaasop *Tylerius spinosissimus*, whereas the other two infected the reticulated puffer, *A. reticularis*. The three taxa differed dramatically from each other, particularly in the morphology and number of testes. However, they also shared some potentially synapomorphic features, including an auxiliary seminal vesicle. Analysis of the partial 28S and ITS2 rDNA regions showed the three taxa form a clade together with the two other tetraodontid-infecting aporocotylids for which sequences are known, to the exclusion of all others. The morphological disparity between taxa in this clade is at odds with the molecular divergence between them, suggesting an episode of rapid morphological diversification. This phenomenon is in contrast to other clades within the Aporocotylidae, which display retention of morphological features despite higher levels of genetic divergence. This issue of morphological diversity *versus* molecular relatedness has implications for the generic-level classification for the taxa in this clade; should they be placed in separate genera, as might be suggested by morphology, or in a single genus as might be suggested by genetics?

O-023

TWO NEW SPECIES OF *MACVICARIA* GIBSON & BRAY, 1982 (DIGENEA: OPECOELIDAE) FROM SPARID FISHES IN THE WESTERN MEDITERRANEAN IDENTIFIED BASED ON MORPHOLOGICAL AND MOLECULAR DATA

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The genus *Macvicaria* Gibson & Bray (1982) is a one of the largest digenean groups parasitic in marine fish throughout the world ocean. As part of a study of the diversity of digeneans parasitising sparid fish off the Tunisian coasts we found that *Diplodus annularis*, *Lithognathus mormyrus*, *Oblada melanura*, *Sparus aurata* and *Spondyliosoma cantharus* were infected with species of the genus *Macvicaria.* This study aims to identify *Macvicaria* spp. using morphological and molecular evidence. A total of 237 fish was sampled in Bizerte Lagoon and the nearby Bay of Bizerte. Live digeneans were fixed in molecular grade ethanol for both DNA isolation and morphological study. Partial sequences of the 28S rRNA gene and/or complete sequences of the ribosomal ITS1-5.8S-ITS2 gene cluster were amplified for 20 isolates. The newly generated sequences were analysed with sequences from GenBank using neighbour-joining, maximum likelihood and Bayesian inference methods. Detailed examination of the digenean morphology allowed us to identify three species of *Macvicaria*: one from *Oblada melanura*, one from *Sparus aurata* and *Lithognathus mormyrus* and one from *Diplodus annularis* and *Spondyliosoma cantharus.* Phylogenetic analyses strongly corroborated the morphological evidence and provided strong support for the distinct species status of the three species. Detailed comparative analyses revealed that specimens from *Sparus aurata* and *Lithognathus mormyrus* and those from *Diplodus annularis* and *Spondyliosoma cantharus* represent species new to science. Our results highlight the need for the use of a combined approach involving morphological and molecular analysis in solving the problems in species delimitation within the genus *Macvica.*

O-024

A COMPLEX OF SPECIES RELATING TO *PARADISCOGASTER GLEBULAE* (DIGENEA: FAUSTULIDAE) IN CHAETODONTID FISHES (TELEOSTEI: PERCIFORMES) OF THE GREAT BARRIER REEF

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A total of 1523 individuals of 34 species of chaetodontids from the Great Barrier Reef were examined for faustulid trematodes. Specimens resembling *Paradiscogaster glebulae* Bray, Cribb & Barker, 1994 were found in nine chaetodontid species at three localities. These species are shown, on the basis of combined morphological and molecular analysis, to comprise a complex of morphologically similar and partly cryptic species. The complex may comprise as many as six distinct species of which three are resolved here. The true *P. glebulae* is identified in *Chaetodon ornatissimus* Cuvier, *C. aureofasciatus* Macleay, *C. plebeius* Cuvier, *C. rainfordi* McCulloch and *C. speculum* Cuvier, and two new species are described. The host specificity of the three species differs strikingly. *Paradiscogaster* type 1 infects just one fish species, *P. glebulae* infects species of only one clade of *Chaetodon*, and *Paradiscogaster* type 2 infects quite unrelated species. The basis of this unusual pattern of host-specificity requires further exploration. Two of the species recognised here, *P. glebulae* and *Paradiscogaster* type 2 showed apparent intraindividual variation in the ITS2 rDNA sequences as demonstrated by clear, replicated double peaks in the electropherograms.

O-025

A NEW SPECIES OF *CARDICOLA* (TREMATODA: APOROCOTYLIDAE) FROM INDO-PACIFIC BUTTERFLYFISHES

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A single blood fluke species, *Cardicola chaetodontis*, is known in butterflyfishes (Chaetodontidae) on the Great Barrier Reef (GBR). Molecular studies have demonstrated a very low host-specificity for this species; it infects 17 of the 18 *Chaetodon* species examined on the GBR. We used ribosomal DNA to explore the composition and host-specificity of *Cardicola* in French Polynesian butterflyfishes relative to that for the GBR. Blood flukes morphologically consistent with *C. chaetodontis* were found infecting chaetodontids in the Society and Tuamotu Archipelagos. A narrower host range was identified in French Polynesia, with infections found in just seven of 18 species examined. Eight *Chaetodon* species, two *Heniochus* species and a single *Hemitaurichthys* species were not infected. Previous reports mainly found adults infecting the heart, whereas in French Polynesia they were found primarily in the gills. Additionally, the French Polynesian specimens regularly infected *Forcipiger*, whereas GBR reports note the absence of *Cardicola* in this genus. Molecular characterisation of French Polynesia specimens showed that, contrary to expectations, two genotypes occur in French Polynesia, one only in *Chaetodon pelewensis* (Genotype 1) and another in four species of two chaetodontid genera (Genotype 2). Genotype 1 matched sequences from the GBR and presumably represents the same species. Genotype 2 differs consistently from the GBR sequences by 3–4 bases and is interpreted as a novel cryptic species. These findings are contrary to the previously reported west-to-east reduction in trematode richness in the Pacific.

O-026

FIRST MOLECULAR DATA FOR DIGENEANS FROM ANTARCTIC FISHES

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Four fish species of the families Nototheniidae and Bathydraconidae were examined from the Prince Gustav Channel off James Ross Island (Weddel Sea, West Antarctica) during two Czech Antarctic expeditions (2013 and 2014) at depths of 5–30 m. Nine digenean species, including eight endemic to Antarctica, were recovered: six in *Trematomus newnesi* (overall prevalence, P = 65%), five each in *Notothenia coriiceps* (P = 57%) and *Trematomus bernacchii* (P = 53%), and three in *Trematomus hansoni* (P = 17%). Representative specimens of each digenean species were characterised morphologically and molecularly; these included the type-species of *Elytrophalloides* Szidat, 1955 and *Neolebouria* Gibson, 1976. Partial fragments of the 28S rRNA gene were amplified for a total of 40 isolates and were analysed together with sequences for related species of the families Opecoelidae Ozaki, 1925 and Lepidapedidae Yamaguti, 1958 and the superfamily Hemiuroidea Looss, 1899 available on GenBank. Phylogenetic analyses confirmed the identification based on morphology and revealed: (i) close relationship between the hemiurid subfamilies Glomericirrinae Yamaguti, 1958 and Elytrophallinae Skrjabin & Guschanskaja, 1954; and (ii) paraphyly of the subfamily Elytrophallinae Skrjabin & Guschanskaja, 1954 (Hemiuridae Looss, 1899) and the genera *Macvicaria* Gibson & Bray, 1982 and *Neolebouria* Gibson, 1976 (both Opecoelidae). The taxonomic implications of these results will be discussed.

This study was supported by the Czech Science Foundation (project P505/12/G112). We acknowledge the Czech Antarctic Station "J. G. Mendel"and its crew for their support.

O-027

PHYLOGENETIC POSITION OF A FISH BLOOD FLUKE (TREMATODA: APOROCOTYLIDAE) INFECTING A BIVALVE

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A fork-tailed cercaria was found developing within minute, spherical sporocysts in the gonad of an intertidal bivalve, *Donax deltoides*, from Stockton Beach, central New South Wales, Australia. The cercaria resembles *Cercaria asymmetrica* Holliman, 1961, which infects *Donax variabilis* in the Gulf of Mexico. In Bayesian inference and Maximum Likelihood phylogenetic analyses of 28S rDNA, the current species formed a strongly supported clade with *Chimaerohemecus trondheimensis* van der Land, 1967, a species that infects holocephalans. All of the marine bony fish aporocotylids for which molecular data are available form a strongly supported clade sister to the present species + *C. trondheimensis*. This phylogenetic topology, considered in the light of known aporocotylid intermediate host identities, predicts that the present species, and perhaps many bivalve-infecting species, are parasites of chondrichthyans; no intermediate host for a chondrichthyan-infecting species has been reported. We speculate that there may be basal distinctions in the identity of the intermediate hosts of aporocotylids of chondrichthyans (which infect bivalves) and those of marine and freshwater bony fishes (which infect polychaetes and gastropods respectively). The identity of the definitive host of the present species is unknown. So far, representatives of four chondrichthyan orders have been identified as hosts of aporocotylids, and representatives from all four orders range off New South Wales.

O-028

PHYLOGENETIC RELATIONSHIPS WITHIN THE BUCEPHALIDAE POCHE, 1907 (PLATYHELMINTHES: DIGENEA) – IMPLICATIONS FOR FUTURE STUDY

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This study is the first to conduct an in-depth phylogenetic analysis of sequence data representing species of the Bucephalidae Poche, 1907 (Platyhelminthes: Digenea). Previous reports using sequencing methods have focused on ITS rDNA to augment and enhance morphology-based taxonomy and link life-cycle stages. Here, we analysed 37 sequences (25 are new), representing the D1-D3 region of the large subunit (28S) of rDNA, from 28 species in nine genera and four subfamilies. Bayesian inference analysis illustrated that i) subfamily classifications based entirely on morphological characters are broadly robust, ii) the sequence representing a new species of *Heterobucephalopsis* Gu & Shen, 1983 resolved as distinct, and basal, to sequences representing the Bucephalinae Poche, 1907, Prosorhynchinae Nicoll, 1914, Paurorhynchinae Dickerman, 1954, and Dolichoenterinae Yamaguti, 1958, iii) the Dolichoenterinae and Prosorhynchinae are monophyletic sister clades, basal to the Bucephalinae and Paurorhynchinae, iv) sequences representing *Grammatorcynicola* Bott & Cribb, 2005, *Prosorhynchus* Odhner, 1905 and *Dollfustrema* Eckmann, 1934 also form monophyletic clades, v) the Bucephalinae is paraphyletic relative to the Paurorhynchinae, and vi) *Prosorhynchoides* Dollfus, 1929, *Rhipidocotyle* Diesing, 1858 and *Bucephalus* Baer, 1827 are each polyphyletic. Establishing the basis of this polyphyly will require further sequencing, especially of type-species, followed by iterative reassessment of morphology, host distribution, and ecology. Because of a combination of morphological and molecular differences observed among the four subfamilies in this study and specimens of a new species of *Heterobucephalopsis*, we propose a new subfamily to accommodate this genus.

O-029

PARASITES CAN ENHANCE INFECTIONS OF FISH WITH BACTERIAL PATHOGENS

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In aquaculture systems, fish are commonly infected by multiple pathogens, including parasites. Parasite *Ichthyophthirius multifiliis* (Ich) and bacterium *Edwardsiella ictaluri* are two common pathogens of cultured channel catfish. The objectives were to 1) evaluate the susceptibility of Ich parasitized catfish to *E. ictaluri*, 2) quantify bacterial loads using quantitative real-time polymerase chain reaction (qPCR) in fish tissues after parasitism by Ich; and 3) determine whether Ich could vector *E. ictaluri* to catfish. Fluorescent tagged bacterium *E. ictaluri* was used to assess the ability of Ich to vector *E. ictaluri*. Ich-parasitized catfish showed significantly higher mortality (91.7%) when exposed to *E. ictaluri* than non-parasitized fish (10%). Bacterial loads in fish infected by Ich theronts was 40 and 2000 fold higher than non-parasitized fish. Ich theronts showed significantly higher fluorescing bacteria (23-39 %) than control theronts (~6 %) after exposure to fluorescent *E. ictaluri*. *Edwardsiella ictaluri* survived and replicated during tomont division. Sixty percent of fish exposed to theronts treated with *E. ictaluri* were positive for *E. ictaluri* by fluorescent microscopy. Fluorescent *E. ictaluri* were observed in skin and gill tissue of dead fish. This study demonstrated that Ich infection resulted in enhanced bacterial invasion, increased fish mortality, and Ich vectored *E. ictaluri* into catfish. Prevention of parasite infection in fish may reduce fish mortality due to bacterial co-infection. The work also highlights the potential role of parasites in vectoring bacterial pathogens. Movement or purchase of new fish should take into account the potential for concomitantly introducing multiple microbial disease agents.

O-030

NON-LETHAL DETECTION OF ECTOPARASITES BY SIMPLEX AND MULTIPLEX PCR OF THE NILE TILAPIA *OREOCHROMIS NILOTICUS*

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Infection of Nile tilapia *Oreochromis niloticus* by ectoparasites is harmful. Currently, diagnosis of this infection is based on invasive techniques and the morphological identification of the parasites. We developed molecular tools to detect genomic of the monogeneans *Cichlidogyrus* spp. and *Gyrodactylus* spp., as well as protozoans *I. multifiliis* and *Trichodina* spp. by simplex and multiplex PCR. DNA was isolated individualy from each ectoparasite and from mucus of tilapia. For the primers design, sequences for each ectoparasite were searched in the Gene-Bank^{TM}, the primers used were Ekgyro1, Ekich5, Ektri3 and Cicly4. The detection limit of the simplex PCR for each ectoparasite was high, for example to *Cichlydogyrus* spp. it was of 3.91x10^{-3} ng/µl. The sensitivity, diagnostic specificity, PVP and PVP were of 100% using DNA from parasites and samples of mucus. We identified six different *Cichlidogyrus* spp. sequences, 16 *Gyrodactylus* spp. sequences, four *I. multifiliis* sequences and one *Trichodina* spp. Sequence, the homology were high with the genus in study. Those results were reinforced with the phylogenetic trees in which was observed that each ectoparasite form a clade with their respective sequences homologous. In the multiplex PCR standardization there was no cross reactions and no false positives or false negatives, the detection limit was high. The multiplex PCR validation in samples of mucus is required to provide a non-invasive test for simultaneous detection. In conclusion we obtained techniques non-invasive diagnosis that can be useful for studies of molecular systematics, phylogeny and genetic variability associated with pathogenicity and virulence of tilapia ectoparasites.

O-031

THE LIFE CYCLE OF *CARDICOLA ORIENTALIS* (DIGENEA: APOROCOTYLIDAE), A BLOOD FLUKE OF PACIFIC BLUEFIN TUNA

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Three species of blood flukes are known to infect bluefin tunas; *Cardicola forsteri*, *C. orientalis* and *C. opisthorchis*. Among them, the life cycle of *C. orientalis* has not been elucidated. Efforts have been made to find the intermediate host of *C. orientalis* for its effective control of infection of cultured Pacific bluefin tuna, *Thunnus orientalis* (PBT). We collected several species of terebellid polychaetes from buoys and ropes attached to the culture cages of PBT. In January and February, we found polychaetes infected with sporocysts in the body cavity, with the prevalence being 1.0% (January) and 3.4% (February) of all terebellids examined. The 28S rDNA and ITS2 sequences of examined sporocysts were almost identical (99.2-100%) with that of *C. orientalis* adults collected from PBT. The polychaete, identified as *Nicolea gracilibranchis* based on morphological characters, was proved to be the intermediate host. This is a third intermediate host of blood flukes of bluefin tunas and a fourth intermediate host of blood flukes infecting marine teleosts, all belonging to the family Terebellidae. Some *C. orientalis* sporocysts contained mature cercariae, which had a small rounded tail, suggesting the cercaria possesses a very limited swimming ability in the water.

O-032

ECOLOGICAL PARASITOLOGY IN RAINBOW TROUT MARICULTURE

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Rainbow trout are cultured in marine net pens along the Danish coastline from stocking in the month of March to harvest and slaughter in November and December. Rainbow trout used for stocking in spring (approximate body weight 1 kg per fish) have been raised in freshwater farms. They carry at the time of sea transfer some freshwater parasites obtained during their freshwater farm period. Ectoparasites may escape after a few months in the marine environment but new infections may be acquired during the marine phase. However, a series of ecological and fish behavioural elements influences the parasite fauna of maricultured fish in these net pens. We have investigated nineteen Danish mariculture systems which are located along a salinity gradient in Danish waters ranging from 24 ppt to 8 ppt. Marine ectoparasites (*Lepeophtheirus salmonis*) were only observed in the high salinity farms. No zoonotic parasites were recorded in any of the mariculture farms but sporadic infections with the cestode *Eubothrium* sp. and *Hysterothylacium aduncum* (adult stage) were found in runts (growth stunted trout excluded from the market) which fed on small fish (sticklebacks) and crustaceans (gammarids) accidentally entering the net pens. Feeding behavior seemed to affect the infection level of endoparasites because fast growing trout, feeding almost exclusively on extruded pelleted feed, did not obtain these infections. Runts on the other hand, which were kept from feeding on pellets, were left with the above mentioned natural feed elements and this type of feeding enhance the probability of infection with *Eubothrium* sp. and *H. aduncum*.

O-033

INFLUENCE OF TEMPERATURE ON HATCHING SUCCESS AND LARVAL DEVELOPMENT OF THE MONOGENEAN *SPARICOTYLE CHRYSOPHRII*, A COMMON PARASITE IN GILTHEAD SEABREAM CULTURES

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Sparicotyle chrysophrii is a harmful monogenean which causes seasonal epizootics and important economical losses in Mediterranean cultures of the gilthead seabream *Sparus aurata*. This seasonality suggests that environmental conditions are determinant for the spreading of this parasite and knowledge about their influence could be useful to manage parasite infections.The aim of this work is to ascertain the effects of some biotic and abiotic factors on oncomiracidial development, hatching, survival and behaviour of *S. chrysophrii* at different temperatures. A total of 1,200 eggs distributed into four 24-well plates were incubated in environmental chambers, at 14, 18, 22 and 26º C under LD 12:12, and monitored periodically. Effects of different factors were analysed using generalized linear mixed models. Experimental results demonstrated that temperature and light had a marked effect on life-cycle timing and reproductive success of *S. chrysophrii*. Egg development and hatching were faster at higher temperatures whereas hatching success peaked at medium temperatures (18, 22ºC) and decreased at extremes. Moreover, most hatchings occurred during dark periods. Concerning oncomiracidia, survival and vertical swimming periods were shorter at higher temperatures. Optimal combination for development and hatching occurred between 18 and 22ºC, in accordance with the range of water temperatures recorded during the months when epizootics in the Western Mediterranean Region are more frequent, spring and early summer. Therefore, water temperature should be considered to establish the treatment application calendar to manage with oncomiracidia recently emerged. However, host condition should also be considered.

Funded by ISIC/2012/003 project. MVT benefits from a PhD fellowship FPU13/05849

O-034

CAPSALID MONOGENEANS REPRODUCE IN ISOLATION

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Parasites use sophisticated life history strategies to ensure their survival in unpredictable environments. These strategies evolved in wild populations where the likelihood of encountering a host is low but in captive populations they can lead to parasite epizootics. This study examined the reproductive biology of *Neobenedenia* sp., a commercially important fish ectoparasite, in a series of laboratory experiments. The ability of hermaphroditic *Neobenedenia* sp. to self-fertilise was evaluated by infecting individual, isolated barramundi (*Lates calcarifer*) with a single oncomiracidium. Single, isolated parasites attained sexual maturity at day 10 post-hatch and laid embryonated eggs. Isolated parasites were fecund and laid an average of 3,229 ± 37 eggs over one week. Egg production rapidly increased from 58 ± 15 eggs on day 10 and peaked at 496 ± 68 eggs on day 15 before gradually decreasing. Eggs laid by isolated parasites were incubated and emerging individual oncomiracidia were used to infect more isolated *L. calcarifer* for three continuous self-fertile generations. This demonstrates that self-fertilisation (or parthenogenesis) is a viable reproductive method for a capsalid monogenean. *Neobenedenia* sp. exhibited a distinct egg laying and egg hatching rhythm. Parasites laid eggs continuously, but egg production increased in the dark (65.7%; peaking between midnight and 0300), while approximately 81% of oncomiracidia emerged from eggs in the first three hours of light. The numerous reproductive strategies exhibited by *Neobenedenia* sp. (i.e. self-fertilisation, high fecundity, egg laying rhythms and hatching rhythms) ensure the persistence of this notorious parasite in captive and wild fish populations.

O-035

EFFECT OF THE EYE FLUKE *TYLODELPHYS CLAVATA* ON THE FEEDING BEHAVIOUR OF EUROPEAN PERCH (*PERCA FLUVIATILIS*) UNDER INTRASPECIFIC COMPETITION

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For numerous fish species the visual system is essential as it provides information from their surroundings. Parasites that occupy the eyes have the potential to impact the sensory performance and consequently alter the host's behavior. In an experimental study, the feeding behavior of European perch infested with the eye fluke *Tylodelphys clavata* was examined. The results showed that an individual's ability to identify and approach food items efficiently was negatively affected by the infestation intensity of *T. clavata*. Additionally, the foraging success of an individual was reduced in competition with a less heavily infested conspecific, when the same food resource was exploited. These alterations in the ability to locate food may have important consequences on the feeding strategy of the fish. First field studies indicated that the intensity of infestation with *T. clavata* has an influence on the prey preference of young of the year perch in Lake Müggelsee (Berlin). As the number of metacercariae increased, fish consumed less daphnids. On the other hand, the opposite pattern was observed regarding the consumption of gammarids. Parasite-induced modifications in the feeding preferences of perch have the potential to induce changes in the abundance of grazing invertebrates, which in turn may have a knock-on effect on the primary production.

O-036

REPRODUCTIVE BEHAVIOUR OF *SCHISTOCEPHALUS* INFECTED STICKLEBACKS DEPENDS ON PARASITE PROVENANCE

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Schistocephalus solidus infections typically impair the reproductive development and behaviour of three-spined sticklebacks, but this varies considerably across populations. Such variability might arise from differences in host or parasite genotypes, or from environmental differences experienced by infected fish. Here we report studies using naturally- and experimentally infected fish, designed to identify the role of environmental conditions and host / parasite provenance on the reproductive phenotype of *S. solidus*-infected sticklebacks.

Wild caught male sticklebacks were collected from three UK populations with endemic *S. solidus* infection, and provided with ideal nesting conditions in laboratory aquaria. All non-infected fish completed nests. However, whilst infected males from both Clatworthy Reservoir (CR) and Inverleith Pond (IP) typically completed nests, those from Carsington Water (CW) failed to nest. We subsequently undertook an experimental cross-infection study to investigate the role of host and parasite provenance (and their interaction) on host reproductive phenotype. Lab-bred sticklebacks, from parental stock from CR, IP and CW were exposed to infective parasites from either their own, or a different, host population. After being reared to adulthood under favourable conditions, males were stimulated to build nests in individual nesting aquaria. Whilst there was no effect of host provenance on nesting probability, parasite provenance significantly affected nesting success. Despite ending the experiment with smaller plerocercoids, males infected with CW parasites typically failed to nest, whereas those infected with CR parasites typically built nests. Our results are consistent with the hypothesis that *S. solidus* genotype influences the reproductive development and behaviour of stickleback hosts.

O-037

THE POTENTIAL ROLE OF PARASITIC CRUSTACEANS IN FEEDING MIGRATIONS AND TROPHIC CONNECTIVITY IN NOCTURNAL REEF FISHES

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Many species of reef fishes in the families Haemulidae (Grunts) and Lutjanidae (Snappers) remain quiescent on reefs during the day and migrate to seagrass beds at night where they feed on benthic invertebrates and small fishes. It has been hypothesized that the advantage of such nocturnal migrations is an increase in food availability or a decrease in predation risk. We tested predictions of an alternative hypothesis that nocturnal migrations reduce exposure to blood-feeding gnathiid isopods. When departure and return times of grunts were compared with diel activity of gnathiid isopods, the departure of grunts from the reef coincided with increasing gnathiid activity, and fish returned to the reef just as gnathiid activity was declining. In field experiments, fish placed in cages and deployed on the reef at night harbored significantly more parasitic gnathiid isopods than those placed in the seagrass. However, this was not the case when the experiment was repeated during the day. These findings suggest that reduction in exposure to parasites constitutes at least one advantage of nocturnal feeding migrations in these fishes. However, the fact that they do not avoid infestation by gnathiids entirely, and that gnathiids are in turn eaten by other organisms, provides a means for ectoparasites to contribute to the transfer of energy between seagrass beds and reef. Future work will examine the extent of the role of gnathiids and other parasitic crustaceans in this trophic connectivity.

O-038

TREMATODE *APATEMON* SP. MANIPULATES BEHAVIOUR OF KILLIFISH *NOTHOBRANCHIUS FURZERI* FROM MOZAMBIQUE

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Tropically transferred parasites often affect the behaviour of their intermediate hosts to increase the probability of transmission to the definitive hosts. In this study, we investigated the behaviour of killifish *Nothobranchius furzeri* from Mozambique naturally infected by larval trematode *Apatemon* sp. This trematode has complex life cycle that requires the ingestion of the second intermediate host (fish) by the definitive host (piscivorous birds) to complete its life cycle. In naturally infected killifish, metacercariae were located in cerebral cavity that may allow a parasite to effectively manipulate its host. We performed two series of experimental observations to compare behaviour of naturally *Apatemon*-infected and control killifish: 1) fish reactions to simulated avian attack and 2) fish position in the aquarium without interruption.

In both series of observations, the behaviour of infected and control killifish significantly differed. Infected fish frequently remained in the upper third of the aquarium and were in contact with the water surface. After simulated attack, they displayed conspicuous swimming behaviour manifested by multiple jumps above the water surface, rotation and uncoordinated movements. On the contrary, control fish spent most of the time in the lower parts of the aquarium and after the attack the fish reaction manifested by an escape response into the deeper water. The results show that killifish naturally infected by larval trematodes *Apatemon* sp. display conspicuous behaviour which may facilitate their predation by trematode definitive hosts.

This study was supported by the Czech Science Foundation P505/12/G112.

O-039

TRACKING TRANSPARENT MONOGENEAN PARASITES ON FISH FROM INFECTION TO SEXUAL DEVELOPMENT

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Capsalid monogeneans are cryptic in nature which can limit quantified data on infection dynamics and ecology. We used fluorescent markers to examine the invasion and infection route of the fish ectoparasite *Neobenedenia* sp. throughout its development. We labelled transparent oncomiracidia with a fluorescent dye prior to infecting Asian seabass, *Lates calcarifer.* Replicate fish were each exposed to 50 fluorescent oncomiracidia and examined for parasites using an epifluorescence stereomicroscope at 10 time intervals post-exposure (15, 30, 60, 120 min, 24, 48 h, four, eight, 12, and 16 days). Parasites retained the fluorescent signal throughout the experiment. *Neobenedenia* sp. larvae settled opportunistically and then migrated to preferred microhabitats. Parasites initially attached (< 24 h) in greater mean numbers on the body surface (13 ± 1.5) compared to the fins (4 ± 0.42) and head region (2 ± 0.41). Once recruitment had ceased (48 h), preferred microhabitats included the eyes, fins, and dorsal and ventral extremities on the main body. Some parasites attached underneath the scales of host fish which may have evolved in response to predation by cleaner organisms. Furthermore, this microhabitat may enable the parasite to be almost entirely secluded from the environment which could impact treatment efficacy in aquaculture. *Neobenedenia* sp. aggregated on the fins within 24 h of attaining sexual maturity indicating that parasite distribution may be influenced by the need to find a mate. Aggregation on the fins may confer other advantages such as a suitable location to disperse eggs.

O-040

MUTUALISM AND COMPETITION IN WITHIN-HOST PARASITE NETWORKS IN MARINE FISH HOSTS

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There is an increasing interest in explaining the ecological mechanisms that determine the patterns of abundance and distribution observed in parasite communities. Since individual hosts are more often than not infected with multiple parasite species, the co-existence of several species within a patch will eventually lead to interspecific mutualistic and competitive interactions, these interact with the simultaneously occurring intraspecific components of competition, immune stimulation, and resource use. The observed community structure will be shaped by the relative intensity at which competition and mutualism act at the inter- and intraspecific level. We use linear mixed models to evaluate the presence and strength of within host parasite interactions in fish hosts from South America, where relationships can be assessed while correcting by host age/size. We also evaluate the effect of host age on the likelihood of co-occurrence of interacting parasite species. We found that parasite communities are highly interactive, with a dominance of positive interactions and few negative ones. There is a higher likelihood to find interactive parasite species pairs or groups at older host ages. This is not the case for species with negative associations; where this parasite species do not follow a particular trend in their co-occurrence likelihood with host age. This network of interactions, where the majority of them is positive, coincide what is found in free-living organisms living in challenging environments, and points out to interspecific interactions as an important structuring force in parasite communities.

O-041

PHENOTYPIC PLASTICITY IN TAPEWORMS (CESTODA: CARYOPHYLLIDEA), PARASITES OF CYPRINID FISHES

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The recent taxonomic research on monozoic tapeworms (Cestoda: Caryophyllidea) revealed unexpected phenotypic plasticity in the critical morphological traits within the most widespread tapeworms of the genus *Caryophyllaeus* Gmelin, 1790, parasites of cyprinid fishes in the Palaearctic zoogeographical region. An extensive tapeworm material was collected and recently evaluated morphologically and by DNA-based methods. Morphological evaluation of the biological material predicted existence of several distinct species or even new taxa. However, molecular analysis of DNA has revealed an unexpected genetic similarity of the studied specimens. Molecular phylogenetic analyses clearly distinguished only two separated clades, which supported validity only two species, the type species *Caryophyllaeus laticeps* (Pallas, 1781) and congeneric *Caryophyllaeus brachycollis* Janiszewska, 1953. Within both species, several morphologically different but genetically highly similar morphotypes were recognised, which largely corresponds to different fish hosts. The obtained results have not supported the traditionally used morphological concept of species-defining characters of the order Caryophyllidea Carus, 1863, especially due to high morphological plasticity of the scolex and the anterior end of the body.

High degree of phenotypic plasticity in the genus *Caryophyllaeus* represents interesting biological phenomenon requiring much more detailed studies.

O-042

SPECIES BOUNDARIES WITHIN A NEW GENUS OF RHINEBOTHRIIDEAN CESTODE FROM ELASMOBRANCHS

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The cestode order Rhinebothriidea Healy, Caira, Jensen, Webster & Littlewood 2009 was created recently to house a clade of elasmobranch cestodes that have several features in common, including bothridia borne on stalks. The Rhinebothriidea includes approximately 125 described species, but the literature includes reference to dozens of additional, undescribed species of cestode that are consistent with the ordinal diagnosis. Among these are several species that were referred to as Rhinebothriinae New Genus 3 by Healy et al (2009). This new genus can be distinguished from other rhinebothriidean cestodes in its possession of bothridia with a combination of complete transverse septa anteriorly and incomplete longitudinal septa posteriorly. This presentation reports on ongoing collaborative work that attempts to characterize species in this new genus using both morphological and molecular (DNA sequence) data. Over 50 specimens have been sequenced for combinations of 18S (V4, V7) and 28S (D1-D3) ribosomal DNA. Sequencing of partial mitochondrial CO1 and ND1 DNA is in progress for these same samples. These cestode specimens were taken from a diversity of stingray hosts from Senegal, Australia, Borneo and Vietnam. Bayesian analyses and examination of genetic distance values suggest that the specimens included in the analysis represent 16 species of the new genus. Morphological study, using light and scanning electron microscopy as well as histological observations, suggest that several of the species recognized using genetic distance values may be cryptic. Putative morphological species boundaries observed for members of this new cestode genus are discussed in the context of the genetic data.

O-043

THE DIVERSITY OF THE CESTODE ORDER TRYPANORHYNCHA DIESING, 1863 FROM NEOTROPICAL FRESHWATER STINGRAYS (MYLIOBATIFORMES: POTAMOTRYGONIDAE)

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The cestode order Trypanorhyncha consists of cosmopolitan parasites infecting elasmobranchs (i.e. sharks and rays) as definitive hosts. Currently, there are more than 300 species described, almost entirely from marine elasmobranch hosts. Only a single species [i.e. *Paroncomegas araya* (Woodland, 1934)] has been reported from Neotropical freshwater stingrays (family: Potamotrygonidae) – a peculiar group of elasmobranchs that is entirely restricted to freshwater habitats of South America. The biogeographical distribution of *P. araya* includes all major river basins of South America. However, each of these river basins (i.e. Orinoco, Amazon and La Plata basins) is known to harbour endemic elements of Neotropical fauna which potentially indicates the presence of cryptic species, which has been observed in other cestode orders infecting potamotrygonid stingrays. In a present study, the entire range (i.e. species diversity and biogeographical representation) of potamotrygonid host species from Neotropical freshwater systems is examined. This not only allows us to refine the systematics of trypanorhynch taxa from the freshwater environment but will furthermore increase the knowledge on host-parasite interrelationships, the processes of diversification and different evolutionary paths, adaptations to different environments as well as the phylogenetic relationships of these freshwater species to related trypanorhynchs infecting marine elasmobranch hosts.

O-044

MYSTERIOUS PARASITES FROM CEPHALOPODS' KIDNEYS: BIODIVERSITY OF DICYEMIDS, A PARTICULARLY PROBLEMATIC GROUP OF MESOZOA.

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Cephalopods harbor a great spectrum of parasites most of which are also infesting other marine organisms. Dicyemid mesozoans and apostome ciliates are however parasites restricted to cephalopods, residing in a very unusual habitat: the renal appendages. Surprisingly, little is known about these 'enigmatic parasites' biodiversity because their morphological identification criteria seem unconvincing to establish species boundaries. This study aims at exploring dicyemids biodiversity, by combining both morphological and molecular approaches. Hence, we collected parasites from more than 200 cephalopods belonging to six species, caught from Concarneau (France) and La Goulette (Tunisia). Molecular analysis (18SrDNA marker) of 470 parasite samples revealed 7 distinct dicyemids' haplotypes corresponding to previously described and novel species. Morphological analysis (slide smears) indicated that each host may harbor up to four dicyemids or Chromidina morphospecies, and allowed further descriptions of morphospecies poorly described in literature. Combining both molecular and morphological tools showed that the morphological diversity is always higher than the molecular diversity. In fact, different morphospecies can have highly similar 18SrDNA sequences. Thus, these parasites diversity cannot be assessed using the two approaches separately. Information from other molecular markers will provide further elements for understanding the diversity and phylogenetic position of these parasites.

O-045

GROWTH PROFILES OF *PARAMOEBA PERURANS*, CAUSATIVE AGENT OF AMOEBIC GILL DISEASE, UNDER VARYING TEMPERATURES AND SALINITIES

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Temperature and salinity are considered important factors in occurrence of amoebic gill disease (AGD), with disease incidence increasing at higher temperatures during late summer and at salinities greater than 32.

These factors will influence survival and proliferation of *Paramoeba perurans*, causative agent of AGD, in the environment and on fish, either directly or indirectly, which in turn may influence disease development.

To determine temperature and salinity ranges for growth and survival of *P. perurans* isolated from Scottish waters, amoebae were cultured *in vitro* over a 15 day period under a combination of different salinities (0, 3, 5, 15, 20, 25, 30, 35, 40, 45, 50) and temperatures (2°C, 4°C, 8°C, 11°C, 15°C, 18°C), each combination tested in triplicate. Growth profiles were analysed using a generalised linear model assuming a negative binomial error distribution. A prominent interaction between salinity and temperature was observed. In particular there was a lack of growth at low salinities (whatever the temperature) and evidence of an increase in amoebae numbers observed from 4°C at the higher salinities, with an indication that the optimal salinity for growth may vary depending on temperatures higher than this. Though subject to culture conditions and associated limitations, results obtained provide a standardised comparison of the growth-behaviour of the pathogen. The results will help inform on potential ability of amoebae populations to “over winter” in the environment and will help predict risk of disease development and shifts in disease patterns with annual and long-term environmental changes.

O-046

A NEW TYPE OF APICOMPLEXAN INFECTION IN THE MUSCLE OF SALMONID FISH

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Coccidia (Phylum Apicomplexa) is a well-known protistan group of obligate intracellular parasites causing important problems in the terrestrial animal industry. Coccidioses have been reported from farmed carp, *Cyprinus carpio*, among other fish species, but so far there are no reports of these infections in salmonid fish in aquaculture.

Increased, unexplained mortalities have been reported from inland rainbow trout, *Oncorhynchus mykiss*, and salmon, *Salmo salar*, farms in Sweden. The external lesions observed in associated with the first mortalities were blackening of the tail region, swelling in the upper part of skull region and/ or external lesions in fins and skin. Histological examination of muscle samples indicated this to be the first reported case in fish of a parasite infection associated with coccidia of the Apicomplexan family Sarcocystidae.

To better understand this type of infection, new researches were initiated in Sweden in 2014. Samples from both wild and farmed salmonid fish were collected in collaboration with the Swedish Fish Health Programme and the regional veterinary health organization. Samples were collected both as fresh muscle tissues and fixed organs in formaldehyde. Samples were mainly collected from dorsal muscle and the heart. Blood smears were also prepared from the heart. Muscle tissues were homogenized and parasitical cyst enrichment was performed by flotation for microscopical examination regarding the presence of cysts. Samples of muscle, gills and heart were fixed in glutaraldehyde for ultrastructural studies. Microscopical, histological and ultrastructural lesions from these investigations will be described.

O-047

FISH COCCIDIANS: WHAT'S GOING ON?

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Coccidians infect fishes worldwide and are common in both marine and freshwater environments. The majority of coccidians infect the intestinal epithelium, but may also infect other tissues such as the kidney, urinary bladder, spleen, liver and swim bladder. Non-intestinal infections can results in the formation of large numbers of sporulated oocysts at the site of development, whereas intestinal infections release sporulated or unsporulated oocysts.

The development, morphology, structure and number of sporocysts in the oocysts are of primary importance when identifying fish coccidians. Release of the sporozoites from the sporocysts can be via a longitudinal suture (*Goussia*) or a Stieda body at the apex of a sporocyst (*Eimeria*).

Until recently there were almost no DNA sequences available for coccidians, however, numerous rDNA sequences have now become available. In this study, we provide additional DNA sequence data for coccidians from marine fishes from cold water and tropical environments. We analyse the position that coccidians occupy within the Apicomplexa and look at the relative grouping of species within the fish clade. Our analyses suggest that it is possible that fish coccidians evolved from species that infect reef-building scleractinian corals and that corals may remain involved as hosts in certain life cycles. Coccidians from corals do not group with others that infect marine invertebrates but consistently group at the base of coccidian clades that infect fish and other higher vertebrates. In addition, molecular phylogenetic data reveal that the genera *Goussia* and *Eimeria* are evenly distributed throughout the fish coccidian clades and that there is no distinction between species from marine or freshwater environments.

O-048

HOW DO TRICHODINIDS (CILIOPHORA: PERITRICHIA) SURVIVE ADVERSE CONDITIONS?

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Trichodinids are mobiline unicellular organisms found worldwide on a wide range of hosts, but the majority are described from fish, being associated with fish hosts only. Some species are also associated with other vertebrate hosts, such as amphibians (adult and tadpoles), as well as some invertebrates. Fish trichodinids such as *T. domerguei* f. *latispina* Dogiel, 1940, *T. nobillis* Chen, 1963, *T. reticulata* Hirschmann & Partsch, 1955, *T. heterodentata* Duncan, 1977 and *T. nigra* Lom, 1961 have also been reported from the skin and gills of different tadpole species, mostly from European countries. In contrast only a single fish trichodinid has been found from tadpoles in southern Africa, i.e. *T. heterodentata.* Transmission experiments by various workers have shown that trichodinids utilise tadpoles mostly as facultative hosts during the spring and early summer months. In the present study *T. heterodentata* was found on tadpoles, where these hosts have not been in contact with any fish hosts, which begs the question where the tadpole infestations came from. Another trichodinid species, *T. diaptomi* Basson and Van As, 1991 not found on fish, but associated with calanoid copepods, was found in an ephemeral river. Mobiline trichodinids are not known to have dormant stages, or any stage able to survive adverse conditions. This poses the question how trichodinids are able to survive either severe habitat changes, such as when a river dries up, or how / where a healthy trichodinid population suddenly appears on amphibians in habitats where there has been no contact with fish hosts.

MODULARITY AND MORPHOLOGICAL INTEGRATION OF HAPTORAL SCLEROTIZED STRUCTURES IN *LIGOPHORUS* SPP. (MONOGENEA: DACTYLOGYRIDAE) IN THE MEDITERRANEAN SEA

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Morphological integration and modularity refer to the patterns and processes of trait interaction and independence. In monogeneans the variability of haptor structures for the control of variational modularity is still relatively unknown. The aims were to describe the interspecific variability in the shape and size in the ventral and dorsal anchors in 14 species of *Ligophorus*, to test the presence of modular partitions using *a priori* hypothesis through the *RV* coefficient: (a) the functional division between roots and points, (b) the opening and closing, and (c) the external and internal part of anchors, and to assess the covariation between the modules detected by two-block partial least square analysis. Data were collected on 526 individuals from the Mediterranean Sea by means of geometric morphometric tools. The results showed no clear separation among *Ligophorus* spp. in the shape space. The analysis of modularity identified the roots and points anchors, as distinct modules for ventral and dorsal anchors and the external and internal part only for ventral anchors. In contrast, we find no support for the hypothesis of opening and closing. The modules detected showed high percentages of covariance explained by the first PLS axis indicated that most of the covariation is concentrated in precise features of shape in the anchors that change in a coordinate manner between modules. Thus, the coordination among anchors in *Ligophorus* spp. and within modular structure may have a functional basis and be a crucial factor for understanding the variability with implications in further systematics and evolutionary studies.

FLUCTUATING ASYMMETRY IN THE HAPTORAL ANCHORS OF *LIGOPHORUS CEPHALI* (MONOGENEA: DACTYLOGYRIDAE) EVALUATED BY GEOMETRIC MORPHOMETRICS

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The morphology of haptoral sclerotized structures is highly preserved in monogeneans. This suggests that anchoring is crucial to parasite survival and it should be under a rigorous control. However, random deviations from the predicted bilateral symmetry axis may appear, this pattern is known as fluctuating asymmetry (FA). Given a sample of individuals, it is expected that differences between left and right sides will follow a normal distribution. FA has been considered as the unique symmetry pattern not determined by genetic factors, hence it can be used as a development instability indicator and the influence of a huge range of factors can be assessed. We assessed the FA degree of dorsal and ventral anchors pairs of the haptor of *Ligophorus cephali* using geometric morphometrics (GM) techniques. GM affords separation of size and shape effects of a structure, based on Cartesian coordinates of landmarks placed over the structure under study. The symmetry pattern was evaluated for size, by means of two-way ANOVA, and shape, with Procrustes ANOVA, of dorsal and ventral anchors, respectively. We also developed Principal Components Analyses (PCA) by ANOVA's factors. Our analyses confirmed the existence of FA for dorsal and ventral anchors in both shape and size. This is consistent with a scenario of morphological coordinated adaptation in anchors for assuring an efficient attachment in the host. To our knowledge this is the first time evidence for FA is reported in monogenean. PCA revealed that variation at individual and FA levels are buffered by different mechanisms.

O-051

COMBINATION OF MORPHOMETRY WITH MOLECULAR METHODS REVEALING NEW PARASITE SPECIES AND INNOVATIVE INSIGHT INTO TAXONOMY OF DIPLOZOIDS

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Monogeneans of family Diplozoidae are common parasites on the gills of cyprinid fish. For parasite species identification, classical morphometric approach uses the size and shape of sclerotized parts of adults´ attachment apparatus. Molecular methods enhance the species discrimination by highly conclusive genome characterization. By combining these two techniques, we described new parasite species and disclosed interesting aspects in taxonomic division of diplozoid monogeneans.

Fish were sampled across several continents during years 2009-2014, put down within 24 hours and dissected to record parasites. Adults or diporpae stages of diplozoids found on the gill apparatus of fish were fixed and stored in absolute ethanol at 4°C until the time of analysis. For morphometric description, one opisthaptor of each specimen was cut off and soaked in 10% SDS, washed in distilled water, mounted on a microscope slide and fixed with GAP. A light microscope equipped with DIC, digital image analysis system and drawing attachment was used. For molecular analysis, gDNA was extracted and along with routinely analyzed ITS2 rDNA marker, two more genome regions were amplified and characterized by sequencing.

The evolutionary distances between relative diplozoid species were computed, phylogeny reconstruction was performed and evolutionary history was acquired by common statistical methods. Morphometry and molecular characterization distinguished several parasite samples from the other valid species in genus *Paradiplozoon* and supported their uniqueness. To explain the resulting division, we took into account the geographical origin of the samples and host-parasite cophylogeny.

The study is supported by Czech Science Foundation – projects P506/12/1258 and P505/12/G112.

O-052

DIPLOZOID MONOGENEANS (DIPLOZOIDAE, MONOGENEA) – A UNIQUE GROUP OF FISH PARASITES IN THE LIGHT OF RECENT KNOWLEDGE

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Diplozoid monogeneans (Diplozoidae, Monogenea) represent a unique monogenean family parasitic cyprinid fishes in so-called “old” world. Until now, more than 70 nominal species have been described from Europe, Asia and Africa, however, due to very limited knowledge related to parasite phenotypic plasticity, correct description of species is still unclear and rather difficult. Large scales of various methods and techniques have been used for parasite investigation. In general, parasites were removed from the gills and fixed for morphometric and ultrastructural analyses and in 96% ethanol for molecular characterization.

During last two decades, diplozoid development, reproductive behaviour, habitat microecology, motility, surface topography including neurobiology and glycobiology have been investigated. This contribution is summarizing the current knowledge of Diplozoidae and showing some directions of a new perspective research. There is no doubt, that significant increase of our knowledge related to diplozoid anatomy, biology, ecology and ultrastructure were achieved. The taxonomic revision of the group is essential; these should be based on combination of corrected and validated morphological and molecular data.

Financial support by Czech Science Foundation, project GAP506/12/1258.

O-053

FARM TO FORK: ARE PARASITES NATURAL TAGS TO TRACE FARMED ATLANTIC BLUEFIN TUNA?

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The Atlantic bluefin tuna (ABFT), *Thunnus thynnus*, is one of the most valuable species for fisheries and aquaculture and its traceability is increasingly important in a “farm-to-fork” approach. The aim of this work is to compare parasite assemblages of wild and cage-reared Western Mediterranean ABFT, to assess their usefulness as biological tags of fish catch. A total of 195 gills of wild and cage-reared tuna captured in 2011-2012 were analysed. Sixteen species of parasites were identified in wild tuna (4 monogeneans, 10 trematodes and 2 copepods), only 11 of these parasites being in captive tuna (1 monogenean, 8 trematodes and 2 copepods). Didymozoidae (Trematoda) was the most abundant and diverse group of parasites in wild and farmed tuna. Most prevalent parasite was *Didymosulcus wedli* (Didymozoidae) in wild tuna and *Cardicola* spp. (Aporocotylidae) in cage-reared tuna. The prevalence of seven of the 11 common species (1 monogenean, 5 trematodes and 1 copepod) was significantly higher in wild than in captive tuna. This reduction is expected as culture conditions are known to be adverse for most parasites. Nevertheless, the prevalence of *Cardicola* spp. was much higher in cage-reared than in wild tunas, probably because the intermediate hosts as part of the biofouling. The marked differences of parasite assemblages between samples indicate that parasites could be used for tuna traceability.

JRL and JPA benefit of doctoral fellowships of the Spanish Government and the University of Valencia, respectively. Funded by projects AGL2010-20892, PROMETEO 795 2015-018 and ISIC/2012/003.

O-054

ASSESING THE ROLE OF HOST SIZE AND AGE AS DRIVERS OF THE ABUNDANCE OF LONG-LIVED PARASITES IN FISH STOCK ASSESSMENT STUDIES

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There is a general consensus that the most important criterion for an effective parasite marker for stock discrimination is its long residence time in the fish. Long-lived parasites tend to accumulate as fish grow, their abundance depending therefore on both fish size and age. Fish size is used as a surrogate of age, however their functional relationship is not linear and large changes in age do not represent proportional changes in size. Therefore, fish assigned to a given size class can belong to different cohorts, and the sample could be heterogeneous in terms of parasite loads. Consequently, the discernment between the effects of these variables could be essential when comparing samples; however, host age is not usually considered in parasitological studies of stock identification, although it undoubtedly deserves more attention and further research. The size, age (otholith aging), sex, parasite species richness and abundance of *Grillotia carvajalregorum*, *Corynosoma australe* and *Hysterothylacium deardorffoverstreetorum* were recorded for 270 specimens of *Percophis brasiliensis* caught in the Argentine Sea. The abundances and species richness were modelled using generalized linear mixed models with a negative binomial distribution. An information theory approach, employing a collection of models with different combination of covariates, was followed to identify the optimal set of factors determining the parasite burdens of *P. brasiliensis*. Results indicated that the covariates length and sex consistently appeared in the most parsimonious models (lowest AIC), suggesting that fish length seems to be a better predictor than age. The biological causes of these patterns are discussed.

O-055

ANISAKIS SPECIES IN ATLANTIC MACKEREL (*SCOMBER SCOMBRUS*) FROM THREE MAJOR EUROPEAN FISHING AREAS; A FIRST GLANCE AT SOME SURVEILLANCE DATA OF THE EU FP7 PARASITE PROJECT

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Atlantic mackerel (*Scomber scombrus*) ranks among the most valuable fish species in Europe. Its stock composition is complex and some components show extensive changes in migration pattern. Although an important food resource, systematic and concerted epidemiological surveys of *Anisakis* species in Atlantic mackerel from European fishing grounds have been lacking. As part of the EU FP7 PARASITE project (GA no. 312068), occurrence and spatial distribution of *Anisakis* species in 1682 mackerel from NE Atlantic waters including the Faroe Islands (n=300), North Sea and British Channel (n=938), off Portugal and NW Spain (n=157), and the Mediterranean (n=287), were recently investigated by applying the UV-press method. Preliminary genetic identification suggests that *A. pegreffii* is the dominating species in mackerel from the Mediterranean, with only a few *A. physeteris* so far detected in/on the viscera. *A. pegreffii* shows generally low prevalence and intensity in the flesh, not exceeding 6% and 1 larva, respectively. In mackerel from the Atlantic areas, *A. simplex* (*sensu stricto*) appears to be the dominating species, commonly exceeding 25% prevalence in the flesh, with mean intensities around 2, ranging 1–19. While *A. simplex (s.s.)* and *A. pegreffii* seem to co-occur in mackerel from off Portugal and NW Spain, a few *A. pegreffii* were also found in the viscera of mackerel from the northern North Sea. Thus, systematic anisakid surveys of Atlantic mackerel throughout its main distribution area, combined with accurate *Anisakis* species identification, may contribute to elucidate stock composition and migration patterns of the fish host species.

O-056

FISH POPULATION STUDIES USING PARASITES FROM THE SOUTHEASTERN PACIFIC OCEAN

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The Humboldt Current Large Marine Ecosystem is one of the most important upwelling systems, with high rates of primary production supporting some of the most massive fisheries worldwide. Large scale fisheries in Southeastern Pacific, especially in Chile, began in the 60`s. Since then, all major “resources” and probably long before had drastic increases and decreases in abundance, as reflected by the landings of anchovy, jack mackerel, sardine and hakes. Studies on parasites of marine fish have increasingly been incorporated into the normal protocols of fish population studies. We summarize studies using parasites as tools for fish population studies in the South Eastern Pacific, whether or not of economic importance. There are ca. 30 such studies and most are on economically important fish species though others on coastal and intertidal fish, or on less or non-commercial species, provide insights on scales of temporal and spatial variation of parasite infracommunities. This case-by-case review shows that there has been a persistent effort to use parasites in fish population studies in SEP. Six out of the top ten Chilean fisheries, have been studied on its stock structure and/or migration. Main aspects considered were sampling site, a less frequent combination of sampling in time and space, and a lower frequency of those dealing with variability along time. Thus, we emphasize on the need for more long-term studies that will permit us to see the variability of these biological tags along time, such as in the jack mackerel.

O-057

THE UTILITY OF PARASITE DATA IN SOUTHERN AFRICAN APPLIED MARINE RESEARCH

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Parasitic species are typically underestimated components of biodiversity and their utility in applied marine research is generally overlooked. We examined the parasites of several commercially exploited southern African marine fish, primarily with the aim of assessing their population structure. South African sardine (*Sardinops sagax*) harboured seven parasite taxa, and spatial and temporal variation in infection indices of two biotag parasites supported a multiple stock hypothesis for this species. Cape horse mackerel (*Trachurus capensis*) from the Northern and Southern Benguela harboured 29 parasite taxa, and whereas fish from each region had similar parasite assemblages significant spatial differences in four parasites were observed, supporting the known existence of two sub-populations in the Benguela ecosystem. Cunene horse mackerel (*T. trecae*) from the Northern Benguela had similar parasite taxa to *T. capensis* from that region but showed differences in some indices, suggesting the existence of subtle interspecies host-parasite relationships. Cape snoek (*Thyrsites atun*) harboured 16 parasite taxa but no spatial variation in parasite communities was found, indicating a single stock of this species in the Southern Benguela. Parasites of St Joseph shark (*Callorhinchus capensis*) were examined for heavy metals and high zinc concentrations in *Gyrocotyle plana* compared to host tissue showed bioaccumulation, emphasising the value of parasites as early warning systems for heavy metal pollution in marine ecosystems. These findings emphasise the importance of documenting marine parasite biodiversity and its utility in multidisciplinary research in marine science.

O-058

USING PARASITE BIOTAGS TO ELUCIDATE POPULATION STRUCTURE OF TWO *MERLUCCIUS* SPECIES OFF SOUTH AFRICA

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Two species of hake, *Merluccius capensis* (shallow-water hake) and *M. paradoxus* (deep-water hake), occur off South Africa where they are targeted by the country's most valuable fishery. The two hakes are currently managed as a single stock that is not differentiated by species and does not take potential stock structure of either species into account. These management practices are changing, however, and investigations into the population structure of both species using genetic approaches have recently been initiated. In 2014 we conducted an initial study to assess the utility of applying the parasite biotag approach to elucidate population structure of the two hake species and identified seven parasite taxa as candidate biotags. Preliminary results from analysis of a larger number of fish show a spatial difference in the parasite assemblage of *M. capensis* off the South African coast, with the monogenean *Anthocotyle merlucci* found attached to the gills of fish collected off the west coast but not in fish from the south coast. In contrast, the copepod *Neobranchiella insidiosa f. lageniformis* was found on the gills of fish from the south coast but not from the west coast. These results suggest the presence of two stocks of *M. capensis* off South Africa, which is also reflected by the genetic results. Further results for *M. capensis*, and on the parasite assemblage of *M. paradoxus* and whether those data indicate the existence of multiple stocks, will also be presented

O-059

LARVAL MIGRATION OF THE ZOONOTIC PARASITE *ANISAKIS PEGREFFII* (NEMATODA: ANISAKIDAE) IN A FISH HOST, *ENGRAULIS ENCRASICOLUS*: IMPLICATIONS FOR SEAFOOD SAFETY

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Anisakid nematodes belonging to the species *Anisakis pegreffii* are distributed in wide variety of fishes from the Mediterranean Sea and they are known to cause the human zoonosis anisakiasis. The present study investigated, for the first time, the response by *A. pegreffii* larvae - identified to species level by genetic/molecular markers – to the storage temperature of anchovies, *Engraulis encrasicolus*. The larval motility of *A. pegreffii* was studied in 1300 fish, captured from a highly infested area (FAO 37.2.1, Central Adriatic Sea), maintained under different storage temperatures (2°C, 5°C, 7°C), and examined at different time intervals (immediately after fishing, 24h, 48h and 72h). Parasitological analysis was carried out with UV-press method. The results showed that the increase of infection values with *A. pegreffii* in fillets of anchovies was statistically positively related to the increase of the temperature (at 5°C and 7°C) and time of storage (after 24h, 48h, and 72h). A significant statistical correlation between the increasing of the worm burden in the fillets and a decreasing of *A. pegreffii* in the viscera was observed. In contrast, those fish constantly maintained at 2°C showed no statistically significant variation in infection either in the viscera or fillets. Our results suggest that temperature plays an important role in the post-mortem motility of *A. pegreffii* larvae in anchovies. The presence of *A. pegreffii* in the fillets inspected immediately after their capture indicates that intra-vitam migration may also occur.

This research received funding from the EU 7FP grant agreement n°312068 PARASITE.

O-060

THE RISK OF HUMAN INFECTION WITH *ANISAKIS* SPP., WHILE CONSUMING ATLANTIC MACKERELS (*SCOMBER COLIAS* AND *S. SCOMBRUS*) CAUGHT OFF PORTUGAL

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Fish dishes are much appreciated in Portugal, one of the European leaders in fish consumption. This could drive the occurrence of anisakiasis, particularly if the ingested fish is not properly cooked. The symptomatology of the associated pathology include acute gastritis and allergies. Chub mackerels, namely *Scomber colias* Gmelin, 1789, are among the top 4 fish most sold in Portuguese fish markets. In this study, the risk of *S. colias* and *Scomber scombrus* Linnaeus, 1758 infection with *Anisakis* was evaluated. Larvae identity was determined by using molecular tools.

The parasitological survey was performed using 40 *S. colias* and 42 *S. sombrus*, fished at FAO area Atlantic Northeast 27, sub area Portuguese waters IX. The isolated worms were preserved in ethanol 70%, mounted in glycerin and identified first on the basis of morphology; later on, some worms were molecularly characterized and their phylogeny evaluated by using software Mega 6.

Recorded prevalence and intensity levels were very high – 85.0% and 21.7 worms/fish for *S. colias* and 83.3% and 16.4 worms/fish for *S. scombrus*. Only occasional infections were detected in the muscle and the large majority of the anisakid larvae were found to infect the body cavity. Still, the risk of infection cannot be discarded. Indeed, the larvae have the ability to move into the muscle, after the host's dead. The anisakid larvae detected in both fish were *A. simplex* (s.s.) (Rudolphi, 1809) (mostly in *S. scombrus*) and *A. pegreffii* Campana-Rouget & Biocca, 1955 (mostly in *S. colias*), with different prevalence.

O-061

NO MORE TIME TO STAY ‘SINGLE’: A MULTI-MARKER APPROACH TO THE IDENTIFICATION OF *ANISAKIS PEGREFFII*, *ANISAKIS SIMPLEX* (S.S.), AND THEIR HYBRIDS (NEMATODA: ANISAKIDAE)

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Species identification remains a challenging task in the study of parasite biodiversity. In anisakid nematodes, genetic markers have been extensively used to identify cryptic species, define population structure, and to study patterns of reproductive isolation and gene exchange between allopatric and sympatric lineages. In this study, we adopted a multilocus genotyping approach to assign numerous anisakid individuals (*N*= 667) to the species *Anisakis pegreffii*, *A. simplex* (s.s.), or to their hybrid classes. Larval and adult samples were collected from allopatric and sympatric areas, and results were compared to those obtained, on the same specimens, using a conventional single-marker approach, based on the analysis of the ITS1 region of rDNA. Bayesian assignment tests identified most of the individuals as belonging to that or the other parental taxa, and a smaller portion (*N*= 12) as F1 hybrids from sympatric areas. Importantly, the results based on the ITS1 rDNA classified as hybrids several individuals that did not show evidence of mixed ancestry; this could be likely the outcome of incomplete lineage sorting at this sole marker. Thus, our results raise concerns on the extensive use of the single-marker approach to identify anisakid species and their hybrids, and invite to a careful reappraise the many inferences that could be derived from its application.

Part of the present research leading to these results has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement n°312068, PARASITE.

O-062

GENOTYPING ANISAKIDS USING HIGH RESOLUTION MELTING ANALYSIS

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Anisakids are parasitic nematodes responsible for a zoonosis caused by their infective larvae in fishes intended for human consumption. *Anisakis simplex* s.l. and *Pseudoterranova decipiens* s.l. are the species mainly involved in human anisakidosis (Arizono et al 2011, Emerg Infect Dis, 17, 555; Mattiucci et al 2013, Emerg Infect Dis, 19, 496) while *Contracaecum* sp. and *Hysterothylacium* sp. (Raphidascarididae) are only rarely found in humans ; the latter is even considered non-pathogenic to humans.

The aim of the study was to develop a new molecular approach to differentiate and identify anisakids based on High Resolution Melting (HRM) of a selected molecular marker (nuclear ribosomal ITS). To date, only the *A. simplex* complex has been discriminated using HRM. Representative species of *Anisakis*, *Pseudoterranova*, *Contracaecum* and *Hysterothylacium* were tested to verify the presence of diagnostic profiles to differentiate nematodes at genus and species level. HRM profiles of *Anisakis simplex* s.l. revealed Tm of 78.0–78.2 °C for *A. simplex* s.s., of 79.4–79.5°C for *A. pegreffii* and of 78.4–78.6 °C for hybrids. Members of the *P. decipiens* complex were tested along with *Anisakis* spp., *Contracaecum osculatum* and *Hysterothylacium aduncum*. Results revealed no amplification for any *Anisakis* spp., but three HRM profiles: one showed Tm of 79.4-79.5 °C for *P. decipiens* s. s. and *P. krabbei*; one showed 79.9-80.0 °C for *P. azarasi* and *P. cattani*; one showed 79.1-79.2 °C for *C. osculatum* and *H. aduncum.* HRM is a relatively new approach for rapid discrimination of parasitic nematodes, with promising diverse applications.

PROTEASES AS VIRULENCE FACTORS: TRANSCRIPTOMIC STUDY OF *CERATONOVA SHASTA* (MYXOZOA) GENOTYPES WITH DIFFERENT VIRULENCE

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Virulence of parasites is still poorly understood. Important components among the arsenal of parasite virulence factors are proteases, essential for parasite invasion, migration and nutrition. Myxozoans are microscopic, parasitic cnidarians that affect fish health in fisheries and aquaculture. *Ceratonova shasta* is a myxozoan pathogen that infects wild and cultured salmonid fishes in the rivers of the Pacific Coast of North America. *C. shasta* shows host-specific genotypes with different degrees of virulence (mortality, proliferation) in the fish host. We extracted RNA from intestines of salmonids infected with single *C. shasta* genotypes: I in Chinook salmon, IIC in coho salmon and IIR in rainbow trout from two different localities. Normalized cDNA libraries were obtained and sequenced on a single lane of high throughput sequencing system Illumina HiSeq 2000. This yielded to 119 million raw reads, which were filtered for low quality data, adaptor sequences and homopolymer repeats, then assembled using Trinity. We obtained ~220,000 contigs, which were annotated using UniProt, Gene Ontology and Taxonomy NCBI databases. Host contamination was filtered using a comparative BLAST search against databases of host proteins (rainbow trout and its closest salmonid relatives) and the non-parasitic cnidarian relative, *Nematostella vectensis*. Preliminary analysis has identified numerous cysteine proteases (cathepsins L, S, Z) and serine proteases. Sequence comparisons revealed greater similarity with cnidarian than fish genes, supporting their annotation as proteases of the parasite *C. shasta*. Identification of proteases is key for development of selective inhibitors for myxozoans in their fish hosts.

MYXOZOAN POLAR CAPSULE CONTENTS AND EJECTION DYNAMICS

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Myxozoans are a large group of parasitic cnidarians that affect wild and cultured fish populations worldwide. Homologous with nematocysts in free-living cnidarians, myxozoans have extrusomes known as polar capsules, which contain a coiled, eversible polar filament (tubule). The infection process is facilitated by polar capsule activation and discharge of the tubule, anchoring the spore to the host and allowing the infective sporoplasm to penetrate. Our study explores the biological and biomechanical processes that occur during polar capsule activation. To gain insight into polar capsule structure and content, we isolated intact capsules from *Ceratonova shasta* myxospores using dielectrophoresis. Next, the proteomic profile of the polar capsules was analyzed by LC-MS/MS using our *C. shasta* transcriptome and other Cnidaria database. The analysis revealed known nematocyst proteins, including both tubule-specific proteins, and enzymes involved in the synthesis of poly-gamma-glutamate, the main capsule matrix component; numerous other detected proteins have yet to be characterized. Using tools that we have developed for analyzing nematocysts, we successfully compared and characterized the discharge dynamics of *C. shasta* and *Myxobolus cerebralis* polar capsules, which represent the marine and freshwater myxozoan lineages, respectively. Our results demonstrate that the two spores have different tubule ejection characteristics. Whereas both tubules performed a possible anchorage function, *M. cerebralis* also displayed rapid injection capabilities that are different from those of nematocysts. These findings indicate that some polar capsules can actively inject their contents, thus suggesting a more complex function of the capsules during the initial steps of myxozoan infection.

O-065

FACTORS AFFECTING SPOROPLASM RELEASE IN *KUDOA SETEMPUNCTATA* AND ITS METALLOPROTEASE

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Kudoa septempunctata has been isolated from cultured olive flounder (*Paralichthys olivaceus*) and was recently identified as a cause of food poisoning in humans. Since the sporoplasm plays an important role in causing diarrhoea by invading intestinal cells, the specific factors affecting the release of sporoplasm from spores should be determined. Thus, we investigated the factors affecting sporoplasm release and characterized a metalloprotease purified partially. Sporoplasm release was observed in the groups treated with Fetal Bovine Serum (FBS) and media containing glucose. In addition, 1,10-phenanthroline (metalloprotease inhibitor) inhibited the release of sporoplasm in the FBS medium. The optimal activity of purified metalloprotease was observed at pH 7 and it is stable from pH 6 to pH 8. These results indicate that *K. septempunctata* uses glucose for releasing its sporoplasm and that a zinc or metalloprotease is related to the release mechanism. The present study provides important information for the development of agents to prevent sporoplasm release and the consequent food poisoning caused by *K. septempunctata*.

O-066

SEATROUT INFECTION BY *KUDOA INORNATA*: DEVELOPMENT IN THE FAST LANE

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Kudoa inornata is a muscle-dwelling myxozoan that commonly infects sciaenid *Cynoscion* spp. on the U.S.Atlantic coast and in the Gulf of Mexico. We determined the temporal pattern of infection by *K. inornata* and the number of degree-days required for myxospores to develop in naïve sentinel seatrout (*C. nebulosus*). During a two-year experiment (2013-2014), two replicates of 10 sentinel fish were exposed monthly to raw seawater from Charleston Harbor, South Carolina, USA. We determined prevalence of infection via both squashed muscle biopsies and PCR. Results showed a seasonal presence of myxospores in seatrout muscle that reflected different development rates of myxospores at various water temperatures. In both years, infection appeared in the spring, peaked over summer, decreased in the fall, with no infections detected in the two coldest winter months. Myxospore development required as few as 489 degree-days post exposure, which we confirmed by experimentally demonstrating development in as little as 16 days post-exposure at 31°C. While 31°C appears to be a temperature threshold, above which *K. inornata* myxospores may not develop well, it is unclear whether the lack of winter infection is due to decreased or arrested actinospore production, or a decrease in density of the putative annelid vector. Future studies will focus on temporal quantification of waterborne actinospores and identification of the alternate host.

O-067

NEW INSIGHTS IN THE HOST SPECIFICITY OF MYXOZOANS: THE ROLE OF FISH BLOOD IN THE EARLY DEVELOPMENT OF TWO *MYXOBOLUS* SPECIES

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Previous *in vitro* viability challenges showed that innate immune system components in fish serum have a key role in the host specificity of myxozoans. While for *Myxobolus pseudodispar*, the sera of non-suitable hosts was able to eliminate the invasive parasite stages only, the number of viable *M. cerebralis* sporoplasm cells was significantly reduced even by susceptible host serum. In *in vivo* exposure trials, we compared the prevalence and intensity of early developmental stages of *M. pseudodispar* and *M. cerebralis* in the blood of susceptible and non-susceptible hosts. SPF fish were exposed individually. The prevalence and the intensity of infections were examined using species-specific nested PCR assay1 d, 1 w and 1 mo post exposure (p.e.). Interestingly, *M. cerebralis* was present in the blood of both susceptible and non-susceptible hosts all over the experiment. The prevalence remarkably increased in the susceptible hosts 1 w p.e., whereas it remained constant in the non-susceptible host. The infection profile of *M. pseudodispar* was in correlation with the findings of previous *in vitro* studies. The parasite stages were present with high intensity in susceptible host (with a notable decrease in the prevalence 1 w p.e.), with low intensity and prevalence in rudd (previously thought to be a susceptible host), and absent in non-susceptible host. As the infection profile of the two examined species was rather different, it seems that blood-related immune components are not the only factors influencing host specificity; furthermore pathogenicity issues may also be considered.

Financial support: Hungarian Scientific Research Fund, OTKA K112301.

O-068

BROMODEOXYURIDINE DNA LABELLING REVEALES THE HOST INTESTINAL PROLIFERATION INDUCED BY A MYXOZOAN PARASITE AND THE TIME-SPACE DEPENDENT PROLIFERATION OF THE PARASITIC STAGES

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Enteromyxum leei is a myxozoan parasite responsible for enteritis in gilthead sea bream (GSB) (*Sparus aurata*). The parasite proliferates in the paracellular space and induces an inflammatory reaction. The infection starts at the posterior intestine (PI), followed by the anterior (AI) and finally the middle (MI). A group of juvenile GSB were infected by anal intubation with *E. leei* infected-intestinal scrapings (RCPT), and another was intubated with PBS (CTRL). At 17 and 64 days post intubation (dpi), 7 fish from both groups were intracelomically injected with 0.1 ml BrdU at a dose of 100 mg de BrdU/kg fish weight. Samples of AI, MI and PI were taken for immunohistochemical localization of BrdU 24 h after injection. For each fish and intestinal segment, ten microscopical fields were photographed and BdrU immunoreactive (BrdU$^+$) cells (either from the parasite or the host) counted. There was a strong effect of the infection on the proliferation rate, as the number of BrdU$^+$ cells was higher for RCPT than CTRL fish in all the intestinal segments. The presence of the parasite at the PI (the first segment infected) seems to induce the proliferation of host intestinal cells even in not yet parasitized segments (AI, MI). The mean ratio of BrdU$^+$ parasites/total parasites in the PI was higher at 17 dpi (0.77) than at 64 dpi (0.30), whereas it was 0.58 and 0.61 at 64 dpi in the AI and MI, respectively. Therefore, the replicating rate of parasites in well-established infections is lower than that in recently invaded tissues.

Acknowledgments: This work was funded by Spanish MINECO through projects AGL2009-13282 and AGL2013-48560 and partially supported by the "Generalitat Valenciana" (projects PROMETEOII/2014/085 and ISI).

O-069

NEW MARKERS FOR RESOLVING MYXOZOAN PHYLOGENETIC RELATIONSHIPS

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Current myxozoan phylogeny relies on single SSU rDNA or concatenated analysis of SSU + LSU rDNA and EF2 genes. These markers, however, are not sufficient to reconstruct the pattern of some early branching nodes that are important for the understanding of the main phylogenetic trends of Myxozoa. These weakly supported nodes show unstable branching patterns or large polytomies.

Multigene analysis with large taxon sampling will resolve most of the polytomic phylogenies as observed in other biological groups and provide greater stability in myxozoan interspecies relationships. We thus used publically available genomic/transriptomic data as well as our own *Sphaerospora molnari* transcriptome to select new candidate genes that are phylogenetically informative and suitable for myxozoan phylogenetic reconstruction. Based on those gene alignments we designed primers that are able to amplify selected genes of other myxosporean representatives.

During preliminary genome/transcriptome mining we selected the following myxozoan genes for further analysis: V-ATPase, PAX-B (paired box), Elongation Factor 1α, PGD (6-phosphogluconate dehydrogenase), SNF (sans fille), TPI (triosephosphate isomerase), as well as three nematogalectins previously identified in Myxozoa. The phylogenetic tree based on V-ATPase and two nematogalectin genes corresponds to known rDNA-based myxosporean phylogeny, validating our hypothesis. Multigene analysis of SSU rDNAs, EF2, V-ATPase and nematogalectins resulted in a well resolved myxozoan tree with highly supported nodes.

Acknowledgement: Funding for the present study was provided by the Czech Science Foundation (P505/12/G112).

O-070

TRACING THE JOINT EVOLUTION OF MYXOZOANS AND THEIR HOSTS

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The combination of exceptionally high species diversity, extreme differences in the degree of host specificity and a complex life cycle raises many questions about the underlying mechanisms triggering speciation and evolution of the Myxozoa. That parasites and their hosts coevolve is a powerful concept with broad theoretical and applied implications. On a basic level, the term ‘‘coevolution’’ is used to describe the extent of congruence of host and parasite phylogenetic trees. In the present study, we used all SSU rDNA sequence data of actinosporeans and their definitive invertebrate hosts as well as of myxosporeans and their intermediate vertebrate hosts to assess the extent of codivergence, i.e. parallel speciation in host and parasite lineages, by examining the degree of congruence between their phylogenies. Using event-based and global fit methods (TreeMap, Jane, ParaFit), we investigated the origin of current associations and determined whether they arose by colonization (host switching) or by descent. We detected strong cophylogenetic signal between myxozoans and their invertebrate hosts, with regard to the deeper branches of the tree. In contrast, we demonstrate that cospeciation with fish hosts happened more recently and resulted in several host-associated species complexes. We also found phylogenetically conserved host-switching to mimic the phylogenetic signature of cospeciation, confounding topology-based programs. We speculate that myxozoans first had a direct life cycle involving only invertebrate hosts and that the acquisition of vertebrate intermediate hosts happened later and was the outcome of the distinct success of alternative transmission and dispersion strategies, resulting in massive specification events in fish.

O-071

HISTOZOIC MYXOSPOREANS INFECTING THE GASTROINTESTINAL TRACT OF CULTURED AND WILD MARINE FISHES

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Traditional studies on myxosporeans have used myxospore morphology as the main criterion for identification and taxonomic classification. This has led to numerous genera becoming polyphyletic in subsequent molecular phylogenetic analyses and it is now known that other features, such as the site of infection in fish, offer a far higher degree of congruence with molecular data than myxospore morphology can provide.

Histozoic gastrointestinal myxosporeans from two elopiform fishes from Malaysia, the Pacific tarpon *Megalops cyprinoides* and the tenpounder *Elops machnata* were identified and described using morphological, histological and molecular methodologies.

Both myxospores had a *Myxidium*-like morphology, but were robustly grouped with similar shaped myxospores from gill monogeneans at the base of the multivalvulid clade in all phylogenetic analyses. A new genus and family are erected to accommodate these taxa. In addition, we propose that the genus *Enteromyxum* is transferred to the new family and we also suggest that *Ceratonova* is better suited to the new family than to the Ceratomyxiidae.

To improve modern systematics of the myxosporeans it is clear that a combination of biological, molecular and morphological data should be used in descriptive studies, and the naming and redistribution of taxa and genera is going to be necessary to achieve this. Here we demonstrate why *Myxidium*-shaped myxospores should not be included in the family Myxidiidae, and create a new framework to accommodate them. Subsequent descriptive works should follow a similar course if we are going to create a prevailing and workable systematic structure for this group.

O-072

MYXOSPORIDIA INFECTING NILE FISH IN EGYPT

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Fish represent one of the most important sources of animal proteins all over the world. Parasitic diseases are the most important health risk facing fish production. Myxosporidian parasites are the most dangerous parasites infecting fish causing muscle and organs degeneration and their death. During the current survey of myxosporidian parasites, a total of 360 fish samples representing six of the most economically important species were collected from different localities along the River Nile, Egypt. These were *Oreochromis niloticus*, *O. aureus* (Cichlidae), *Barbus bynni*, *Labeo niloticus* (Cyprinidae), *Syodontis schall*, and *Clarias lazera* (Siluridae). Number of 180 fish specimens (50%) were found to be naturally infected with these parasites. The recovered parasites were morphologically described and identified according to light and transmission electron microscopic studies. Myxosporidian spores of *Myxobolus* four species, one species for *Myxidium*, and one for *Henneguya* were identified and described. Host-parasite relationship and pathological effect were recorded.

O-073

IS *NEOBENEDENIA MELLENI* (CAPSALIDAE: MONOGENEA) REALLY THE CULPRIT IN GLOBAL AQUACULTURE?

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Neobenedenia spp. pose a serious threat to global aquaculture through host-fish mortality and lost productivity. *Neobenedenia* have highly conserved morphology making morphology-based identification difficult. Recent research indicates that *Neobenedenia melleni*, may comprise a complex of morphologically indistinguishable but genetically distinct (cryptic) taxa. Cryptic species can exhibit variability in host-specificity, pathogenicity, and epidemiology which necessitates the accurate identification of *Neobenedenia* isolates from wild and farmed fish from around the world. In this study, we constructed the phylogenetic relationships of 36 *Neobenedenia* isolates from 27 host-fish species in 12 countries using nucleotide sequences from three genes; two nuclear (*H3* and *28s*) and one mitochondrial (*cytochrome b*). Our analysis recovered four separate clades, three of which correspond to *Neobenedenia longiprostata*, *Neobenedenia pacifica* and *N. melleni sensu stricto*. The fourth clade, which was the most diverse and contained the majority of the isolates examined, is probably '*Neobenedenia girellae'* which was synonymised with *N. melleni* in 1996. This study identifies nine new countries in which *N. 'girellae'* is present and 19 new host records that have not been recorded for *N. girellae*. These include fish species that support important commercial fisheries, ornamental trade, and aquaculture. Unfortunately, accurately identifying original sources of infection is complicated by global trade of ornamental fishes and because many studies have not accessioned research specimens for further study. This research begins the process of defining clear species limits and host records for *Neobenedenia* and represents one of the most comprehensive investigations into the phylogenetics of a capsalid genus to date.

O-074

SPECIES IDENTIFICATION OF DIPLECTANID MONOGENEANS PARASITISING WILD AND FARMED ASIAN SEABASS (*LATES CALCARIFER*) BY MORPHOLOGICAL AND MOLECULAR TOOLS

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Diplectanid monogeneans can induce morbidity and mortality in cultured fish due to destructive feeding mechanisms and high fecundity and short generation time. Eight species of diplectanids have been reported to parasitise wild and farmed Asian seabass, *Lates calcarifer*, in the Asia-Pacific region, but the species assemblage on *L. calcarifer* in Australia is poorly known. The aim of this study was to accurately identify diplectanid species infecting *L. calcarifer* in northern tropical Australia, where aquaculture farms have experienced production issues from persistent diplectanid infections. Parasite isolates were collected from wild and farmed fish across tropical northern Australia and a combination of morphological and molecular methods used to diagnose species. Our results indicate that a single, dominant species of diplectanid, *Laticola paralatesi*, infects farmed and wild *L. calcarifer* in tropical Australia. Two species of *Laticola* were also synonymised based on the combination of host infected, geographic distribution, morphological similarity and in sharing identical 28S rRNA sequences. Overall, this study highlights that species of *Laticola* reported infecting *L. calcarifer* in the tropical Indo-West Pacific may not be as diverse as previously thought. Further studies on Laticola paralatesi biology and ecology will contribute to effective and efficient strategies for managing these parasites in aquaculture.

O-075

DIPLECTANID MONOGENEANS IN GROUPERS OF THE MEDITERRANEAN AND EASTERN ATLANTIC

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The biodiversity of diplectanids on groupers of the Mediterranean and Eastern Atlantic is already known for some species, but certain grouper species were completely unexplored. The aim of our study was to establish a complete list of diplectanid monogeneans from all groupers of the region, especially those off Tunisia and nearby Western Libya. We collected gill monogeneans from the following grouper species: *Epinephelus aeneus*, *Mycteroperca costae*, *M. marginata*, *M. rubra*, and *Hyporthodus haifensis*. Fish were caught in the Mediterranean off Tunisia and Libya and specimens from off Senegal (Eastern Atlantic) were also examined. Fish identification was performed using barcoding methods (COI). The diplectanids included *Pseudorhabdosynochus americanus*, *P. beverleyburtonae*, *P. bouaini*, *P. dolicocolpos*, *P. enitsuji*, *P. hargisi*, *P. regius*, *P. riouxi*, *P. sinediscus*, *P. sosia*, and *Echinoplectanum echinophallus*. In addition, we found possibly new species on *M. rubra* and *H. haifensis*. Some of these species are abundant, but certain diplectanid species appear to be rare. Morphological identification of diplectanid species was mainly based on a comparative study of sclerotised reproductive organs, especially the vaginae. Our preliminary results show that the vaginal structures in several species of *Pseudorhabdosynochus* from *Mycteroperca* spp. in the Mediterranean are similar, thus suggesting a common origin for some of the diplectanid species of this geographic area.

O-076

DIVERSITY OF MONOGENEANS (MONOGENEA: DACTYLOGYRIDAE) PARASITIZING GOATFISHES (PERCIFORMES: MULLIDAE) OFF NEW CALEDONIA: ARE *HALIOTREMA* SPECIES FROM GOATFISHES MONOPHYLETIC?

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Percomorph fishes in tropical to warm-temperature seas associated with coral reef environments are known to harbour monogeneans of *Haliotrema* (Dactylogyridae), a genus that is currently considered to be a polyphyletic taxon containing numerous morphologically diverse species. While a strict definition of *Haliotrema* remains wanting, several new genera have been proposed for species groups embedded within this genus based on morphological and/or molecular characteristics. Moreover, recent studies show that some species of *Haliotrema*-like dactylogyrids from hosts of specific families appear to form monophyletic group. The aims of our study are to interpret the taxonomic data obtained for dactylogyrids from goatfishes off New Caledonia in a phylogenetic context and to test the hypothesis that *Haliotrema*-like dactylogyrids from goatfishes form a monophyletic lineage. A total of 13 species of goatfishes were examined. As a result, nine new and eight previously described species of *Haliotrema*-like dactylogyrids have been recognized to parasitize eleven species of New Caledonian goatfishes. Morphological analysis of sclerotized structures suggests that there are several groups within the dactylogyrids parasitizing these fishes, with regard to the basic structure of the male copulatory organ. The division into morphological groups is also supported by the results of a phylogenetic analyses using nuclear rDNA sequences. The twelve *Haliotrema*-like dactylogyrids from New Caledonian goatfishes were found to form a monophyletic lineage.
Acknowledgements: This study was supported by the Czech Science Foundation (project No. P505/12/G112).

O-077

ANNULOTREMA (PLATYHELMINTHES: MONOGENEA) FROM AFRICAN TETRAS (CHARACIFORMES: ALESTIDAE) IN LAKE TURKANA, KENYA: MORPHOMETRIC AND MOLECULAR ANALYSES

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The Alestidae, often called African tetras, are known to be parasitized by monogeneans representing three genera, namely *Afrocleidodiscus, Annulotrema* and *Characidotrema*, from which *Annulotrema* includes the highest number of species. The aims of the study were to: (1) investigate species diversity of *Annulotrema* species from alestids in Lake Turkana; (2) evaluate the relative taxonomic importance of the characters of the reproductive organs and those of the haptoral sclerites in accordance with molecular data; and (3) investigate the relationships between *Annulotrema* species and other representatives of Dactylogyridae based on nuclear and mitochondrial gene sequences. Our taxonomical evaluation revealed four new and four previously described *Annulotrema* spp. on the gills of *Alestes baremose, A. dentex, Brycinus minutus* and *Hydrocynus forskahlii*. Morphological analysis of sclerotized structures suggests that there are several different groups within the dactylogyrids parasitizing these fishes, with regard to the basic structure of the male copulatory organ.

Acknowledgements: Czech Science Foundation (project no. P505/12/G112).

O-078

GYRODACTYLUS PARASITES ON AFRICAN CICHLIDS: A VIEW ON SPECIES DIVERSITY, DISTRIBUTION, INTERSPECIFIC RELATIONSHIPS AND ECOLOGICAL PATTERNS

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Gyrodactylus von Nordmann, 1832 (Monogenea: Gyrodactylidae) includes highly diverse ubiquitous viviparous monogeneans. These ectoparasites feed on mucus and epithelial cells of host fishes that can induce hyperplasia of affected tissues and increase the potential of subsequent secondary infections. To date, 33 species of *Gyrodactylus* from hosts originating from nine host families have been described in Africa, but at least two species should be regarded as invalid. The present study summarizes ongoing research on *Gyrodactylus* spp. parasitizing African cichlids with emphasis on its diversity using an integrative approach (molecular sequences supported by morphometrical analysis), distribution and ecological patterns in the host-parasite system taking into account diverse environmental factors. Our findings of 10 undescribed species of *Gyrodactylus* on cichlid hosts in different parts of Africa significantly extend the knowledge on these parasites in terms of species richness and distribution. The observed “diversity” of *Gyrodactylus* spp. on cichlids differs considerably between the host species. The highest number of species was observed from the invasive *Oreochromis niloticus* and the commonly widespread *Pseudocrenilabrus philander*. The overlap in theirs habitats and distribution together with the artificial spreading by humans undoubtedly have contributed to the current situation. The phylogenetic analyses resulted in clustering of *Gyrodactylus* spp. that share similarities in the morphological type of the hard parts of attachment organs. Species of one morpho-group can parasitize cichlid species of up to 5 tribes, and to date we have found *Gyrodactylus* spp. on cichlid hosts of 7 tribes. Our results show clear evidence for intensive host-switching in this *Gyrodactylus*-cichlids system.

O-079

CICHLIDOGYRUS (MONOGENEA: DACTYLOGYRIDAE) FROM LAKE TANGANYIKA CICHLIDS: A PHYLOGENETIC AND EVOLUTIONARY APPROACH OF THE PARASITE FAUNA OF LAKE TANGANYIKA CICHLID TRIBES

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Lake Tanganyika, the oldest lake in Africa, harbors the most genetically, morphologically and ecologically diverse cichlid assemblages of the African Great Lakes. Its mostly endemic cichlids are considered as a model to study adaptive radiation and rapid diversification. Because of the apparently high host specificity of gill monogeneans, phylogenetic studies using molecular data of these parasites offer an interesting additional approach to study their hosts' diversity and evolution. Cichlids in Africa are known to host five different genera of monogeneans, *Cichlidogyrus* being the most diverse with more than 95 species recorded from more than 70 cichlids. Members of *Cichlidogyrus* are gill ectoparasites. To recognize different species of *Cichlidogyrus*, we use morphological data (haptor and male copulatory organs (MCO)) in addition to molecular phylogenetic analyses. The goal of this study is to investigate the phylogenetic and evolutionary affinities between the *Cichlidogyrus* fauna of the different Tanganyika cichlid tribes using different molecular markers (28S rDNA, 18S rDNA, ITS1 and COX1). In addition, we will morphologically characterize the new species of *Cichlidogyrus* encountered. An evaluation of the phylogenetic relationships of cichlid mononeneans parasitizing the different Lake Tanganyika cichlid tribes may help us to elucidate the historic relationships between these cichlid tribes and to determine the origin of *Cichlidogyrus* of Lake Tanganyika cichlids.

"This study was supported by Czech Science Foundation, project No. P505/12/G112 (ECIP)."

O-080

COPHYLOGENY OF *LIGOPHORUS* (MONOGENEA: DACTYLOGYRIDAE) AND THEIR MULLET (MUGILIDAE) HOSTS IN THE MEDITERRANEAN AND BLACK SEAS

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We evaluated the phylogeny of *Ligophorus* spp., and of their hosts in the Mediterranean, Black and Azov Seas, assessing their cophylogenetic relationships. We used partial 28S and complete ITS1 sequences from 14 *Ligophorus* species and 16S partial sequences from 6 species of mullets available at GenBank, and new 28S and ITS1 sequences from *L. parvicirrus*. The phylogeny of hosts (16S) and parasites (concatenated genes) was determined by maximum likelihood (ML) and Bayesian analyses. PACo (Procrustes Approach to Cophylogeny) was used to assess the congruence between the ML and Bayesian phylogenies of *Ligophorus* spp. and mullets, as well as the contribution of each host-parasite association to the global fit. The host and parasite phylogenies were more congruent than expected by chance. The most divergent host-parasite associations concerned the *Ligophorus* spp. on *M. cephalus*. These *Ligophorus* spp. formed a crown group, sister to species occurring on *Liza haematocheilus*. Given that this host is an introduction from the Pacific, this points to an ancient host-switch to *M. cephalus* before colonization of the Mediterranean. Additional phylogenetic evidence from outside the Mediterranean would be most valuable to fully understand the coevolutionary history of this host-parasite system.

O-081

MONOGENEA IN DEEPWATER CICHLIDS: DECREASE IN HOST SPECIFICITY OR ONGOING SPECIATION?

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Lake Tanganyika contains unique species flocks of vertebrate and invertebrate taxa and harbours the most diverse cichlid species assemblage of all African lakes. This makes it an unrivalled study area in the field of biodiversity and investigations of parasite evolution processes and mechanisms. Whereas in littoral Tanganyika cichlids the host-specificity of *Cichlidogyrus* species (Monogenea, Dactylogyridae) is relatively strong, a previous study indicates reduction of this specificity in deepwater habitats. In our study we examined and analysed monogeneans occurring in deepwater cichlids from three different tribes (Bathybatini, Trematocarini, Benthochromini). Samples originated from many localities throughout the lake. Hypotheses were tested by three different techniques including morphometrics, geomorphometrics and genetic characterization using multivariate statistical approaches. We confirmed the reduction of host-specifity of *Cichlidogyrus casuarinus* in the deepwater realm; this parasite is widespread throughout the tribe Bathybatini. On the other hand, there is evident intraspecific variation in parasite sclerotized structures influenced by host preference. Since intraspecific genetic variation is independent from the host species, this pattern seems to be caused by phenotypic changes during ontogenetic development. Moreover, analyses of parasite demographic history on the basis of COI sequences indicate recent population increase of *C. casuarinus*.

O-082

GYRODACTYLUS SPP. ON AFRICAN *CLARIAS GARIEPINUS*: PARASITES WHO PREFERRED CO-EVOLUTION INSTEAD OF HOST SWITCHING?

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Sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), is widely distributed in Africa with the exception of Maghreb, Upper Guinea and Cape provinces of South Africa. On the African continent, *C. gariepinus* is currently known to be a host to seven species of the genus *Gyrodactylus* von Nordmann, 1832.

During the period August 2011 – November 2014, several localities were sampled to establish the diversity of gyrodactylid parasites in the southern African region. In total 31 specimens of *C. gariepinus* were collected on several localities in South Africa (Flag-Boshielo Dam, Loskop Dam, Sand and Barota River in Limpopo Province; Mooi River and Barberspan Dam, North-West Province) and Zimbabwe (Zambezi River and Lake Kariba). The prevalence of *Gyrodactylus* spp. was 45%. Species identification based on opisthaptoral hard parts morphometry and nuclear rDNA ITS sequences identified the presence of 10 different species of *Gyrodactylus*, of which four are described, i.e. *G. alekosi* Přikrylová, Blažek & Vanhove, 2012; *G. gelnari* Přikrylová, Blažek & Vanhove, 2012; *G. rysavyi* Ergens, 1973 and *G. transvaalensis* Prudhoe & Hussey, 1977. Present findings reveal unexpected *Gyrodactylus* species diversity and their wide distribution across different regions. Phylogenetic analyses based on the ITS rDNA sequences assisted in revealing interspecific relationships. Analysis of 18S rDNA revealed identical sequences for four species with distant geographical origin which also differed substantially in morpho-metrical characters. The observed monogenean species richness in a single host seems to be a signal that co-evolution with intensive diversification occurred in this host-parasite system

O-083

ANISAKIDS DOWN UNDER

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Anisakid nematodes are well known parasites of aquatic and aquatic associated animals, including fish, with a global distribution. Their larvae cause a disease in humans known as Anisakidosis which occurs after infected fish is consumed. Employing advanced morphological and molecular techniques to study and elucidate various biological and ecological aspects of anisakids has had a later start in Australia than in the Northern hemisphere, nevertheless great biodiversity has been found and unique species have been discovered. This presentation will review the recent advances in our knowledge of Australian anisakids. Some of the new species recently discovered in Australia, life cycles of selected taxa and phylogenetic relationships between them and other members of anisakids reported from elsewhere in the world will be presented. Research on Australian anisakids is essential to have a better understanding of the co-evolution of these parasites as well as their biology and ecology worldwide. The development of seafood safety guidelines and establishment of control and preventive strategies to protect consumers and workers are also highly dependent of the outcomes of this research.

O-084

ANISAKID INFECTION IN COMMERCIAL CEPHALOPODS FROM THE SPANISH MEDITERRANEAN COAST

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This study aims to investigate the occurrence of anisakid larvae in commercial cephalopods in the Western Mediterranean Sea. A total of 202 animals comprising 123 *Illex coindetii*, 34 *Loligo vulgaris* and 45 *Octopus vulgaris* were analysed using enzymatic digestion. Isolated larvae were identified at species level using molecular methods. A total of 31 larvae were recorded (prevalence: 14.6%) parasitizing *I. coindetii* corresponding to the genus *Anisakis* (P= 12.2%) and *Hysterothylacium* (P= 4.1%). The nematode identified were *A. pegreffii* (P= 11.4%), *A. physeteris* (P= 1.6%), a hybrid larva between *A. simplex* and *A. pegreffi* (P= 0.8%) and *Hysterothylacium* sp. (P= 4.1%). All *Anisakis* larvae were found within the visceral cavity; in contrast, most of the *Hysterothylacium* larvae were isolated from the mantle. A significant correlation was found between total anisakid prevalence and depth, explained by the presence of largest squids inhabiting deeper depths; also between anisakid prevalence and geographical sector of capture. Results obtained in the present study improve the picture of the occurrence of anisakid species in *I. coindetii* from the Spanish Mediterranean Sea, confirming the role of the broadtail shortfinned squid as paratenic host in the life cycle and its potential hazard due to the parasite's ability to cause anisakidosis and associated allergic reactions.

PREVALENCE AND IDENTIFICATION OF ANISAKIDAE PARASITES IN FISH: THE FISH-PARASITES PROJECT

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Among parasites frequently present in edible fish, nematode larvae belonging to the *Anisakidae* family occur in numerous fish and cephalopods. These larvae may induce digestive or allergic symptoms in human. To better define the impact of fish parasites on consumers' health and to improve the safety of fish products, we set up the French national Fish-Parasites network (ANR-10-ALIA-004, http://fish-parasites.com/en/). One axis of this program was to collect prevalence and identification data on *Anisakidae* nematodes in fish.

Fifteen species of fish were selected according to a risk-ranking analysis. A total of 1 781 fish were sampled. Anisakid identification relied on 2 methods: individual Sanger sequencing of a *Cox2* fragment or pooled High Throughput Sequencing based on a *Cox2* fragment, method developed and validated during the project.

No *Anisakidae* were isolated from 44 % of all the fish sampled whereas 33 % of all fish had *Anisakidae* only in their viscera and 23 % had *Anisakidae* either in their fillets only or in their fillets and viscera. Nematodes isolated from 15 fish species caught in North East Atlantic and Mediterranean Sea were mostly identified as belonging to the genus *Anisakis*, mainly *A. simplex*, but also *A. pegreffii*. Species belonging to other genera such as *Pseudoterranova*, *Contracaecum* and *Hysterothylacium* were also identified.

All data were gathered in a database (PARAFISH) specifically designed for the project. Other outcomes of the Fish-Parasites project, such as the internet site, the professional training sessions and the fish parasite identification platform will also be presented.

PARASITE RISK IN COMMERCIAL FISH FROM ATLANTIC IBERIAN PENINSULA AND GRAN SOLE FISHING GROUNDS

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As part of the ambitious Surveillance Program within the EC Project PARASITE (http://parasite-project.eu/), we analyzed for nematode presence 1516 fish of 8 species from 3 ICES areas: ICES VII (Gran Sole), ICES VIII (Galician coast) and ICES IX (Portuguese coast). Upon capture, fish were placed in ice until landing and transport to the lab, where they were immediately frozen to prevent nematode migration. After thawing, fish were dissected, pressed, examined under UV light and digested enzymatically. Nematode samples were stored frozen. Data for every fish and nematode sample were saved and coded in a BioBanking platform for further molecular species identification.

For analyzing risk associated to parasites we used the SADE inspection rating scheme. Results were translated to the scoring Fish Parasite Rating (FPR) Standard, allowing the classification of the fish lots in five categories: POOR, FAIR, GOOD, VERY GOOD and EXCELLENT. The application of the above risk ranking tool rendered the following classification of the fish lots:

- **Poor:** *Lophius budegassa, Lophius piscatorius* and *Merluccius merluccius* from ICES VII; *M. merluccius* from ICES VIII.
- **Fair:** *Lepidorhombus boscii* from ICES VII; L. *budegassa, L. piscatorius* and *Micromesistius poutassou* from ICES VIII; *M. merluccius* from ICES IX.
- **Good:** *Scomber scombrus* from ICES VIII; *L. budegassa, M. poutassou* and *S. scombrus* from ICES IX
- **Very good:** *Engraulis encrasicolus* and *L. boscii* from ICES VIII; *L. boscii, L. piscatorius* and *S. japonicus* from ICES IX
- **Excellent:** none

Conclusions are discussed under epidemiological, industrial and food safety perspectives.

O-087

GETTING MYXOZOAN PARASITES HOT UNDER THE COLLAR: A REAL-TIME PCR ASSAY TO QUANTIFY PROLIFERATIVE BLOOD STAGES OF *SPHAEROSPORA MOLNARI* IN *CYPRINUS CARPIO* IN A RANGE OF TEMPERATURES

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Climate change is affecting the environment of many fish species globally with increased temperatures, rainfall and extreme weather. Parasites are also coping with these changes and are likely to adapt their transmission, reproduction rates and disease effects accordingly. Myxozoan parasites will have to adjust to these changes in both their vertebrate and invertebrate hosts. Higher temperatures are often linked to faster parasite replication rates, host susceptibility or altered host immune function. How will Myxozoa react to increased temperatures? How will hosts respond? Will their relationship be negatively affected? We used *Sphaerospora molnari*, an emerging myxozoan pathogen in common carp, to answer some of these questions experimentally. Following entry into the host, the parasite proliferates in the blood prior to spore formation in the gills. The blood stages of this species are purely proliferative, increasing the number of circulating parasites in the host. We tested the effect of temperature on the prevalence of these blood stages. SPF carp fingerlings were acclimatized to temperatures mimicking winter to peak summer temperatures Czech ponds. Fish were infected with *S. molnari* blood stages and bled repeatedly over 100 days, parasetemia was measured with real-time PCR using host and parasite markers. We saw differences in parasite proliferation rates between temperatures, revealing the complexity and interplay between the host immune system, myxozoan parasitemia and water temperature. These are the initial steps to understanding the biology behind infection and make some projections about the future of aquaculture in areas affected by myxozoans and climate change.

O-088

MONITORING A MYXOZOAN PARASITE FOR MANAGEMENT AND MODELS: LONG-TERM SURVEILLANCE OF A FISH PATHOGEN BY MOLECULAR QUANTIFICATION OF RIVER-BORNE STAGES

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Ceratonova shasta causes enteronecrosis in juvenile salmonids in the Pacific Northwest of North America and is associated with population-level impacts in the Klamath River. Transmission occurs through waterborne stages: actinospores released from polychaete worms develop into myxospores in salmonid fishes. In response to the high prevalence and severity of *C. shasta* infection in Klamath salmonids, we developed a parasite monitoring program that included sentinel fish exposures, invertebrate host sampling and molecular quantification of parasite DNA in water samples. In 2006, we established 5 mainstem index sites, which spanned 240 river kilometers, and 4 sites in tributaries. Water samples were collected approximately weekly, filtered, total DNA extracted and *C. shasta* quantified using a TaqMan qPCR (ssrRNA gene). Relative abundance of host-specific *C. shasta* ITS-1 genotypes was determined using a SYTO9 qPCR and sequencing. Parasite abundance varied significantly among the 9 years. Direct, regular measurement of waterborne parasite stages was a pragmatic alternative to host sampling and facilitated semi-real-time reporting via weekly, online updates. These data inform epidemiological and predictive model development and host management strategies. A management goal for the system is to reduce salmonid mortality below 40%. Parallel water sampling and sentinel fish exposures provided disease thresholds for Chinook and coho salmon (10 and 5 spores/L respectively). In 2014, when our real-time monitoring data parasite levels surpassed these thresholds, water was released from the dam in a ‘pulsed flow event’ in an effort to reduce disease severity in outmigrating salmonids; we monitored parasite levels before, during and after the event.

O-089

FIRST OUTBREAK OF PROLIFERATIVE KIDNEY DISEASE IN AUSTRIA LINKING TO THE AETIOLOGY OF “BLACK TROUT” THREATENING AUTOCTONE BROWN TROUT POPULATION

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Proliferative Kidney Disease (PKD) was diagnosed for the first time from juvenile autoctone brown trout (*Salmo trutta*) in Austria during the summer 2014. A highly immuno-compromised condition was indirectly shown due to a massive ectoparasitosis. *Tetracapsuloides bryosalmonae* sporoblasts were seen on stained kidney impression smears, while sporogonic (coelozoic) and extrasporogonic (histozoic) proliferations were assessed by histopathology. Malacosporean ribosomal SSU detection by PCR from DNA, revealed a closely related strain to other European isolates. Infection and strain high pathogenicity were confirmed following a small scale pre-restocking test with juvenile SPF brown and rainbow trout (*Oncorhynchus mykiss*). The farm trial resulted in 100% morbidity, mortality occurred between 28 and 46 days post exposure. Fish showed grade 2 of the kidney swelling index and grade 3 of the PKD histological assessment. Further locations were inspected along the river Kamp, infected sites were demonstrated up to 6 km upstream the infected farm. *Fredericella sultana* colonies were collected from these locations and cultivated in laboratory. Malacospores released elicited PKD in exposed SPF brown trout. This study represents the first isolation of a highly pathogenic myxozoan parasite from an Austrian river. The immunosuppressant action played by this highly pathogenic *T. bryosalmonae* strain could link PKD as a key factor in the context of the syndrome locally so-called “Black Trout” (BT). Further research is needed to assess the impact of the spread of PKD and BT in this region and to several other European countries via the river Danube, to which river Kamp is a tributary.

O-090

DESCRIPTION OF A NEW *TETRACAPSULOIDES* SPECIES AND ITS LIFE CYCLE – WHAT ARE THE FEATURES THAT BEST DEFINE A MALACOSPOREAN SPECIES?

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In contrast to approx. 2400 described species of Myxosporea, that utilize annelids as definitive hosts, the evolutionary older Malacosporea develop in freshwater bryozoans and presently include only three nominal species. However, recent molecular studies strongly indicate that malacosporean biodiversity is unexpectedly high.

Malacosporeans alternate between bryozoan and fish hosts. To date, three malacosporean life cycles are known, with only that of *Tetracapsuloides bryosalmonae*, having been completed *in vitro*. Two further species (*Buddenbrockia plumatellae* and *Buddenbrockia* sp.) have been transmitted from bryozoan to fish. We recently detected *in vitro* transmission of a new malacosporean species belonging to the genus *Tetracapsuloides* from *Fredericella sultana* to SPF common carp *Cyprinus carpio* and vice-versa., in cohabitation experiments of the two hosts. In fish, this new species produces monosporous pseudoplasmodia within the renal tubules, whereas a motile, vermiform stage is produced in the bryozoan. Previously, vermiform stages were only known from the genus *Buddenbrockia*. All stages from the different hosts were confirmed by SSU rDNA sequencing.

During compilation of morphological, host and molecular characteristics for the species described herein, we have noticed that, despite major advances in our understanding of the Malacosporea brought about by studies on morphology, cellular ultrastructure, development and especially by recently emerged molecular data, a consensus definition of ‘species’ or even ‘genus’ remains a notoriously difficult concept in this group. We hereby point out the flaws in malacosporean taxonomy and provide guidelines for the future description of malacosporean taxa.

O-091

MONOGENEAN PARASITES OF CICHLID FISHES: FROM ADAPTIVE RADIATION TO A TOOL IN INTRODUCED SPECIES RESEARCH

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Studying cichlids enhanced our understanding of speciation and radiation. In contrast, little is known about the evolution of their parasites. Lake Tanganyika's (cichlid) biodiversity and its importance as cradle and reservoir of ancient lineages seeding other radiations has resulted in a significant body of literature. Remarkably, until recently virtually no research was carried out into the Tanganyika parasite fauna. We investigate the monogenean flatworm species flock infecting Tanganyika cichlids, focusing on the endemic tropheine cichlids and report high host-specificity in monogeneans belonging to *Cichlidogyrus* (Dactylogyridae). Congruence between the flatworm and cichlid phylogenies seems the result of concomitant divergence. Within-host speciation caused this parasite genus to surpass the species richness of its hosts. Furthermore, we applied *Cichlidogyrus* species distributions when revising a tropheine cichlid genus. Investigating other Tanganyika host lineages demonstrated that *Cichlidogyrus* saw different speciation pathways in different cichlid tribes, e.g. with variable levels of host-specificity, underscoring the uniqueness of Lake Tanganyika's cichlid flock from a parasitological point-of-view as well. Apart from the Tanganyika biodiversity hotspot, several primarily riverine cichlids of the Congo Basin are important to fisheries, aquaculture and aquaristics. We explore their monogenean fauna and its potential as threat and tool in introduced species research: the risk of introduction of non-native monogeneans together with Nile tilapia, and the potential of monogeneans in stock identification.

Research supported by the Czech Science Foundation (P505/12/G112 – ECIP), the Austrian Science Fund, the Research Foundation–Flanders, the King Leopold III Fund for Nature Conservation and Exploration, and the Belgian Federal Sciency Policy (BR/132/PI/TILAPIA).

O-092

PRELIMINARY RESULTS ON THE CO-PHYLOGENY OF THE DIPLOZOIDAE AND THEIR CYPRINID HOSTS

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The Diplozoidae are some of the most intriguing monogenean parasites as a result of their unique reproductive strategy. However, very little is known about their phylogenetic relationship with their hosts. In an earlier study, the phylogeny of these parasites appeared to mirror that of the hosts, but due to the increased amount of molecular data available for the Diplozoidae, it became possible to study this co-phylogeny in more detail. This study aims to further investigate the phylogenetic relationship of the parasites and their hosts by increasing the number taxa included in the analysis. Sequences where acquired experimentally from diplozoid taxa for which molecular material was available in the form of internal transcribed spacer 2 (ITS2), large subunit rDNA (LSU) and cytochrome c oxidase subunit 1 (COI) markers, as well as COI and cytochrome b (cyt b) for the hosts. In addition, representative sequences available from GenBank for both the parasites and their hosts were retrieved, with mostly ITS2 sequences available for diplozoids and both COI and cyt b for the hosts. The resulting cyprinid phylogeny showed the expected grouping of taxa according to both taxonomic and geographic traits. The diplozoid phylogeny also grouped into similar clades, but not as strictly as in the hosts. Thus, the results show some interesting differences between the phylogeny of these two groups which might add to the understanding of the evolution and speciation of the Diplozoidae.

Funding from National Research Foundation and University of Johannesburg is acknowledged.

O-093

PHYLOGENY OF SPECIFIC GILL MONOGENEANS: A TOOL FOR STUDY HOST BIOGEOGRAPHY?

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Species of Dactylogyrus are gill parasites almost specific to cyprinid fish. This highly diversified genus includes many host-specific species. Many *Dactylogyrus* spp. coexist on the same host species. *Dactylogyrus* spp. evolve by intrahost speciation and the species coexisting in the same host differ in their niche position. Cyprinids originate from Asia and are classically divided into several subfamilies: Cyprininae, Rasborinae, Acheilognathinae, Cultrininae, Gobioninae, Tincinae, Leuciscinae and Alburninae. The cyprinids show a specific pattern of biogeographical distribution. Whilst the members of all subfamilies are widely distributed in Asia, some cyprinid groups are absent in Europe. Only the species of Cyprininae in Africa and only the species of Leuciscinae in North America are present. Previous study focusing on molecular phylogeny using European cyprinid fish species showed that Cyprininae represent plesiomorphic host group. The aims of this study were (1) to analyze the phylogenetic position of *Dactylogyrus* spp. parasitizing African cyprinid fish, and (2) to estimate the role of *Dactylogyrus* spp. for inferring historical biogeographical patterns of cyprinid fish species. We showed that *Dactylogyrus* spp. from cyprinins are not monophyletic. Surprisingly, *Dactylogyrus* spp. from common carp (*Cyprinus carpio*) and gibel carp (*Carassius gibelio*) originated from Asia and recently distributed across Europe together with *Dactylogyrus* spp. from *Labeo* spp. and *Barbus* spp. (i.e. African cyprinins) form a monophyletic group. *Dactylogyrus* spp. from other Asian cyprinids clustered together with *Dactylogyrus* spp. of European Gobioninae and Leuciscinae. We demonstrated the nested position of *Dogelius* and *Dactylogyroides* in one of two main lineages of *Dactylogyrus* which suggests the need of a taxonomic reevaluation.

This study was supported by Czech Science Foundation, project No. P505/12/G112 (ECIP).

O-094

COMPARATIVE PHYLOGEOGRAPHY OF TWO MONOGENEAN SPECIES (MAZOCRAEIDAE) ON THE CHUB MACKEREL, *SCOMBER JAPONICUS*, ALONG THE COAST OF CHINA

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In the present study, the phylogeography of two monogenean species, *Pseudokuhnia minor* and *Kuhnia scombri*, parasitising *Scomber japonicus*, was studied. Fragments of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene were sequenced for 264 individuals of *P. minor* and 224 individuals of *K. scombri* collected from 10 localities along the coast of China. Genetic diversity of *K. scombri* was higher than that of *P. minor*, which may imply that *P. minor* has a low evolutionary rate and/or is a younger species. The neighbour-joining trees for both parasites were divided into two clades without any association to sample sites, which might be the signature of remixing populations following past division. Analyses of molecular variance (AMOVA) and pairwise F_{ST} revealed different genetic structures for these two closely related species: *P. minor* exhibited a lack of genetic structure in the range investigated, while *K. scombri* showed genetic differentiation along the coast of China, which might have resulted from differences in evolution, life histories and dispersal strategies of the two species. Both neutrality tests and mismatch distribution suggested that the populations of these two species along the coast of China experienced population expansion in the late Pleistocene due to the glacial-interglacial cycles induced by climatic oscillations.

O-095

OF SNAILS AND WORMS – TREMATODE COMMUNITIES IN A RESERVOIR SYSTEM IN GERMANY

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Digenean trematodes are important and integral elements in aquatic ecosystems, especially in eutrophic systems which create favourable conditions for diverse host and parasite communities. To better understand their role in man-made waterbodies, we assessed the trematode diversity and community composition and structure in snails in the Ruhr River reservoir system (Germany). Snails were collected from five reservoirs in 2012 and 2013 and examined for trematode infections. Trematode species were identified based on morphology and, in cases of questionable taxonomy, using molecular methods. In total, 6,507 snails belonging to 19 species of eight families were examined. Most abundant were the lymnaeids *Lymnaea stagnalis* (n = 245), *Radix auricularia* (n = 1,909), *R. peregra* (n = 349) and *Stagnicola palustris* (n = 668), and planorbid species *Gyraulus albus* (1,919) and *Segmentina nitida* (195). Altogether, these six snail species harboured a diverse trematode fauna with 34 distinct species. Overall prevalence in these hosts varied considerably and ranged from 1.4 % (*S. nitida*) to 37.4 % (*R. auricularia*). Molecular identification of taxonomically controversial groups revealed the presence of cryptic species, including groups utilising fish as second intermediate hosts (*Diplostomum* spp., *Petasiger* spp.). Component community analyses revealed distinctive trematode community composition in different snail hosts and different reservoirs that can serve as indicators of the presence of the parasites' hosts. Our study shows that this reservoir system offers ideal conditions for a species-rich and diverse trematode fauna, including important pathogens of fish. The community composition analyses show that trematodes can be valuable bioindicators in ecosystems.

O-096

PARASITE BIODIVERSITY IN CORAL REEF FISH AS SHOWN BY RESULTS IN NEW CALEDONIA, SOUTH PACIFIC

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More than 3,000 fish were sampled off New Caledonia, South Pacific, in an 8-year program. Parasites from various groups were collected, and included helminths (digeneans, monogeneans, cestodes, nematodes) and crustaceans (isopods and copepods). Thanks to international collaborations with several outstanding colleagues, more than 120 papers, representing more than 1,700 published pages, were written in an attempt to take account of the exceptional biodiversity of parasites in coral reef fish. Review papers have already been published about groupers (Serranidae), emperors (Lethrinidae), snappers (Lutjanidae) and breams (Nemipteridae); these include long parasite-host and host-parasite lists and a numerical evaluation of parasite biodiversity. Results about several other fish families have been published as individual descriptions of new parasite species but were not assembled in a review. Many parasites are still undescribed or unidentified and currently published results represent only a part of the available information about fish parasite biodiversity in the coral reef lagoon of New Caledonia. However, minimalist calculations based on four families of fish suggest a mean number of 10 parasite species and 30 host-parasite combinations per fish. These are only mean numbers and some large species might have more than 35 species of parasites. Comparisons with other reefs in the world are difficult since no comparable study is available in the literature. Comparisons with fish of similar families in other, non-coral reef environments, suggest, again, that fish in coral reefs are exceptionally rich in parasites.

O-097

DIDYMOZOID TREMATODES OF THE HEAD OF THE ATLANTIC BLACK SKIPJACK *EUTHYNNUS ALLETTERATUS* (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN SEA

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The Atlantic black skipjack *Euthynnus alletteratus* (Rafinesque, 1810) is a pelagic fish distributed in the Atlantic Ocean and Mediterranean Sea, where it has a commercial interest for coastal and artisanal fisheries. Nevertheless, the information on its parasite fauna is limited to the southwestern Atlantic Ocean, and no information is available for the Mediterranean Sea. The aim of this study is to describe the didymozoid parasites (Trematoda) of the head of *E. alletteratus* from the western Mediterranean Sea. Between 2008 and 2011, 105 heads of *E. alletteratus* from the western Mediterranean Sea were examined for parasites. Didymozoids were identified using both morphological and molecular methods.Five didymozoid taxa were found: *Melanocystis* sp. (prevalence 33%) in the pharynx; Nematobothriinae gen. sp. (31%) in the opercula and pharynx; *Oesophagocystis* sp. 1 (43%) in the inner margin of the gill filaments; *Oesophagocystis* sp. 2 (10%) in the gill rakers; *Oesophagocystis* sp. 3 (6%) in the nostril and the tip of the gill filaments. None of the parasite herein found has been previously reported in this host from the Atlantic Ocean. Parasite populations of *E. alletteratus* from the Mediterranean Sea seem to be isolated from the Atlantic ones, partly because of the limited dispersion of parasite species due to environmental conditions, but also because of the scarce migratory behaviour of this tuna, as confirmed by these results.

Research supported by the projects funded by Spanish government (MINECO, GPM12-13; MICIN, PARATUN AGL2010-20 892) and the Regione Autonoma della Sardegna grant (S.M., PRR-MAB-A2013-17 605).

O-098

GASTROINTESTINAL PARASITES OF FREE-LIVING INDO-PACIFIC BOTTLENOSE DOLPHINS (*TURSIOPS ADUNCUS*) IN THE NORTHERN RED SEA, EGYPT

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The present study represents the first report on the gastrointestinal parasite fauna infecting the free-living and alive Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) inhabiting waters of the Red Sea at Hurghada, Egypt. A total of 94 individual faecal samples of the examined bottlenose dolphins were collected during several diving expeditions within their natural habitats. Using classical parasitological techniques, such as SAF-method, carbol-fuchsin stained faecal smears, coproantigen-ELISA, PCR and macroscopical analyses were applied, the study revealed infections with 21 different parasite species belonging to protozoans and metazoans with some of them bearing zoonotic potential. In detail the study indicate stages of ten protozoan species (*Giardia* spp., *Sarcocystis* spp., *Isospora* (like) spp., *Cystoisospora* (like) spp., Ciliata indet. I, II, Holotricha indet. Dinoflagellata indet., *Hexamita* (like) spp., *Cryptosporidium* spp.), seven trematode species (*Nasitrema attenuata*, *Nasitrema* sp. I, II, *Zalophotrema curilensis*, *Zalophotrema* spp., *Pholeter gastrophilus*, Trematoda indet.), one cestode species (*Diphyllobothrium* spp.), two nematode species (Ascaridida indet, *Capillaria* spp.) and one crustacean parasite (Cymothoidae indet.). Additionally we molecularly identified adult worms of *Anisakis typica* in individual dolphin vomitus samples by molecular analyses. The present results may be used as a baseline for future monitoring studies targeting the impact of climate or other environmental changes on dolphin's health conditions and therefore contribute to the protection of these marine mammals.

O-099

PRELIMINARY ANALYSIS ON OCCURRENCE OF GILL ECTOPARASITES (PLATYHELMINTHES, MONOGENOIDEA) ON COMMERCIAL MARINE FISH FROM THE SOUTHWESTERN GULF OF MEXICO

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The Campeche Bank (southwestern Gulf of Mexico) constitutes an important marine ecosystem characterized by its high biodiversity, living resources and energy resources from which this region is considered as strategic in the national plans for social and economic development of Mexico. However, although the increasing economic importance of fish species in this region, there is limited information on its parasite fauna in order to understand host–parasite interactions. The purpose of this study has been to carry out a parasitological analysis on 5 fish species in the context of the presence of gill monogenoidean ectoparasites. Fish were captured from January to March 2015 from 3 sites along the Campeche Bank by local fisherman and euthanized by thermal shock in ice. After euthanasia, gills were removed, fixed in 4% formaldehyde and subsequently examined for the presence of monogenoideans. In this study were found monogenoidean species of *Euryhaliotrema* and *Haliotrematoides* on *Lutjanus synagris* (100 individuals collected/ mean total length of 28cm), *Lutjanus griseus* (Lutjanidae) (65/28), *Haemulon plumieri* (Haemulidae) (105/26), *Archosargus rhomboidalis* (Sparidae) (49/23) and species of *Hargitrema* and *Neotetraonchus* on *Ariopsis felis* (17/34) and *Bagre marinus* (Ariidae) (15/34). Co-ocurrence of parasites on its respective hosts seems strongly to be drived by the host phylogeny instead of local factors. Data obtained until now significantly enhance our knowledge of the distribution and specificity of species of these monogenoideans infecting marine fishes mentioned above.

O-100

MORE TAXONOMY NEEDED: A SURVEY OF OTSEGO LAKE FISH PARASITES (NEW YORK, USA)

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A survey of the intestinal parasites of fishes of Otsego Lake and its tributaries (Cooperstown, New York) took place from 2008 to 2014. Over 500 individual fish were collected by hook and line, seine, gill net, or ElectroFisher, and examined for intestinal parasites, and in many cases, for parasites in other fish organs. Helminths were prepared as whole mounts using conventional methods and examined with light microscopy and scanning electron microscopy. DNA sequence data (mitochondrial cytochrome oxidase I) was also obtained for a subset of the helminth species, and those data were compared with other published sequence data. The survey included a total of 27 fish species. Thirteen of the 27 fish species examined were infected with parasitic worms in the alimentary canal, including four species of acanthocephalans, nine species of cestodes, six species of digenetic trematodes, and at least five species of nematodes. Additional species of helminths were encountered as larvae in the body cavity, or the viscera, in most of the fish species examined. Among the intestinal parasitic worms, the most prevalent and least host specific was the cryptic acanthocephalan, known as *Leptorhynchoides thecatus* 'Large form.' At least one new species was discovered, of nematode genus *Spinitectus.* Generic identifications of helminths were possible based on the published literature, but unequivocal species identifications were often not tenable owing to a deficiency of reference information. This was the case for species of *Neoechinorhynchus*, *Proteocephalus, Crepidostomum* and *Spinitectus,* signifying the need for revisionary work in North America on these taxa.

O-101

METAZOAN PARASITES OF PANAMA SPADEFISH *PARAPSETTUS PANAMENSIS* (PERCIFORMES: EPHIPPIDAE) FROM PUERTO PIZARRO, TUMBES, PERU

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Parapsettus panamensis (Ephippidae) is a marine fish species distributed from Mexico to Peru. The aim of this study was to identify the metazoan parasites of *P. panamensis* on the north coast of Peru. 43 specimens of *P. panamensis* were acquired in 2014 from Puerto Pizarro, Tumbes, Peru. Parasites were catalogued and evaluated using standard protocols. A total of 2662 metazoan parasites were collected with a total average abundance of 61.9. 11 parasites were identified: Monogenea: *Sprostoniella* sp. and *Parancylodiscoides* sp. Trematoda: *Aponurus* sp., *Multitestis* sp1. and *Multitestis* sp2. Nematoda: *Anisakis* sp. and nematoda gen. sp. Copepoda: *Caligus* sp, *Lernanthropus*. sp. and *Bomolochus* sp. Isopoda: *Cymothoa exigua*. The average species richness was 4.49 (0-8). Endoparasites dominated to ectoparasites. 81.82% of metazoan parasites collected were mature forms of gill and gastrointestinal location and 18.19% correspond to larval forms. The prevalence, intensity and mean total abundance of infection were 100%, 61.9 and 61.9, respectively. The four most importance parasites were *Aponurus* sp., *Lernanthropus* sp., *C. exigua* and *Multitestis* sp2. During this research the infective stage of *Anisakis* sp, nematode of zoonotic significance was found in the Peruvian coast. The parasites *Sprostoniella* sp., *Parancylodiscoides* sp., *Aponurus* sp., *Multitestis* sp1. and *Multitestis* sp2. are considered new records for Peru and *P. panamensis* is a new host for all parasites, except for *C. exigua*.

O-102

HUNTING FOR THE ALTERNATE HOST OF *KUDOA INORNATA* (MYXOZOA) AND DOCUMENTING MARINE MYXOZOAN DIVERSITY IN SOUTH CAROLINA, USA

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Although *Kudoa* spp. are cosmopolitan myxozoan parasites of marine fishes, no *Kudoa* life cycle is known. We hypothesise that they have complex life histories, which involve annelid alternate hosts. *Kudoa inornata* infects spotted seatrout *Cynoscion nebulosus* on the south-eastern coast of the USA. To find its alternate host, we sampled annelids from 0-8m deep water in Charleston Harbor, South Carolina, USA. We individually examined 2553 polychaetes and 931 oligochaetes. We found a low diversity of oligochaetes (2 families, 5 species), but the prevalence of overt myxozoan infections was relatively high: 42/931 (4.5%). In contrast, polychaete diversity was very high (>20 families), but we found myxozoans only in 1 species of spionid at low infection prevalence: 6/592 (1%). These are the first myxozoan records from North American marine annelids, and only the fifth report of infection in a marine polychaete. We found 30 unique 18S rDNA sequences from 34 oligochaete myxozoans, which indicated high local myxozoan diversity. No sequences had similarities >98% with GenBank myxozoan records. The 6 polychaete myxozoan sequences were identical, and were most similar to basal Kudoids (i.e. not *Kudoa inornata*). Phylogenetic analysis showed a striking correlation between polychaetes as hosts of “marine” lineage myxozoans and oligochaetes with “freshwater” myxozoans. We have now developed a SYTO9 qPCR assay for detection of *K. inornata* in water, to better locate ‘hot spots’ in the harbor for future polychaete sampling. We are developing sampling and sequencing protocols to estimate the total myxozoan diversity from eDNA.

O-103

DO CLINID FISH FROM THE ROCK POOLS IN SOUTH AFRICA SHARE *CERATOMYXA* SPP.?

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Intertidal rock pools harbour large numbers of fishes and invertebrates, which are well adapted to life in harsh conditions with a strong fluctuation of water temperature and salinity. These rock pools with close contact of fish and invertebrates may represent ideal conditions for myxosporean life cycles. We focused on different host species - clinid fish (*Clinus acuminatus*, *Clinus brevicristatus*, *Clinus cottoides*, *Clinus superciliosus*, *Muraenoclinus dorsalis*) collected from the rock pools from different localities along the temperature gradient of the South African coast.

Species of the genus *Ceratomyxa* were found in host gall bladders. *Ceratomyxa*, the second most numerous myxosporean genus, has crescent shape of spores with two polar capsules. Its species are generally strongly host specific. Ceratomyxids are morphologically uniform and individual species commonly differ by host preference and sequences. The intraspecific variability in ceratomyxids based on their SSU rDNA is lower (up to 0.3%) than in the remaining myxozoan genera (1%).

We morphologically and molecularly characterise *Ceratomyxa* species from several clinid fish species and localities. Based on molecular data (rDNA), seven species were found in five fish hosts in single as well as in co-infections. Four of *Ceratomyxa* species were strictly host specific, on the contrary, one ceratomyxid was found in four fish hosts. Interestingly, *Clinus superciliosus* was infected by six *Ceratomyxa* species in three different localities. Phylogenetically, all ceratomyxids from clinid fish are closely related and sister to ceratomyxids from labrid fish.

Acknowledgement: The present study was funded by the Czech Science Foundation (P505/12/G112).

O-104

BIODIVERSITY OF MYXOZOA INFECTING GILTHEAD SEABREAM AND EUROPEAN SEABASS REARED IN A PORTUGUESE AQUACULTURE FACILITY

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The exponential growth of fisheries and aquaculture industries worldwide raises interest in the parasitic groups that may be detrimental for production, such as the case of the phylum Myxozoa Grassé, 1970. The gilthead seabream *Sparus aurata* Linnaeus, 1758, and the European seabass *Dicentrarchus labrax* Linnaeus, 1758, are fish species of high commercial value for the South European aquaculture industry. Twelve myxozoan species have been reported infecting these two fish species, some causing serious growth delays and increased morbidity and mortality. In Portugal, however, there's little information regarding myxozoan infections in aquaculture environments, with no species registered in gilthead seabream and European seabass. As such, during 2 years, our group conducted a parasitological survey for myxozoans in a Portuguese facility, having microscopically analyzed the organs and tissues of 260 specimens of gilthead seabream and 154 specimens of European seabass. The results revealed high biodiversity of myxozoans. In gilthead seabream, *Sphaerospora sparis* was recorded from the kidney, and were performed new records of *Zschokkella* and *Ceratomyxa* from the gallbladder and of *Ortholinea* from the urinary bladder. In European seabass, *Ceratomyxa labracis* and *C. diplodae* were recorded from the gallbladder, *Sphaerospora testicularis* from the testis and *S. dicentrarchi* from several organs. A new record of *Ortholinea* in the urinary bladder was also performed. Parasitic material was processed for light and electron microscopy, as well as for sequencing of the SSU rRNA gene. The combined use of microscopic and molecular methodologies allowed the apprehension of several aspects of these parasites morphology, phylogeny and evolution.

O-105

DIVERSE MYXOZOAN COMMUNITY IN A SMALL RIVER RUNNING AT THE TYPICAL SEMI-RURAL AREA IN JAPAN

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Myxozoans are receiving a lot of attention recently in Japan not only as the pathogens of fish but also as a cause of food poisoning. Because of the lack of effective drags and vaccines, control of the myxozoan infections in fish is basically relied on breaking the transmission cycles. However, the lifecycle has been elucidated for only about 40 species out of more than 2,000 described species. Therefore, comprehensive field surveys are needed for the better understanding of myxozoan lifecycles.

In this study, the community of myxosporeans and actinosporeans inhabiting a small river in front of Yamaguchi University, Japan, was investigated. We examined 195 oligochates obtained in the river (Kuden River, Yamaguchi City) from March 2013 to June 2014 for the investigation of actinosporeans. We also examined 59 fish containing 9 species in the same river from June 2012 to August 2014. Five actinosporean types belonging to the collective groups of triactinomyxon, raabeia and neoactinomyxum were found to be released from oligochaetes. On the other hand, 7 morphologically distinguishable myxosporeans belonging to the genus *Myxobolus* were identified from various organs of 6 fish species. BLAST search revealed one of the neoactinomyxum type actinosporean is the alternate stage of *Thelohanellus wangi* described in China. Phylogenetic analysis based on the sequences of 18S rDNA could not find any pairs of myxosporean and actinosporean stages in the collection site. This result indicates the unexpectedly diverse myxozoan community exists in a small river running at the typical semirural area in Japan.

O-106

EGG DISPERSION STRATEGIES IN APOROCOTYLID TREMATODES AND PATHOLOGICAL IMPLICATIONS

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The blood-flukes (Trematoda, Aporocotylidae) are parasites specific of fish. Adults live into blood vessels or heart where they release their eggs into the blood stream. Blood-fluke infections can be very pathogenic as eggs accumulate in gills until hatching, what provokes the rupture of the gill tissues, bleeding and, sometimes, asphyxia and death. Eggs have different shapes and sizes among species, what affects their options to reach to the gill, and their capability to penetrate and disperse along the capillaries or to remain attached in afferent arteries. In fact, final infection-site of the aporocotylid eggs vary between species; whereas relatively large eggs of *Aporocotyle simplex* get individually trapped in the lamellar arterioles of *Merluccius merlucciu*s, small eggs of *Cardicola* spp. in *Thunnus* spp. penetrate and spread into the lamellar capillaries. However, the developmental degree of released eggs must be considered as intrauterine eggs are generally crumple and smaller, getting their final shape and size out of the uterus. Therefore, the period that shells need to get rigid and enlarge temporarily allows penetrating in smaller vessels. Eggs of *Paradeontacylix balearicus* would be too large to advance through the lamellar capillaries of *Seriola dumerili*, however the adults in gills release soft, crumple and small eggs, which penetrate into capillaries. In contrast, the intrauterine eggs of *Skoulekia* spp. from sparid fish are already elongated and large, hampering their dispersion up to distal vessels. The degree of egg dispersion would be related with the extension of damages.

JPA benefits from a PhD fellowship, University of Valencia.

O-107

HOST LIFE TRAITS AND PARASITE LOAD IN FISH HYBRIDS

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Hybridization is a very common evolutionary process. Physiology and life traits have been changed in the hybrids when compared to parentals, consequently this may also affect the qualitative and quantitative composition of parasite communities in hosts. In addition, it is supposed that hybridization may interrupt the system of host-parasite gene co-adaptation, which may affect parasite load in hybrids. In this study, common bream (*Abramis brama*), roach (*Rutilus rutilus*) and their respective hybrids from Hamry reservoir (Czech Republic) were collected from 2011 to 2013 and investigated for the presence of metazoan parasites. Identification of the fish individuals was performed using morphological characters and molecular markers (partial cytochrome *b* gene and microsatellite loci). Fish condition, vigour and immunocompetence were measured using basic physiological indexes. The aim of this study was to compare fish life traits, selected physiological and immune parameters as well as parasite infection between each of two parental species and hybrids. In hybrids, the diversity of parasite communities was higher but parasite abundance was lower when compared to parental species. Specialist and generalist parasites from both parental species were present in parasite communities of hybrids. Regarding maternal origin of hybrids the predominance of individuals with common bream maternal origin was found. The presence of some parental species specific parasites in hybrids was determined by maternal origin of hybrids. The significant effects of the species and sex on fish condition were found on physiological conditions. The non-specific immunity measured by lysozyme activity and oxidative burst activity was affected only by season.

O-108

CYSTATIN: THE CYSTEINE PEPTIDASE INHIBITOR FROM *EUDIPLOZOON NIPPONICUM* (MONOGENEA)

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Eudiplozoon nipponicum (Monogenea, Diplozooidae) typically inhabiting the gills of carp (*Cyprinus carpio*) is widely distributed Eurasian blood-feeding ectoparasite.

The foregoing research of diplozoid Monogenea has been predominatly oriented towards understanding morphology and ecology of particular species. Nevertheless there is a lack of information regarding functional molecules produced by this taxonomic group.

Among important molecules produced by various parasitic species belong cystatins (inhibitors of cystein peptidases). Besides regulation of endogenous processes in parasite bodies they play a substantial role e.g. in manipulation of the host immune system and/or blood digestion.

The aim of our experimental work is to reveal the presence, structure and function of *E. nipponicum* cystatin DNA/protein molecules using bioinformatic, molecular and proteomic methods.

The transcriptomic data of *E. nipponicum* was screened for the presence of cystatin sequences. The partial nucleotide sequence was identified, amplified, completed using 3’and 5’ RACE PCR, cloned and sequenced. Gene coding 98 amino acid cystatin of *Eudiplozoon nipponicum* with predicted molecular weight 10,85 kDa and theoretical pI 6,27 was inserted into pET19b expression vector a cloned into *E. coli* competent cells (BL 21 strain). Expression was induced by IPTG at 37 °C. The production of recombinant cystatin by *E. coli* host cells was analysed on SDS-PAGE (Coomasie staining) and verified by mass spectrometry (Orbitrap). Recombinant protein was produced in the insoluble form - incorporated into inclusion bodies. The cystatin from *E. nipponicum* will be subsequently solubilized and further tested for its functional and structural properties.

Acknowledgements: Czech Science Foundation (P506/12/1258, GBP505/12/G112), grants of the Masaryk University (MUNI/A/1484/2014) and grants of the Charles University in Prague (UNCE 204017, PRVOUK P41, SVV260074/2014).

O-109

CHEMICAL VARIABILITY IN GILL MUCUS OF BUTTERFLYFISH FROM FRENCH POLYNESIA AND RELATIONSHIP TO THEIR MONOGENEAN LOADS

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Chaetodontidae is one of the most diverse family of coral reef fish and its ecology has been extensively studied. However, ecology of their gill monogeneans and parameters explaining parasite specificity between sympatric butterflyfish species is not well understood. Fish mucus is often described as the first line defense against external attacks. Several studies have reported molecules present in fish mucus with potential protective roles against parasites such as antimicrobial peptides or immune-related molecules, but until nowadays no study have focused on the bioactive specialized metabolites which might also play a defensive role against pathogens. We aimed to develop a non-targeted metabolomic approach to study the chemical fingerprint from gill mucus of several butterflish species in order to elucidate if there are chemical parameters controlling differences in monogenean loads between different species. Three sympatric butterflyfish species from French Polynesia have been chosen: 1) *Chaetodon ornatissimus* who always presents a high load of parasites, 2) *Chaetodon lunula* who presents a middle parasite load and 3) *Chaetodon lunulatus* who has never been found parasitized. Gill mucus was recollected and extracted through a biphasic extraction (DCM/ MeOH / H$_2$0) and then both extracts were analyzed by LC/MS (ESI +) to obtain gill mucus chemical fingerprints. Raw data were treated with XCMS software and multivariate statistical analyses were performed. An initial method validation was performed, confirming the suitability of the developped method to study intra-specific and inter-specific gill mucus chemical fingerprints. Preliminary results show differences in chemical composition between different Chaetodon species.

O-110

NITROGEN (^{15}N) AND CARBON (^{13}C) STABLE ISOTOPE SIGNATURES OF TROPHIC TRANSMITTED PARASITES: EXPERIMENTAL STUDY ON LIFE CYCLE OF *SCHISTOCEPHALUS SOLIDUS* FROM ITS STICKLEBACK HOST (*GASTEROSTEUS ACULEATUS*)

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Trophically transmitted parasites experience one or several trophic shifts during their life cycles. As their transmission occurs by predatory-prey relationships, it could be expected that parasites permanently adjust to the respective trophic level of their host during their life span. Insights into trophic relationship between organisms can be gained analyzing the signatures of stable isotopes of nitrogen (^{15}N) and carbon (^{13}C).

In order to study the trophic position of trophically transmitted parasites and their hosts, three-spined sticklebacks (*Gasterosteus aculeatus*) experimentally infected with the cestode *Schistocephalus solidus* were used as a model system. Tissues of first intermediate host (copepods), second intermediate host (stickleback) as well as the tapeworm's larval stages (procercoids and plerocercoids) were analyzed using elemental analyser (EA) coupled to isotope ratio mass spectrometer (IRMS). In order to evaluate the possible trophic shift during parasite development, plerocercoids from fish hosts collected at 30, 60, 90 and 120 dpi (days post infection) were taken into account. Additionally, the stable isotope signatures of non-infected (control) groups of first and second intermediate hosts were analysed, in order to evaluate the influence of the parasite on the composition of stable isotopes in host.

Results of the analyses confirm the assumption that trophically transmitted parasites adjust to the trophic level of their hosts, whereas no significant changes in the signatures of host liver and muscle tissues were observed.

O-111

STABLE ISOTOPE ANALYSES REVEAL VARIOUS TROPHIC LEVELS AMONG DIFFERENT GROUPS OF PARASITES

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It is assumed that parasitism evolved from predator-prey relationships and from a variety of probiotic relationships (e.g. commensalism) or from a combination of both. During their life history, parasites evolved various adaptation traits, which allow them to utilize different energy resources of their hosts. Stable isotope signatures of nitrogen (^{15}N) deliver insights into the trophic interactions within food webs. It was empirically shown that consumers are enriched in nitrogen on average by 3.2 ‰ with respect to their diet. Using stable isotopes signatures of nitrogen we could clearly distinguish parasitic from predator-prey and different commensalistic relationships and determine different degrees of adaptation to a parasitic life style among the most common taxa of parasites. Several parasitic taxa (e.g. cestodes, trematodes, acanthocephalans) as well as larval nematodes were usually depleted in ^{15}N. Parasitic crustaceans (copepods, isopods, cirripedia) and gastropods showed similar or only slightly lower isotope values, whereas parasitic insects, arachnids, fish as well as adult nematodes were usually enriched in ^{15}N (in average 4,6 ‰), which points on a completely different strategy/mechanism of nutrient assimilation. Accordingly, it appears that monophyletic taxa (taxa with only one parasitic ancestor) such as Cestoda, Trematoda and Acanthocephala, i.e. those groups which mainly take up their nutrients via their body surface evolved in a completely different way of nutrition than polyphyletic taxa (exhibiting more than one parasitic ancestor) such as parasitic Crustacea, Insecta and Arachnida. These differences are critically evaluated in terms of our common understanding of a parasitic mode of life.

O-112

FROM BENTHOS TO SEAGULLS: ON HOST SPECTRA AND TRANSMISSION STRATEGIES OF *CARDIOCEPHALOIDES LONGICOLLIS* (STRIGEIDAE, TREMATODA) IN THE MEDITERRANEAN

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Trophically-transmitted parasites are essential elements in most aquatic food webs, playing an important role in host community structure and even exerting strong effects on food web structure when a parasite species affects a high number of host species. However, information on the full spectrum of host species used by parasites is scarce and unavailable for most parasite species. The trematode *Cardiocephaloides longicollis* is distributed mainly in the Mediterranean; it has a complex three-host life cycle, including a snail, a fish and a definitive bird host. In the present study, we aimed to compile large-scale information on the stage-specific host spectra and transmission strategies that characterize the life cycle of this trophically-transmitted parasite and that allow it to be so successful. We sampled 3351 molluscs (24 species), 2108 fish (25 species) and 154 birds (17 species), and found 13 new host records at the first and second intermediate host level, with the latter including two new host families. Statistical analyses of the differences in infection levels among fish species from different habitats allowed us to determine the marine microhabitat targeted by infective cercariae. Furthermore, we determined the effect of fish size on the accumulation of metacercariae. The patterns uncovered in this study will be discussed in relation to factors likely to enhance transmission and parasite numbers in the food web. These include anthropogenic effects such as aquaculture installations as well as discarded bycatch from the extensive Mediterranean fisheries.

O-113

CURRENT STATUS OF KNOWLEDGE ON FISH DIGENEANS ASSOCIATED WITH FRESHWATER BIVALVE MOLLUSKS IN EUROPE

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Widespread fish digeneans of the families Bucephalidae, Allocreadiidae and Gorgoderidae, using freshwater bivalves of the families Unionidae, Sphaeriidae and Dreissenidae as primary intermediate hosts, were studied in Central and Northern Europe. New karyological and molecular data revealed the insufficient and/or mistaken knowledge on species variety and life cycles. The reports on *Bucephalus polymorphus* in unionids are misleading because of confusion with *Rhipidocotyle fennica*, which occurs not only in Finland but also in Central and Eastern Europe. The adults in fish are still confused with *R. campanula*. Data on cercariae are lacking for majority of allocreadiids. The ITS2 and 28S sequences of cercaria of type-species *Allocreadium isoporum*, described by Wiśniewski (1958), were identical to those of adult *Bunodera luciopercae*. Cercariae of *B. luciopercae* sensu Wiśniewski (1958) infesting *Pisidium amnicum* were shown to be larvae of newly described *B. acerinae*. Molecular analysis support the allocation of *Cercariaeum crassum* from *P. amnicum* to *Allocreadium*, but its adult remains unknown. Our data disproved life cycle of *Phyllodistomum folium* described by Sinitsin (1905). Cystocercous cercariae of *P. folium* were recorded in sphaeriids. Closely related cercaria of *P. umblae* was found in sphaeriids in Norway. Larval and adult of *P. elongatum* and *P. simile* showed no karyological and sequence differences from *P. folium*. The studies revealed the need for reconsidering some of the accepted but untested tenets regarding life-cycles and species variety of well-known fish digeneans.

This research was funded by a grant (No. MIP-52/2013) from the Research Council of Lithuania.

O-114

JELLYFISH AS INTERMEDIATE HOST OF DIGENEAN PARASITES OF FISH

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In the last 20 years jellyfish have been revalorized as important food items of marine fish. In this context, its importance as intermediate host of digeneans has increased. Since the 90s I have been examined jellyfish for parasites mainly from two Marine Ecoregions, Temperate Northern Atlantic and Temperate South America. During these years, more than 50,000 jellyfish were examined. Different metacercariae of Lepocreadiidae, Hemiuridae, Fellodistomidae and Baccigeridae were found in addition to larval nematodes, metacestods, trichodinid and crustaceans, and four digeneans life cycles were studied. Some changes in the relation digenean-jellyfish were observed probably associated with increases in the jellyfish blooms worldwide. At the moment were observed: 1-As the transmission of parasites often depends on the host densities, during blooms certain parasites such as amphipods, anemones and digeneans can spread more easily in the population of jellyfish and then the prevalence may increase. 2-The parasites of jellyfish can have deleterious effects on host populations. 3-There are still few studies that evaluate the effects of jellyfish parasites on host populations over time. 4-Prevalence values observed in the Gulf of Mexico and southwest Atlantic, contrast with the idea that the helminth infections is low in the marine zooplankton due to the dilute nature of the pelagic environment. 5- Carnivorous habits of many jellyfish can transfer parasites among them. 6- The presence of metacercariae encysted of some digeneans could indicate a change in the host-parasite relationship.

O-115

ASSOCIATION BETWEEN ABUNDANCE OF GREY SEALS (*HALICHOERUS GRYPUS*) AND INFECTION OF BALTIC COD WITH *CONTRACAECUM OSCULATUM* AND *PSEUDOTERRANOVA DECIPIENS* (ZOONOTIC NEMATODE LARVAE)

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The abundance of grey seals (*Halichoerus grypus)* in the Baltic Sea has increased significantly during recent years. The grey seal population has recovered from almost extinction before 1999 and has shown a rapid increase from the year 2000 to 2015. Now heavily colonized haul out sites are observed near the main spawning area east of the island of Bornholm (Bornholm basin, ICES SD 25) and the total Baltic grey seal population has been estimated to count approximately 40,000 individuals. During the same period the infection level of the local Baltic stock of the Atlantic cod (*Gadus morhua*) increased for third stage nematode larvae comprising *Contracaecum osculatum* and *Pseudoterranova decipiens*. We investigated (by using full digestion by HCl and pepsin) cod samples caught by local fishermen in SD 25 during 2013 and 2014 and found that the liver worm (*C. osculatum*) reached 100% prevalence and a mean intensity of 84.6 parasites per fish. Intensities up to 320 parasites per fish were recorded. *P. decipiens* occurred with 28.7 % prevalence and a mean intensity of 5.2 parasites per fish. Infection was size dependent which was reflected by larger fish (>50 cm) showing a prevalence of 55 % and up to 56 *P. decipiens* worms per fish. The impact of the accelerated infection levels on health of the individual cod, the cod stock size and consumer safety will be discussed.

O-116

PARASITES OF COD *GADUS MORHUA* IN THE BARENTS SEA

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The early results from the parasite component of a major new research project are presented. The project is TIBIA (Trophic Interactions in the Barents Sea – Steps towards an Integrated Ecosystem Assessment), the primary objective of which is to improve understanding of trophic interactions and food web structure in the Barents Sea. One of the main aims of the parasite component is to search for evidence that parasites might be affecting survival and/or reproduction of North-east Atlantic (NEA) cod. NEA cod feed in the Barents Sea and migrate in spring to the west coast of Norway to spawn, mainly around the Lofoten area. A feature of this cod population is the annual occurrence of a significant proportion of mature female fish that miss a year's spawning. These fish are known as "skippers". The practical parasite work started in January-February 2015 with a research vessel cruise in the Barents Sea, followed by another in March-April 2015 to the Lofoten area. The main purpose of these cruises was to identify parasites that may contribute to the "skipping" phenomenon. One hundred and ninety-two female cod were therefore examined for a number of parasites selected for their known or suspected pathogenicity. The selected parasites are *Anisakis simplex*, *Pseudoterranova bulbosa, Contracaecum osculatum* and *Hysterothylacium aduncum* (Nematoda), *Abothrium gadi* and *Pyramicocephalus phocarum* (Cestoda), *Echinorhynchus gadi* (Acanthocephala), *Lernaeocera branchialis* and *Cresseyus confusus* (Copepoda Parasitica) and *Myxidium* spp. (Myxozoa). The data collected on these initial cruises are presented and discussed.

O-117

IN SITU EVIDENCE OF *PONTOPOREIA FEMORATA* (KROYER 1842) ROLE IN INFECTION WITH ACANTHOCEPHALAN PARASITE *ECHINORHYNHUS GADI* IN COD *GADUS MORHUA* L. IN THE BALTIC SEA

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Cod *Gadus morhua* is one of the most important fish species in the Baltic Sea. Its diet preferences are changing during the life time and are dependent on the fish capacity to catch and eat the prey. Young cods mostly occur nearby the coast and feed on the invertebrates, especially crustaceans. Older and bigger cods prefer deeper parts of the Baltic Sea, where prey mostly on fish. The presence of acanthocephalan parasite *Echinorhynhus gadi* in digestive tract of cod is well documented. The general life cycle of that parasite has been also described. Despite that fact there is still little known about the particular species of crustacean representatives, that play the role of intermediate host in life cycle of *E. gadi* in the Baltic Sea. The main aim of the study was to examine *Pontoporeia femorata* (Kroyer 1842), obtained from the stomach of Baltic cod, for the presence of the acanthocephalan parasite *E. gadi*. In October 2013 and February 2014 the total number of 932 cods stomachs has been sampled and analyzed. The studies revealed the relation between the presence of amphipods and parasites *E. gadi* in cods' stomachs. The presence of both, amphipods and acanthocephalans were dependent on the length and the age of fish, that determine the availability of diet components for cods. It is the first evidence *in situ* that 80 individuals of *P. femorata* found in the stomach of Baltic sea cods has been infected with *E. gadi.*

O-118

INGENUITY IN *CHONOPELTIS AUSTRALIS* (CRUSTACEA) FEMALE

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Chonopeltis differs from other Branchiura in that they lack antennulae, their maxillulae are relatively larger, and their carapace shields are relatively short which affects their ability to swim. In order to study their biology, live adult specimens of *C. australis* Boxshall, 1976 collected in the Vaal River in South Africa were transported to the laboratory to start a breeding colony. The female's behaviour during sperm transfer and egg deposition were studied using observation and photography. The female reproductive system was studied using light microscopy, histology and scanning electron microscopy (SEM). Histological sections were reconstructed to describe the anatomy of the reproductive system. SEM specimens were either studied whole or after dissection. Despite differences in morphology, all branchiuran males transfer sperm via a spermatophore and the females store the sperm in spermathecae. The female reproductive system of *C. australis* is similar to that of other Branchiura, it consists of an ovary within a gonocoel where egg development occurs and a pair of oviducts, one of which functions at a time. The species' feeble swimming ability and thus inability to quickly re-attach to a host has changed their behaviour in egg deposition. While the host fish is resting, females deposit eggs on to the surrounding substrate by leaning off, attached only by their large maxillulae. In this way the eggs and thus the larvae are at the same level as the fish, enhancing infection opportunity.

Chonopeltis australis thus compensates for feeble swimming by a changed behaviour which supports a parasitic existence.

O-119

WHY DO *SPIRONUCLEUS* TROPHOZOITES SWARM, STICK, AND CLUSTER? LIFE CYCLES INSIGHTS FROM *IN VITRO* CULTURE

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The diseases caused by *Spironucleus* species, intestinal flagellates of salmonids, gadids and cichlids, and systemic invaders of salmonids, are well known, and ultrastructure of trophozoites and cysts is documented. However, little is known about trophozoite behavior: how do they react to their environment, and interact with each other; what roles do these behaviors play in the direct life cycle? We addressed these questions by observing *Spironucleus* trophozoites in culture. *Spironucleus vortens* (ATCC 50386, from Angelfish *Pterophyllum scalare*), were maintained in modified LYI medium, at 23 °C. Trophozoites were observed in a hemocytometer chamber and on a slide, and video recordings made. *Spironucleus salmonis* from rainbow trout, *Oncorhynchus mykiss*, were maintained in minimal essential medium supplemented with newborn calf serum, at 10 °C. Trophozoites were observed in flasks and on slides. In *S. vortens*: (i) trophozoite distribution eventually conformed to the shape of the liquid in the hemocytometer chamber, with a 1.5 – 2.0 mm boundary, (ii) spherical swarms of trophozoites, 200 mm in diameter, formed, persisted for several minutes, and then disassociated. In *S. salmonis*, trophozoites adhered to each other by their posterior flagella, forming clusters prior to encystment. We interpret, for *S. vortens:* (i) adjustment in distribution as response to chemoreception, probably of oxygen concentration, and (ii) swarming as intercellular communication leading to coordinated movement (possibly indicative of quorum sensing). For *S. salmonis*, multifunctional flagella initiate cell adhesion, leading to clusters of cysts, enhancing buoyancy and ingestion of numerous cysts. These qualities of *Spironucleus* spp. are rare in flagellates.

O-120

FISH IMMUNE RESPONSES AGAINST PARASITES ELUCIDATED BY INTEGRATED RESEARCH TOOLS

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Recent progress within fish immunological research allows a deeper insight into the antiparasitic responses raised by fish. Due to the wide diversity within the teleost group it may be worthwhile to focus on selected parasite host systems where access to reproducible experimental models and molecular tools secures a broad approach to both cellular and humoral responses of the host following infection with a specific parasite. The main aquacultured species including common carp, sea bass, sea bream, Atlantic salmon, rainbow trout, Atlantic cod and eels have already contributed considerably to our increased knowledge about antiparasitic immune responses towards various types of parasites. The present work provides an overview of the intricate network in rainbow trout with regard to immune cells, signal molecules and effector systems put in action upon infection with different types of parasites.

O-121

ULTRASTRUCTURAL AND TRANSCRIPTOMIC ASPECTS OF THE ATLANTIC BLUEFIN TUNA *THUNUS THYNNUS* (LINNAEUS, 1758) GILL EPITHELIUM INFECTED WITH *DIDYMOSULCUS KATSUWONICOLA* (POZDNYAKOV, 1990)

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Didymosulcus katsuwonocila (syn. *Didymocystis wedli*) is the most abundant didymozoid species infecting farmed Atlantic bluefin tuna (*Thunnus thynnus*, Scombridae). In most cases infections terminate without any gross pathology, characterised by a significant decrease of cysts per host after couple of months of tuna rearing, however under certain conditions inflammatory and necrotic changes in the gill tissue may be induced, making it an interesting case of host-parasite interaction. The main goal of our study was to analize ultrustructural changes of tuna gill tissue parasitised with *D. katsuwonicola* and infer functional interpretation at the transcriptomic level. Infected and uninfected gill samples were collected from *T. thynnus* at harvest after having been farmed for two years and prepared for transmission electron microscopy and transcriptomic profiling by DNA microarrays according to standardized protocols. Micrographs showed numerous transiting vesicles in didymozoid's tegument in direct contact with host's loose collagen connective tissue capsule comprising fibrocytes, fibroblasts, eosinophilic-granulated mast cells, eosinophils and plasma cells. The capsule encompassed a nerve composed of three neuronal axons, numerous anastomosing capillaries and was overlaid with multilayered squamous epithelium abundant with mucous goblet cells. The transcriptional profiling revealed moderate gene regulation in both directions with fold changes ranging from -10.38 to 4.56 (N=768 statistically selected features). Pathway analyses based on KEGG sets showed the pertubrations of components of innate immunity, complement and coagulation cascades, as well as endocrine, digestive and nervous functional pathways. The findings elucidate vivid cellular and molecular cross-talk between the host and the parasite.

O-122

ATLANTIC SALMON IMMUNE RESPONSE TO AMOEBIC GILL DISEASE AND INSIGHT INTO THE BIOLOGY OF THE AMOEBA

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Amoebic gill disease (AGD) is an emerging disease in North European Atlantic salmon (*Salmo salar*) aquaculture caused by the amoeba *Paramoeba perurans*. Non-optimal environmental conditions may affect AGD progression and, to understand the role of predisposing environmental conditions on the biology of the parasite, amoebae were exposed *in vitro* to different temperatures and salinities. Outputs from these experiments are 1) an improved understanding of the impact of temperature on amoeba growth rate during conditions that potentially influence disease development and 2) the monitoring of amoeba recovery after exposure at low salinities relevant to current freshwater bath treatments (e.g. 3 ppt).

The host immune response to AGD infection is still not well understood. Atlantic salmon smolts were exposed for 3 weeks to either 500 or 5000 cells l^{-1} of two cultures of *P. perurans*. Gene expression analysis of cytokines involved in the different T_H pathways was performed on the interbranchial lymphoid tissue of the 1st gill arch. T_H1, T_H17 and Treg pathways were found to be significantly down-regulated, mainly in samples from fish given the highest dose. In contrast, the T_H2 pathway was found to be significantly up-regulated by both cultures. Regression analysis of the gene expression data and the amoeba load, assessed by qPCR of the 18S rRNA, has been investigated. The present results seen during late stage AGD suggest that either an activation of humoral immunity or an immune evasion strategy is occurring, similar to the responses driven by helminthic parasites to avoid cell-mediated killing mechanisms.

O-123

CYTOKINE EXPRESSION WITHIN THE INTERBRANCHIAL LYMPHOID TISSUE OF ATLANTIC SALMON (*SALMO SALAR*) DURING AMOEBIC GILL DISEASE

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Amoebic gill disease (AGD) is caused by the marine protozoan *Neoparamoeba perurans*, an ectoparasite that affects the gills of farmed marine fish. This disease is considered a serious health and welfare challenge, mainly for marine farmed salmonids, and is emerging in geographical areas where it has not been previously reported. The role of the interbranchial lymphoid tissue (ILT), which has recently been described in the gills of salmonids, is not fully understood. Histological studies have shown ILT is composed mainly of T cells supported by a meshwork of epithelial cells, and it has been suggested that it could function as a secondary lymphoid organ. It is a unique tissue found in fish and does not resemble any lymphoid tissues previously described in mammals. This investigation aimed to characterise this tissue during AGD, by measuring immune gene transcription and histopathological changes within the ILT during AGD progression. Atlantic salmon were sampled prior to exposure and at 5 and 10 days post exposure to N. perurans trophozoites. Immune gene expression including selected cytokines relevant to innate and adaptive immune responses was determined using Realtime-PCR. The results from this project will be presented and will contribute towards our understanding of the role of the ILT in Atlantic salmon AGD.

O-124

FISH CELLS INVOLVED IN MUCOSAL IMMUNITY AGAINST ENTERIC HELMINTHS

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In recent decades, the mucosal immunology of higher vertebrates has been an intensively explored area of research and yet little is known regarding this in fish. The intestinal mucosa is continuously exposed to a broad spectrum of antigens derived from ingested food, water, commensal flora and pathogens. Recent comparative histological and ultrastructural studies on the uninfected intestines of chub *Squalius cephalus*, European eels *Anguilla anguilla*, and catfish *Silurus glanis* and in conspecifics naturally infected with acanthocephalans and platyhelminths, however, have begun to explore this. Endoparasitic helminths frequently cause intestinal inflammation inducing the recruitment of various immune cells to the site of infection. In each of the fish-helminth systems that were studied, a massive hyperplastic granulocyte response involving mast cells (MCs) and neutrophils in the epithelia, lamina propria and submucosa was seen in close proximity to the point of parasite attachment. An increase in the number of rodlet cells (RCs) in the intestinal epithelium of parasite-infected specimens was also seen. Hyperplasia and hypertrophy of mucous cells in infected intestines was evident. In transmission electron microscopy sections of all three infected fish hosts, MCs and mucous cells were frequently seen in contact with one another with evident degranulation of those MCs in close proximity to mucous cells. In fish the mechanism by which mucosal MCs might induce the secretion of excess mucus is unknown and is the main focus of this study. The role of MCs, neutrophils, RCs and mucous cells in the mucosal immune system of fish will be discussed.

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INTERACTION BETWEEN *TRICHODINA TRUTTAE* INFECTION AND MUCOUS CELLS IN THE SKIN EPIDERMIS OF CHUM SALMON ALEVIN AND FRY

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Trichodina truttae is a parasitic ciliate, causing mortalities of salmonid fishes reared at hatcheries in Japan. The abundance of *T. truttae* on the alevin and fry of chum salmon (*Oncorhynchus keta*) reared at the Nijibetsu Salmon Hatchery was bi-weekly monitored. The first parasite infection was observed on salmon alevin two weeks before leaving the gravel. The abundance increased from 3 to 42 parasites on chum salmon fry during 2 weeks after leaving gravel. The density of mucous cells in the skin epidermis drastically decreased during their transition from alevin to fry stage. It is known that mucus secretion helps to protect fish skin from parasite infections and physical injury. We have supposed the reduction of mucous cell density triggers *T. truttae* infection.

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LECTIN HISTOCHEMISTRY OF INTESTINAL MUCOUS AND MAST CELLS IN HELMINTH-INFECTED FISH

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Lectins, proteins that bind the specific carbohydrate structures, are ubiquitous and are found in animals, plants and microorganisms. Eight biotinylated lectins linking carbohydrates were used on the deparaffinised, hydrated uninfected and helminth-infected intestinal sections of chub *Squalius cephalus* and brown trout, *Salmo trutta.* The reaction of each lectin was determined through the use of avidin DH-biotinylated horseradish peroxidase and the DAB chromogen. A diverse array of lectin/carbohydrate responses were seen across the two fish species. DBA, PNA, UEA I and WGA were seen on trout mucous cells, while only DBA and PNA were seen on the mucous cells in chub. Parasite presence was seen to induce changes in the mucus carbohydrate composition of the fish's intestine. Numerous mucous cells reactive to DBA-PNA and UEA I subsequently appeared in parasitised chub and trout. The responsiveness of the mucous cells, however, is not dependent on the type of parasite infecting the host, as similar lectin patterns were seen in trout intestines infected with acanthocephalans and cestodes. Lectin histochemistry also highlights the responsiveness of mast cells (MCs), a type of granulocyte which are present in most tissues and are strategically positioned near blood vessels, in the connective tissue of chub (PNA-SNA) and trout (SNA). This comparative study between cestodes and acanthocephalans and their effects on the distribution of various lectins in the intestines of two fish represents a first record.

O-127

POTAMILUS ALATUS (MOLLUSCA: BIVALVIA: UNIONIDAE) GLOCHIDIA-INDUCED HISTOPATHOLOGY IN FRESHWATER DRUM, *APLODINOTUS GRUNNIENS* RAFINESQUE, FROM THE KANAWHA RIVER, WEST VIRGINIA, USA.

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The gills of 37 of 173 freshwater drum, *Aplodinotus grunniens* Rafinesque, caught by anglers from two locations on the Kanawha River, West Virginia, USA, were infected with glochidia of the freshwater mussel, *Potamilus alatus* Lea. All infections (37 infected hosts of 85 sampled) were observed between 25 May and 21 June, 1986. No glochidia were recovered from 88 hosts in monthly collections from 22 June through mid-October. Infections gave rise to granulomas that caused a distal “clubbing” of the parasitized gill filaments. The resulting lymphohistocytic response was comparable to a type IV hypersensitive reaction seen in human tissues. Increased vascularization within the granuloma was not observed. Glochidial development of the defintive and larval mantles, and the gut and digestive glands, corroborated findings of previous investigators. The transitory larval adductor, pedal retractors, heart, and gill buds were prominent as well, but the number of glochidia at various stages (ages?) of development was insufficient to completely describe the origin and subsequent development of these glochidial tissues.

O-128

POTENTIAL PATHOLOGY OF PARASITES FROM THE OROPHARYNGEAL CHAMBER IN OCEAN SUNFISH, *MOLA MOLA* (L.)

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The different potential pathological effects of three parasites from the oropharyngeal chamber in the ocean sunfish *Mola mola* (L.) is herein reported. Each species live in different microhabitats, with different attachment mechanisms and live strategies: *Gonapodasmius squamata* (Pozdnyakov, 1993) (Trematoda: Didymozoidae), an endoparasite encapsulated in gills; *Accacoelium contortum* (Rudolphi, 1819) Monticelli (Accacoeliidae Odhner, 1911) (Trematoda: Accacoeliidae), an ectoparasite living within pits of epithelial swallows; and *Cecrops latreillii* Leach, 1816 (Copepoda: Cecropidae), a mobile ectoparasite attached with hooked appendages to the gill surface. Fresh samples of parasitized gills were studied with standard histological methods and observed under light microscopy. Some sections were stained with Giemsa in order to detect the presence of secondary bacterial infections. The tissues close to the capsules of *G. squamata* were not observed to be related to a noticeable inflammatory response, but the tissues grew together with the parasite, showing neovascularization. The damage related to *C. latreilli* depended on the stage and sex of the copepod with more extensive erosion and inflammatory response in areas parasitized by adult females. The most pathogenic parasite appeared to be *A. contortum.* The induction of prominent alterations and intense proliferative and inflammatory response related to these parasites seem to be an adaptation to the external environment, an unusual location for trematodes with “endoparasitic morphology”.

Funded by AGL2010-20892, ISIC/2012/003 & PROMETEO 795/2015/018 projects.

O-129

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF HISTOPATHOLOGICAL IMPACTS OF THE MONOGENEAN *MACROGYRODACTYLUS CLARII* ON THE GILLS OF THE CATFISH *CLARIAS GARIEPINUS* AND POSSIBLE HOST RESPONSE

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Scanning (SEM) and transmission electron microscopy (TEM) were used for the first time to study the histopathological effects of the monogenean *Macrogyrodactylus clarii* on the gills of the catfish *Clarias gariepinus*. SEM revealed the footprint of the haptor to be elevated above the general gill surface and bordered by furrows and depressions produced by the anterior, posterior and lateral flaps indicating a suction force during attachment. No evidence was found of the insertion of the marginal hooklets into the gill tissue. TEM revealed blood capillaries and sinuses with compressed erythrocytes and some types of agranular and granular leucocytes. Erythrocyte contents were observed outside the cells , indicating haemorrage. Cells adjacent to the lateral flaps appeared to be compressed in some regions and widely spaced with large intercellular spaces in other regions. Desquamation of the surface epithelia adjacent to the lateral flaps was revealed. Some of the widely spaced epithelial cells have extensions (pseudopodia-like processes). Many vacuoles were found inside the cells of the upper surface epithelial layer. Necrotic tissue, damaged cells and cells with pyknotic nuclei were also detected. Probably accumulation of gland cells was detected in the surface epithelial layer of the gill tissue. The host response represented in the appearance of cells with fibrotic cytoplasm, presence of granular cells which may act as phagocytes and accumulation of probably mucous cells in the surface epithelial layer were discussed.

O-130

COMPETITION AMONG INVASIVE PARASITES: SWIM BLADDER NEMATODES AS AN EXAMPLE

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Anguillicola crassus is the most invasive species of its genus and a successful colonizer of different eel species worldwide. It is the only species of the genus whose life cycle has been studied completely under laboratory conditions so far. In order to analyze if life cycle characteristics may explain invasion success, we collected the congeneric species *Anguillicola novaezelandiae* from naturally infected Short-finned eels in New Zealand. Infrapopulations in eels of the North and the South Island were dominated by larval parasites. This unique composition of infrapopulations has never been described before for any *Anguillicola* species and suggests a seasonal occurrence as a possible reason. Apart from studying *A. novaezelandiae* individuals under natural conditions, we investigated their development in experimentally infected European eels. Again, *A. novaezelandiae* showed a more synchronized development than *A. crassus*. No density-dependent effect in parasite development could be found for *A. novaezelandiae*. Finally, stress markers such as serum cortisol and heat shock proteins were analyzed in eels experimentally infected with *A. crassus* and *A. novaezelandiae*, respectively. Taken together, our results show that the life cycle of *A. crassus* differs on final host level compared to *A. novaezelandiae*, which may result in a more successful invasion of new host species. However, if both *Anguillicola* species are experimentally introduced into a single eel, they can mature together in the same swim bladder. Furthermore, hybridization of *A. crassus* males with *A. novaezelandiae* females occurs as shown by microsatellite analysis, which might also contribute to the invasion success of *A. crassus.*

POMPHORHYNCHUS LAEVIS AS AN INVASIVE ACANTHOCEPHALAN IN THE RIVER RHINE?

O-131

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During the past 20 years, many studies have described the acanthocephalan *Pomphorhynchus tereticollis* as a common fish parasite in European water bodies including the river Rhine. However, recent research based on molecular analysis of Acanthocephala sampled in the Rhine River shows that *P. tereticollis* was most likely displaced by the invasive acanthocephalan *Pomphorhynchus laevis*. In order to investigate this hypothesis, we analyzed ITS genes of *Pomphorhynchus* parasites that infected eels (*Anguilla anguilla*), sampled from different sites in the River Rhine and from some of its tributaries in 2003, 2004, 2005 and 2014. Molecular analyses revealed that *P. tereticollis* and *P. laevis* coexisted during the years 2003 and 2004 at some of the most southern sampling sites. In the same period, an invasion of several gammarid species from the Ponto-Caspian region into the river Rhine has been described in various studies. Spreading of these gammarids within the Rhine River began at the confluence of the rivers Main and Rhine following the inauguration of the Main-Danube canal. After successful invasion of these gammarids only *P. laevis* was detected in the sampled area. As it is well known that gammarids like *Dikerogammarus villosus* are infected by Acanthocephala, this gammarid invasion could possibly be linked to the occurrence of *P. laevis*, which is naturally found in the Ponto-Caspian region. Upstream stretches of rivers in the Rhine catchment which are still not invaded by the Ponto-Caspian gammarids are still inhabited by *P. tereticollis*.

ASIAN FISH TAPEWORM, *KHAWIA JAPONENSIS* (YAMAGUTI, 1934), HAS EXPANDED ITS EUROPEAN INVASIVE RANGE

O-132

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The invasive fish tapeworm, *Khawia japonensis* (Yamaguti, 1934) originally described in Japan, is reported for the first time in Slovakia. The tapeworm was found in farmed common carp (*Cyprinus carpio* L.) from East-Slovakian breeding fish pond. This finding is registered four years after the first announcement of this parasite in Europe (Po River Basin in Italy) in 2010 and increases its distribution area within this continent. Morphological characterization of *K. japonensis* supplemented with DNA sequences of *cox*1 and ribosomal *lsr*DNA genes is provided. Specimens from Slovakia phenotypically corresponded with those from feral and farmed carps from China, Vietnam and Italy. Moreover, 100% and 98.7% identity of partial ribosomal *lsr*DNA gene and mitochondrial *cox*1 genes, respectively, were detected with *K. japonensis* from Japan. In addition, other two tapeworms of the Asian origin, a congeneric *Khawia sinensis* Hsü, 1935 and *Atractolytocestus huronensis* Anthony, 1958 occurred in mixed infections with *K. japonensis* in the inspected fish samples. The invasive and pathogenic potential of *K. japonensis* in commercial breeding fisheries and its possible further spread in natural habitats is difficult to estimate for now. As yet, *K. japonensis* appear to be without a major impact on commercial breeding fisheries, but call for more attention to the problem of biological invasions.

This study was supported by the Slovak Research and Development Agency (projects nos. APVV-0653-11 and LPP 0171-09), Grant Agency VEGA (No. 2/0129/12).

ANGUILLICOLA CRASSUS IN THE EUROPEAN EEL: 15 YEARS MONITORING IN LAKE MÜGGELSEE (BERLIN, GERMANY)

O-133

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The infection of European eels (*Anguillicola crassus*) with the neozoan swim bladder nematode *Anguillicola crassus* was monitored in Lake Müggelsee (Berlin, Germany) from 2000 to present. The extensive dataset was used to test hypothesis on the temperature-induced seasonality and density-dependent regulation of the infrapopulation of the parasite as well as the correlation between infection intensity and host size, and the occasionally assumed development of increased resistance in the host.

Although suggested by previous experimental studies on the effect of the water temperature on the development and survival of *A. crassus*, the preset field data do not indicate a seasonality of the infection parameters. Despite of slight annual fluctuations, prevalence, intensity and abundance were largely constant all over the period of surveillance. At a mean prevalence of 88 %, the mean intensity fluctuated around a value of 7.6 (L3, L4 and adults) and an average of 5.0 adult worms per host. However, the present data do not support the hypothesis of a regulation of the infrapopulation size by gravid females, but the intensity is slightly correlated with the mass of the host. The frequency distribution of *A. crassus* does also not support the hypothesis that the host develops increased resistance. Thus, in conclusion, the present long term monitoring indicates that the new host-parasite system has stabilized at a high prevalence and moderate intensity values.

METAZOAN PARASITES OF THE BARBUS FISHES FROM A HYBRID ZONE

O-134

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Recent introduction of widely distributed *Barbus barbus* from the Durance River into the habitats of the native and endangered *Barbus meridionalis* of the Argens River resulted into the hybridization between these two species. Generally accepted scenarios (i.e. lower parasite prevalence and species richness in newly established populations compared to the source populations or higher parasite abundance in the native hosts than the newly arrivals) can be modified due to the hybridization since hybrids represent the “bridges” for the parasite infection. In 2011-2014, *Barbus* individuals sampled from the Durance and Argens Rivers were examined for the presence of metazoan parasites. Overall, significantly lower prevalence and species richness of metazoan parasites were revealed in *B. barbus* on the Argens River compared to the Durance River. Moreover, metazoan parasite abundance found in local *B. meridionalis* was significantly lower in contrast to the introduced *B. barbus* individuals within the Argens River. In hybrids, intermediate values of abundance and trend towards lower species richness of metazoan parasites compared to the both parental species was revealed. However, different scenarios of hybrid susceptibility to metazoan parasites in contrast to the parental species on different localities were detected which might result from the different host-parasite interactions. Therefore, diversity of MHC II*B* genes as a result of co-evolutionary processes between hosts and their parasite has been analysed in *B. meridionalis* and *B. barbus* hybrid zones.

Acknowledgement: This study was founded by the GACR project No P505/12/0375.

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THE EVOLUTION OF DIPLOID-POLYPLOID COMPLEX OF GIBEL CARP (*CARASSIUS GIBELIO*): THE ROLE OF PARASITES AND DIFFERENT INVESTMENT IN PSYSIOLOGICAL TRAITS

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Carassius gibelio is a unique fish species in which two kinds of reproduction are known i.e. gynogenesis and gonochoristic reproduction. The former populations of this invasive species entered in the rivers of Czech Republic about 1975 and were composed of triploid females with gynogenetic reproduction. Recently, the coexistence of gynogenetic form (mainly triploid females, but occasionally also triploid males and tetraploid specimens have been identified) and gonochoristic diploid form have been reported. In this study the potential effect of parasitism on the evolution of diploid-polyploid complex of *C. gibelio* was analyzed. In addition, the selected measures of immunity and physiology were analyzed. We hypothesized that some traits in gynogens are weakened in order to compensate the two fold cost of reproduction in sexual form.

All specimens were determined using morphological traits and mtDNA (D-loop). The presence and intensity of infection for metazoan parasite species was compared between two different reproductive forms. The parameters of non-adaptive (i.e. respiratory burst, lysozym activity and complement activity) and adaptive immunity (IgM antibodies) were compared between two forms in three consecutive years.

A weak difference in parasite load was found between two reproductive forms. This study showed that the basic physiological parameters were primarely affected by sex and were not affected by reproductive form. Blood innate immunity of gibel carp was not affected by reproductive form. However, trend of higher IgM in gynogenetic females when compared to sexual diploids was found.

This study will be funded by the Czech Science Foundation, Project No. P505/12/0375.

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PARASITES OF THE LESSEPSIAN SPRINTER *FISTULARIA COMMERSONII* (OSTEICHTHYES: FISTULARIIDAE): AN UPDATE AFTER 15 YEARS SINCE ITS ARRIVAL IN THE MEDITERRANEAN SEA

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Fistularia commersonii is a Lessepsian sprinter recorded for the first time in the eastern Mediterranean Sea (Israel) in 2000. Within a few years, this species reached the central Mediterranean (2004, Sicily) and, subsequently, the western Basin (2007, Iberian Peninsula).

Merella et al. (2007) and Pais et al. (2007) described its parasites in the Mediterranean Sea from one fish caught off the Sardinian coast and six from North Africa, finding native generalist species, but also two Indo-Pacific digeneans: *Allolepidapedon fistulariae* and *Neoallepidapedon hawaiiense*.

From 2007 to 2014, the capture of 24 specimens of *F. commersonii* in Sardinian waters allowed to add information about its parasites in the Mediterranean, and to evaluate their dynamics throughout the host migration path.

Twenty parasite species/taxa were found, adding 13 taxa to the former Mediterranean list. The results confirmed earlier data: *A. fistulariae*, *Nybelinia africana* and Phyllobothriidae (Cestoda), *Hysterothylacium* spp. (Nematoda) and Gnathiidae (Crustacea) were among the most frequent parasites (P>15%); besides, two other taxa showed prevalence higher than 15%: *Pseudogrillotia* sp. (Cestoda) and *Breizacanthus* cf. *ligur* (Acanthocephala). *Neoallepidapedon hawaiiense*, previously recorded in North Africa, was not found in the present specimens.

The results suggest that a non-indigenous species is not always subjected to the release from its natural enemies. Actually, the parasite fauna of *F. commersonii* increased throughout the host migration path, acquiring new generalist species, but also conserving the natural parasite *A. fistulariae*, that seemingly has been able to close its life cycle in the Mediterranean Sea.

Thanks to R.Bray, J.Hernández and H.Palm.

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HISTORY OF DISCOVERY OF FISH PARASITIC CRUSTACEA WITH A FOCUS ON THE CONTRIBUTIONS BY PAST AND PRESENT LEADING PARASITOLOGISTS IN THIS FIELD

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Parasitic Crustacea comprise a large and diverse group of parasites on aquatic host species. The copepods are undoubtedly the most dominant in the group and frequently studied. Isopods which infect fish and other crustaceans as well as branchiurans infecting mostly freshwater fish, are some of the other well-known parasitic Crustacea. Some of the lesser known groups like the minute tantulocarids; barnacles (including Thoracica and Rhizocephala); amphipods which infect numerous hosts (including medusae, siphonophores, ctenophores, and thaliaceans); and the Ascothoracida which infect various echinoderms and cnidarians, are also important parasitic groups. This presentation revises the history of discovery in each of these different groups with the focus on those parasitising fishes and highlights some of major historical influences as well the knowledge development which influenced the group up to the present. The significant contributors to each parasitic crustacean group are introduced in a “Who’s Who” review depicting leading parasitologists in this field. This basic review of these crustacean parasites will provide a substantial background to these abundant organisms and will contribute to a better understanding of their unique niche in the aquatic environment.
This review of the history of discovery of parasitic Crustacea will be part of the book Crustacean Parasites by Nico Smit and Niel Bruce.

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PATTERNS OF HOST SPECIFICITY IN PARASITIC COPEPODS

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Host specificity, the extent to which a parasite species can exploit different host species, is a fundamental property of parasites and is a key measure of the ability of a parasite to colonise novel hosts. Host specificity data have, correctly, been treated with great caution since sampling bias is known to be a significant problem. Copepod parasites of marine invertebrates, for example, utilise fewer hosts than parasites of vertebrates, but baseline parasitological surveys of potential invertebrate hosts are few and far between. Data on fish parasites are more extensive, however, and a comparative approach reveals differences in host specificity between certain families: sea lice (Caligidae), for example, utilise a significantly wider range of hosts than gill parasites such as the Lernanthropidae and Hatschekiidae. Comparisons between all families of parasitic copepods utilising on fish hosts are presented and analysed with respect to host taxon, microhabitat and degree of association with the host. The lack of robust phylogenies at the appropriate levels for parasites and for hosts is identified as an obstacle hindering analysis of the main drivers.

ADAPTATIONS AND EVOLUTION TOWARDS A PARASITIC WAY OF LIFE

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This paper is a summary of a chapter in a book on Parasitic Crustacea.

Crustacean symbiotic associations range from facultative to very specialised parasitism where the parasite undergoes total morphological adaptation, becoming completely reliant on the host for its survival. Many crustaceans are temporary parasites, only associated with hosts for short period when feeding, such as in the branchiuran genus *Argulus*. In some obligate parasites the adults are parasites and larvae free-living, in others the larval stages are parasitic and adults not. Obligate permanent parasites include ecto- and meso-parasites, some copepods penetrating deep into vital organs of their fish hosts. Some amphipods colonising sponges display eusocial behaviour where a single queen reproduce with casts of sexless workers and soldiers collectively rearing larvae.

Parasitism can and probably did evolve at any point between these extremes and the progressive complexity of the type of parasitism does not mean that temporary parasites will eventually evolve to become permanent parasites or mutualists. Complex adaptation is not necessarily a more successful strategy. Any kind of association between crustaceans and their very large spectrum of aquatic hosts existing today is successful and could have come about in many different ways. The prerequisite for an association to be initiated is that the potential parasite and potential host must be found in the same habitat and there must be some pre-adaptive morphology that could facilitate the initiation and eventual consummation of the symbiotic association.

FISH PARASITIC CRUSTACEANS AS DEFINITIVE, INTERMEDIATE AND PARATENIC HOSTS, AND AS VECTORS

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Parasitic crustaceans are found among the Class Maxillopoda, Subclass Copepoda, and its constituent families; they are also found among the subclasses Branchiura, Tantulocarida and Thecostraca of this same class (Class Maxillopoda). Further parasitic examples occur among crustaceans in the Class Ostracoda and particularly in the large Class Malacostraca, the latter containing, for example, the orders Amphipoda, Isopoda and Decapoda. In assessing the role of parasitic crustaceans themselves as definitive, intermediate and paratenic hosts, and as vectors, it is interesting to note that the relevant literature appears limited, but focussed on the following: Copepods of the genus *Caligus* and *Lepeophtheirus* (Copepoda, Caligidae) may be mechanical vectors or alternative hosts of a number of viral diseases between fishes and *Lepeophtheirus* may also transfer some bacteria between fishes. Ergasilids (Copepoda, Ergasilidae) parasitic on the gill filaments of fishes can support the replication of shrimp viruses, and likely act as viral vectors; they may also transmit lymphocystis virus of fishes. Branchiurans of the genus *Argulus* are thought to serve as mechanical vectors of several viruses to fishes, especially carp, as well as acting as intermediate hosts for dracunculoid nematodes of fishes. Isopods of the genus *Gnathia* (Family Gnathiidae) likely act as definitive hosts and vectors of fish blood parasites of the genus *Haemogregarina*; they may also transmit some fish viruses, and may be paratenic or intermediate hosts for nematode larvae. Cymothoids (Isopoda, Family Cymothoidae) may transmit the lymphocystis virus to fishes.

This review will detail the examples cited above and identify areas of future research and will form part of the book Crustacean Parasites by Nico Smit and Niel Bruce.

OVERVIEW OF HYPERSYMBIONTS/PARASITES FOUND ASSOCIATED WITH PARASITIC CRUSTACEANS

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Hypersymbionts have been found on parasitic crustaceans (Branchiura and Copepoda) from different freshwater fish hosts. *Doropygus* (Notodelphyidae), found in the branchial chamber of ascidians also harbour hypersymbionts. Hyperparasitic isopods (*Cabirops* sp.) are found on Bopyroidea, occurring on penaeid shrimps. *Udonella* spp. (Monogenea) are recorded from the carapace of caligids found on marine fishes. In free-living plankton water currents formed by the crustaceans carry food to the epibiont, thus the question that arises is what will the situation be for hypersymbionts found attached to parasitic crustaceans? Hypersymbionts may compete with the basibiont (parasitic crustacean) for food. It will not be a problem for the branchiurans that feed on fish blood as the attached peritrichs are filter feeders. Udonellids feed directly of the fish host using the caligids as a vehicle and feeding platform. With free-living crustaceans the epibiont may leave the basibiont during moulting. Crustaceans discard epibionts attached to the carapace through ecdysis. Epibionts adapt their lifecycle that telotrochs (of peritrichs) form when the crustacean moults, rapidly re-infecting a basibiont. Amongst parasitic crustaceans the situation is different, in most cases it is the adult stages that are parasitic; thus moulting has no effect on infestation.
This review will be part of the book "Crustacean Parasites" by Nico Smit and Niel Bruce.

THE LIFE CYCLE OF SEA LICE (*CALIGUS MINIMUS*) ON SEABASS (*LATES CALCARIFER*) FROM FLOATING CAGE CULTURE

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Caligus minimus, known as a sea louse, is a very important pathogen of sea floating cage culture. This paper describes the life cycle of *Caligus minimus* isolated from seabass culture in cages in Penang, Malaysia. The observations were made at 30°C, with ambient photoperiod under laboratory conditions. Females of *Caligus minimus* with matured eggs were collected and incubated in a beaker with slow aeration. The life cycle consists of nine stages: 2 nauplii, 1 copepodid, 4 chalimi, pre-adult and adult. The copepodid is infective and all subsequent stages were attached on fish and do not require an intermediate host for its development. The eggs hatched out within 13.83±3.03 hours and two nauplii stages were recorded after 1.88±0.74 hours. Copepodid stage was observed after 24.91.83±2.24 hours. A single experiment conducted by exposing an uninfected sea bass fingerling to copepods showed the copepodid immediately attached to the body, fins and gills of the fish. The chalimus stage was observed after 20.95±3.70 hours followed by pre-adult stage after 45.88±5.95 hours. Within 44.5±1.08 hours, the adult was observed and showed the female became mature and laid the eggs after 43.25±3.11 hours. Conclusively, 5.5-6.9 days were needed for *Caligus minimus* to complete its life cycle.

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A HITCHHIKER *ERGASILUS* FROM SOUTH AFRICA

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Ergasilus Nordmann, 1832 is the largest genus of the family Ergasilidae and comprises more than 180 species. Larvae and males are free swimming and it is only after copulation that inseminated females attach to the gills of their hosts and then produce eggs. The males remain free swimming. Following attachment, females undergo metamorphosis and the antennae are transformed to hooks used for attachment to the gills by embracing the filament, causing hyperplasia and feeding of the gill epithelium. Two previous records reported *Ergasilus* form the skin of hosts, in both instances the parasites was not identified to species level and the occurrences were suspected to be incidental. Recently large numbers of *Ergasilus* was found attached to the fins of a large variety of fish species from the Vaal and Olifants rivers in South Africa. Both these rivers have been studied extensively in the preceding 15 years. The parasites and host tissue were collected and fixed in acetoformaldehyde alcohol and stored in 70% ethanol. This copepod is not host specific and has been recorded from at least seven different host species from diverse localities. Prevalence ranges from 21 – 100% with a mean intensity of 1.0 to 66.1, with the highest mean intensity recorded for *Hypophthalmichthys molitrix* (Valenciennes, 1844) at Flag Boshielo Dam. Females attach with their antennae through insertion of the antennal tip into the epidermis of the hosts. This attachment has a very limited pathological effect and no evidence of inflammation occurs.

O-144

FIRST RECORD OF *PARABRACHIELLA INSIDIOSA* AND *PARABRACHIELLA MERLUCCII* FROM EUROPEAN HAKE, *MERLUCCIUS MERLUCCIUS* IN TURKEY

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In this study, *Parabrachiella insidiosa* (Heller, 1865) and *P. merluccii* (Bassett-Smith, 1896) (Copepoda; Lernaeopodidae) were reported for the first time on European hake in Turkey (*Merluccius merluccius*). Morphological characters of these parasitic copepods are given in photographs and drawings. Eight species of the family Lernaeopodidae are reported from marine habitats, *Clavellotis fallax*, *Clavellisa scombri*, *Clavellotis strumosa*, *Lernaeopoda galei*, *Parabrachiella impudica*, *Parabrachiella bispinosa*, *Parabrachiella exigua*, *Clavellotis briani* in Turkey. One hundred and fifty European hakes, *Merluccius merluccius* (Linnaeus, 1758) (Pisces; Merluccidae) were collected by local gears from Aegean Sea of Turkey in 2014. The parasites were fixed in 70% ethanol. Some of specimens were later cleared in lactic acid before dissection of the appendages of copepods. The drawings of appendages were carried with the aid of camera lucida. Also, the photos were taken with the aid of camera attached to a microscope. The shape of the trunk, longer and width and morphologic features of all dissected parasites as structure, size of exopod and endopod of maxillule, dental formula of mandible, denticulation and location of seta on maxilliped, size of exopod and endopod of antenna; number of segment and seta on antennule, second maxillae with lobate processes permitted identification of these copepods as *Parabrachiella insidiosa* and *P. merluccii* (Copepoda; Lernaeopodidae). The prevalence values of *Parabrachiella insidiosa* and *Parabrachiella merluccii* are found as 9.33% and 6.6% respectively. *P. insidiosa* was found on gill filaments of host, while *P. merluccii* on gill rakers of host.

O-145

BAIKAL COTTOID FISH ACANTHOCEPHALANS: DYNAMICS OF INFECTION AND POPULATION STRUCTURE IN THE CHANGING CONDITIONS OF ENVIRONMENT

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Endemic ichthyofauna of Lake Baikal is represented by cottoid fish, 29 species of them inhabit the lake from littoral zone to maximal depth. Acanthocephalans are one of the dominant groups of parasites of cottoid fish. The aim of the research was to study the dynamics of changes of infection of cottoid fish by Acanthocephala.

We studied 512 specimens of 18 species of cottoid fish. The species composition of Acanthocephala of cottoids include *Echynorhynchus salmonis*, *E. borealis* and *E. truttae*. A total of 834 acanthocephalans were found. The highest level of infection was observed in benthic littoral fish species: *Leocottus kesslerii* (prevalence 63.4%, abundance - 8.1 sp.) and *Paracottus knerii* (62.1 - 4.2). Pelagic fish (*Cottocomephorus grewingkii* (8 - 0.2) and *C. inermis* (8 - 0.2) is weakly infected by Acanthocephala, pelagic fish (*Comephorus baicalensis and C. dybovski*) are infected by Acanthocephala only accidental.

Infection of *L. kesslerii* by Acanthocephala in the spring is significantly lower than in the autumn. Interannual fluctuations in the relative abundance of parasites in *L. kesslerii* are small. Infection of *L. kesslerii* in shallow-water bay (Chivyrkui Bay) in comparison to the coastal part of the open lake was significantly higher.

Sexual structure of parasite population varies significantly, but in general, females are more numerous. The main part of acanthocephalan population is localized in the second half of the digestive tract, but acanthocephalans inhabit the pyloric caeca also.

O-146

BIOLOGY AND VARIABILITY OF ACANTHOCEPHALANS DESCRIBED BY OMAR AMIN

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Unique and unusual features in the many species of acanthocephalans described and/or studied by Amin from fish, amphibians, reptiles, birds, and mammals, in various parts of the world including South America, Vietnam, Japan, the United States, the Middle East, and North and East Africa, are described. The presentation is in five parts. (1) An introductory section dealing with the classification, general morphology, ecology, and life cycles of the Acanthocephala. (2) Unusual anatomical features of taxonomic or of questionable taxonomic importance addressing variations in the proboscis, proboscis hooks, male and female reproductive organs, and lemnisci. Newly described structures including (a) Para-receptacle structure (PRS) and hoods in certain species as well as a new order of Acanthocephala from Vietnamese birds, are also featured. (3) Structural and functional relationships explaining the relationship between the metamorphosis of the giant nuclei in Eoacanthocephala and worm reproductive cycle. (4) Host-parasite relationships elucidating the relationships between worm anatomy and biology during worm growth. (5) Curiosities in reviews and revisions highlighting taxonomically based zoo-geographical patterns and trends in the genera *Neoechinorhynchus*, *Polymorphus*, and *Pallisentis*. A comprehensive treatment of the acanthocephalans of South America and those marine forms off the Eastern United States is also included here. A look at the September, 2013 classification scheme of the Acanthocephala is included covering 4 classes, 26 families, 157 genera, and 1298 species are included.

O-147

STRUCTURAL-FUNCTIONAL RELATIONSHIPS AND CURIOSITIES IN THE ACANTHOCEPHALA

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This treatment of variability in the Acanthocephala is in 3 parts (1) Structural and functional relationships explaining the relationship between the metamorphosis of the giant nuclei in Eoacanthocephala and worm reproductive cycle. (2) Host-parasite relationships elucidating the relationships between worm anatomy and biology during worm growth. (3) Curiosities in reviews and revisions highlighting taxonomically based zoo-geographical patterns and trends in the genera ***Neoechinorhynchus***, ***Polymorphus***, and ***Pallisentis***. A comprehensive treatment of the acanthocephalans of South America and those marine forms off the Eastern United States is also included here. A look at the September, 2013 classification scheme of the Acanthocephala is included covering 4 classes, 26 families, 157 genera, and 1298 species.

O-148

ANATOMICAL VARIABILITY IN THE ACANTHOCEPHALA

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Unique and unusual features in the many species of acanthocephalans described and/or studied by Amin from fish, amphibians, reptiles, birds, and mammals, in various parts of the world including South America, Vietnam, Japan, the United States, the Middle East, and North and East Africa, are described. The presentation is in 2 parts. (1) An introductory section dealing with the classification, general morphology, ecology, and life cycles of the Acanthocephala. (2) Unusual anatomical features of taxonomic or of questionable taxonomic importance addressing variations in the proboscis, proboscis hooks, male and female reproductive organs, and lemnisci. Newly described structures including (a) Para-receptacle structure (PRS) and hoods in certain species as well as a new order of Acanthocephala from Vietnamese birds, are also featured.

O-149

COMPARATIVE FUNCTIONAL ULTRASTRUCTURE OF MATURE VITELLOCYTES OF FOUR SPECIES OF TRYPANORHYNCH CESTODE PARASITES OF ELASMOBRANCHS

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Cestode vitellocytes function in formation of protective coverings and nutrients for embryos and larvae. These functions vary among different taxa depending on the type of embryonic development, degree of ovoviviparity and life cycles. This study provides comparative analysis of data on the ultrastructure and cytochemistry of mature vitellocytes of *Parachristianella trygonis* and *Dolfusiella spinulifera,* (Eutetrarhynchidae), *Progrillotia pastinacae* (Progrillotiidae) and *Grillotia erinaceus* (Lacistorhynchidae), to assess if these features have phylogenetic variability or represent adaptations to different hosts and/or life cycles. The general pattern of vitellogenesis in *P. trygonis* is similar to that in other lower cestode taxa, showing no trace glycan vesicles. The most important differences in the mature vitellocyte of *P. trygonis* and those *D. spinulifera* and *P. pastinacae* relate to glycogenesis. Only *P. trygonis* has large amounts of glycogen. In *D. spinulifera*, cytochemical staining indicated a strongly positive reaction for membrane-bound glycoproteins in all membranous structures. Similar staining revealed β -glycogen scattered in the cytoplasm of maturing vitellocytes. Typical cytoplasmic β-glycogen particles appeared seldom during early vitellocyte maturation and were seldom visible in mature vitellocytes of *D. spinulifera* and *P. pastinacae*. Different species also varied in content and chemical nature of lipid droplets. In *P. trygonis*, massive concentrations of saturated lipids were like those of *G. erinaceus* and *P. pastinacae*. Lack of data on the life cycles and development of Trypanorhyncha makes speculation difficult regarding functional ultrastructure of vitellogenesis and vitellocytes. Differing concentrations of glycogen and lipids may support adaptations that enhance transmission, but further research is needed.

O-150

CRYOTECHNIQUES REVEAL NOVEL ULTRASTRUCTURAL FEATURES OF EMBRYONATED EGGS OF THE MONOZOIC BASAL CESTODE *GYROCOTYLE URNA* (CESTODARIA)

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The cestodarian, *Gyrocotyle urna*, is a primitive monozoic cestode that live in the spiral valve of holocephalan fishes. Unlike the well-studied polyzoic eucestodes that have diverse hexacanth larvae, cestodarians produce unique decacanth larvae within their thick-shelled eggs. Ultrastructure of eggs of this basal taxon with fully formed shells and early embryonic stages is described for the first time. Multiple techniques used included cryomethods of high-pressure freezing with freeze substitution (HPF/FS) combined with field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). After 20 days of *in vitro* maturation, eggs are composed, from the exterior to the interior, of a thick egg shell of vitelline origin, an early embryonic envelope, a ring of numerous large vitellocytes, and a group of differentiating blastomeres situated in the centre, forming the early presumptive larva. The thick resistant egg shell is similar to that reported from other polylecithal neodermatan flatworms. The embryonic envelope is poorly formed at this stage, and not yet divided into inner and outer envelopes as occurs in all neodermatans described to date. Vitellocytes are separate, not forming a syncytium, and are filled with diverse vitelline materials as well as lipid droplets and associated endomembrane components having ultrastructure consistent with macroautophagy systems seen among many animals in apoptotic or autophagic cells. These results largely corroborate the basic observations and interpretations of previous TEM studies on other neodermatans, while showing additional details and providing insight into powerful cryotechniques that can enhance our work with these challenging subjects.

COMPARATIVE ULTRASTRUCTURE OF EGGS AND HEXACANTHS OF *BOTHRIOCEPHALUS CLAVIBOTHRIUM* AND *EUBOTHRIUM SALVELINI* (CESTODA: BOTHRIOCEPHALIDEA)

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Data on cestode ontogeny represent useful criteria for studying phylogeny. Oviparous *Bothriocephalus clavibothrium* releases eggs at early cleavage into seawater. The embryonated egg consists of the eggshell, outer envelope, and coracidium, each comprising a hexacanth larva within a ciliated envelope. Hexacanths include subtegumental, nerve, germinative, penetration gland and flame cells, somatic and hook myocytons, and small embryonic cells. Conversely, the entire embryonic and larval development of *Eubothrium salvelini* occurs in the uterus; eggs comprise three non-ciliated envelopes surrounding the infective hexacanth. The nonoperculated eggshell is thin, with thickness of the capsule the same as in proteocephalideans, perhaps because embryonic development entirely in the uterus reduces the need for embryo protection, whereas oviparous *B. clavibothrium* require robust eggshells for embryo protection in the open marine environment. Infective *E. salvelini* hexacanths contain all the other cell types as *B. clavibothrium*, plus a unique interstitial cell, but lack flame cells. These results and literature on oncospheres of other bothriocephalideans and diphyllobothriideans, suggest phylogenetic criteria for these taxa. By definition (Conn and Świderski 2008) a coracidium is a hexacanth enclosed by a ciliated inner envelope. Coracidia generally have a protonephridial system with flame cells. Besides lacking these features, the ultrastructure of *E. salvelini* oncospheres is more similar to cyclophyllideans than bothriocephalideans and diphyllobothriideans. Thus the term coracidia is inappropriate for *E. salvelini*. Currently, *E. salvelini* and *B. clavibothrium* are regarded as confamilial. Differences in ultrastructure of the oncospheres presented here, along with some molecular data, indicate need for phylogenetic revision of *Eubothrium*.

METAL SEQUESTRATION AND REACTIVE OXYGEN INTERMEDIATES IN *PARADIPLOZOON ICHTHYOXANTHON* AVENANT-OLDEWAGE, 2013 FROM THE VAAL DAM, SOUTH AFRICA

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Accumulated metals can be removed and detoxified through binding to specific proteins and structures within the bodies of exposed organisms. Parasites accumulate trace elements and heavy metals at levels which are orders of magnitude higher than their hosts. This study aims to determine where in the body of *Paradiplozoon ichthyoxanthon* metals are sequestered and to further determine if accumulation of metals by these monogeneans is associated with the production of reactive oxygen intermediates. *Labeobarbus aeneus* were collected from the Vaal Dam, South Africa with the use of gill nets. Gills were dissected out, assessed and *P. ichthyoxanthon* were removed, placed into Eppendorf tubes and flash frozen. Cryosections (5 - 10 µm) of parasites were made and stained with Phen-Green^{TM} FL Cell permant diacetate, CellROX® Deep Red and NucBlue® fluorochromes. Results of the present study indicate positive reactions for metals throughout the tissues of *P. ichthyoxanthon*, with the vitellaria and clamp sclerites being principle sequestration sites for metals, while egg shells indicated negative results for metals. Positive reactions for reactive oxygen intermediates were noted for the tegument epithelium while no reactions were found for the visceral tissues. This study indicates that diplozoid parasites possibly accumulate metals and certain tissues could function as sequestration sites in a manner so as to regulate metal concentrations within their bodies. The tegumental epithelium may be the route for metal accumulation, resulting in high levels of oxidative stress while due to the sequestration mechanisms present the production of oxidative by-products in the viscera is reduced.

O-153

MONOGENEAN PARASITE *MAZOCRAES ALOSAE* FROM ITS HOST THE PONTIC SHAD (*ALOSA IMMACULATA*) AS BIOINDICATOR FOR METAL POLLUTION

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Many studies demonstrated that various fish heminths are applicable as sensitive accumulation indicators. Due to their remarkable accumulation properties, metal concentrations in parasites can exceed many times those in host tissues, water and sediments as well as in different free living sentinels. Until now most of the investigations focused on acanthocephalans, cestodes and nematodes, whereas information concerning the metal accumulation capacity of fish monogeneans is completely missing. Therefore, the aim of our study was to evaluate the indication potential of the fish monogenean (*Mazocreas alosae*) recovered from its Pontic shad host (*Alosa immaculata*).

Fish were caught in 2012 and 2013 between May and June in the Danube River in Bulgaria during their spawning migration. They were infected with *M. alosae* and the nematode *Hysterothylatium aduncum*. Both parasites as well as fish tissues (gills, muscle, liver, intestine) were collected for metal analyses. Samples were digested and subsequently the nine elements (As, Cd, Co, Cu, Fe, Mn, Pb, V, Zn) were analyzed using inductively coupled plasma mass spectrometry (ICP-MS).

Six elements (Cd, Cu, Fe, Mn, Pb, Zn) were found in higher concentrations in monogeneans in comparison to host muscle, with the elements Pb and Zn being significantly higher accumulated in the parasite compared with the other host tissues and nematodes. The results demonstrated that the monogeneans exhibit excellent metal accumulation capacity and could therefore be used in future metal monitoring studies.

O-154

USE OF *SERRASENTIS SAGITTIFER* (ACANTHOCEPHALA) INHABITING THE BENTHIC FISH *NEMIPTERUS PERONII* (NOTCHEDFIN THREADFIN BREAM) AS INDICATOR OF METAL BIOACCUMULATION IN THE EASTERN COASTAL WATER OF SOUTH CHINA

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The eastern states of peninsular Malaysia receive intensive effluents of industries along the coastline. Sewage effluents, tourism and oil pollution are major anthropogenic stressors that contribute to environmental degradation. The purpose of the present research is to determine the effectiveness of *Serrasentis sagittifer,* inhabiting the benthic fish species *Nemipterus peronii*, as indicator to provide synoptic information of marine ecosystem health; discriminating between polluted and non-polluted sites located at the east coast of South China sea, possibly helping to monitor trace element contamination in a tropical ecosystem. During northeast monsoon period up to 40 bream were collected each month from four selected sites located at the east coast of the South China Sea. All selected sites were quite distant from each other and impacted by different types of anthropogenic activities. The concentrations of ten metals were determined in different tissues of hosts and in their parasites using inductively coupled plasma mass spectrometry (ICP-MS).The present findings showed that all the metals were found in higher concentrations in the acanthoceplans than in tissues of the host. As, Al, Cr and Sr were found in higher concentrations in all samples collected from Besut, samples of P.kambing showed highest concentration of Pb. On the other hand, Zn and Ni were found in maximum concentration in samples collected from Kuantan. *S. sagittifer* is extremely sensitive in indicating environmental differences in metal uptake. It can be used as a promising biomonitor of metal contamination in tropical ecosystems as it shows a consistent bioaccumulation behaviour and high tolerance to contamination, wide abundance, is easy collected and identified.

O-155

ENVIRONMENTAL AND ANTHROPOGENIC DRIVERS AFFECTING THE GEOGRAPHICAL DISTRIBUTION OF *ONCOMEGAS WAGENERI* (CESTODA:TRYPANORHYNCHA) IN THE SOUTHERN GULF OF MEXICO

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The aim of this study was to determine whether physicochemical variables, polyaromatic hydrocarbons or sewage discharge affect the spatial distribution and individual numbers of the larval cestode *Oncomegas wageneri*, infecting the shoal flounder *Syacium gunteri*, in the southern Gulf of Mexico. We used boosted generalised additive models (boosted GAM) and MaxEnt to examine potential statistical relationships between the environmental variables and the probability of occurrence of this parasite. The models were calibrated using all of the sampling sites with (29/162) and without parasite occurrences. The boosted GAM accurately predicted the probability of occurrence of *O. wageneri* in the study area. By contrast, poor probabilities of occurrence were obtained with the MaxEnt models for the same areas. The variables with the highest frequencies of appearance in the models (proxies for the explained variability) were the polyaromatic hydrocarbons of high molecular weight (PAHH, 95%), followed by a combination of nutrients, spatial variables and polyaromatic hydrocarbons of low molecular weight (5%). The PAHH contribution to variability was explained by the fact that these compounds together with N and P are carried by rivers discharging into the ocean enhancing growth of hydrocarbonoclastic bacteria, productivity and the number of intermediate hosts. The poor performance of the MaxEnt models was not improved by changing the size of the background area for *O. wageneri*. Overall, the results indicated that PAHH apparently affect the geographical distribution and number of individuals of this helminth parasite.

O-156

RELATIONSHIPS BETWEEN WATER QUALITY AND THE INFESTATION OF *CONTRACAECUM* (NEMATODA: ANISAKIDAE) LARVAE IN *OREOCHROMIS* SP. FROM THE NORTH EASTERN REGION OF SOUTH AFRICA

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The culture of *Oreochromis* spp. constitutes about 80% of the global tilapia production. Species from this genus have been investigated to establish their suitability for intensive culture in the northern South Africa. However, increased infestation of farmed fish by *Contracaecum* spp can impact meat quality and productivity when the fish's immune system is compromised due to poor water quality. In this study we investigated the relationship between infestation of *Contracaecum* larvae and water quality in a species representative of the genus *Oreochromis*, *O. mossambicus* from ten localities in northern South Africa.

Fish were caught using gill nets, sacrificed and examined for parasites. An environmental database was compiled and regression analyses performed to determine relationships between water variables and the level of infestation by *Contracaecum* larvae. At all localities *Contracaecum* spp. were present in fishes sampled. At one locality no *O. mossambicus* were found to be infected with *Contracaecum* spp. Initial findings indicate that concentrations of calcium and copper display a positive correlation (R = 0.8) with regard to parasite burden. Similarly, conductivity and chlorine concentrations were positively correlated (R = 0.7). Conversely, dissolved oxygen (R = - 0.8), pH (R = - 0.7) and phosphorus (R = - 0.7) were negatively correlated in relation to parasite burden. Results, therefore, indicate these variables to be important in determining *Contracaecum* larvae infection levels in *O. mossambicus.*

POSTERS

P-001

EFFECT OF DIETARY SUPPLEMENTATION WITH PROPOLIS AND *ALOE BARBADENSIS* EXTRACTS ON PARASITISM IN NILE TILAPIA

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This study evaluated the influence of diet supplementation with propolis and *Aloe barbadensis* on parasitism in tilapia. One hundred and eighty fish were distributed among 12 water tanks forming four treatments: fish supplemented with a 1:1 mixture of 0%, 0.5%, 1% and 2% propolis and aloe extracts. After the fish had been fed on the experimental diets for 15 and 21 days, parasites were collected. The monogeneans *Cichlidogyrus sclerosus*, *C. halli*, *C. thurstonae* and *Scutogyrus longicornis* were identified in the gills. Supplementation with the mixture of propolis and aloe for 15 days showed the highest efficacy against the parasites. This was possibly due to the association between the two compounds, that provided in the diet for 21 days, negatively influenced the results from this study, possibly due to stress containment and/or accumulation of the products. The results demonstrated that supplementation with mixtures of extracts favored a significant reduction in the number of gill parasites. The best results were achieved after 15 days of feeding with a diet with 0.5% and 1% supplementation with the extract mixture, wich increased efficiency by 83 and 85% respectively. However, it is known that one of the main factors that influence fish health is dietary balance, such that a combination of compounds is needed in order to meet the appropriate nutritional requirements. Dietary supplements can also stimulate the action of nonspecific defense mechanisms and immune responses in fish.

P-002

A SURVEY OF ECTOPARASITES IN FARMED NILE TILAPIA (*OREOCHROMIS NILOTICUS* L.) IN BRAZIL, WITH DESCRIPTION OF PARASITE INTENSITY SCORE

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Parasitic diseases are responsible for important economic losses in tilapia culture, favoring infections by opportunistic bacteria. We performed a parasitological study in 28 tilapia farms to investigate the cause of mortality in different culture cycle stages and we also describe the use of parasite intensity score. Parasitological analyses were performed in fish obtained from hatchery and grow out farms in different regions of Brazil. We diagnosed 20 parasite taxa in Nile tilapia cultured in ponds and net cages. Ciliate protozoa and Monogenea were the most prevalent groups of parasite in all Nile tilapia culture cycle stages, affecting all studies fish farms. On the other hand, parasites like *Myxobolus* sp., *Lamproglena monodi*, Lasidium larvae and Nematoda larvae had diagnosis restricted to some culture cycle stages and and were detected in just a few farms. We verified that 84.2% of the diversity of parasite taxa diagnosed in the larvae, masculinization, juvenile and grow out stages were also present in the broodstock of hatchery farms. Therefore, we suggest that the main source for dissemination of the most prevalent parasites in tilapia culture are hatchery farms that provide parasitized juvenile to the further stages of the culture cycle.

P-003

ESSENTIAL OILS OF *LIPPIA SIDOIDES* AND *MENTHA PIPERITA* AGAINST THE MONOGENEAN PARASITES AND THEIR INFLUENCE ON THE HEMATOLOGY OF NILE TILAPIA, *OREOCHROMIS NILOTICUS*

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Therapeutic bathes with essential oils of *Lippia sidoides* (pepper rosemary) and *Mentha piperita* (peppermint) against the monogeneans *Cichlidogyrus tilapiae*, *C. thurstonae*, *C. halli* and *Scutogyrus longicornis* and hematological parameters of tilapia were studied. 320 juvenile fish were distributed in 16 tanks, 20 fish per tank, divided in 4 treatments in quadruplicates: fish exposed to 20 $mg.L^{-1}$*L. sidoides* bath; 40 $mg.L^{-1}$*M. piperita* bath; fish exposed only to water bath and to water + DMSO (dimethyl sulfoxide) bath. Three bathes for 10 min were realized with intervals of 24 h. After the third bath the parasitological and hematological analysis were performed and calculated the efficacy of the oils. Parasite prevalence showed a reduction of 70% in essential oils treated fish. The efficacy obtained in *L. sidoides* treated fish compared to water and water + DMSO was 1.96% and 14.16%, respectively, and in *M. piperita* treated fish was 33.33% and 41.63%, respectively. Total numbers of erythrocytes and thrombocytes were lower in *L. sidoides* treated fish. Glucose concentration and the number of neutrophils were significantly higher in *L. sidoides* treated fish. Due to efficacy and positive hematological results, the authors suggest the use of 40 $mg.L^{-1}$*M. piperita* bathes as anthelmintic.

P-004

THE POSSIBILITY OF TREATMENT AND PREVENTION OF PARASITE DISEASES IN AQUACULTURE IN CZECH REPUBLIC

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Prosperity of aquaculture in Czech Republic directly depends on health condition of fish. According to the Act on veterinary care (No 166/1999 Col., as amended) the breeder must monitor state of health of animals and give skilled veterinary aid. Prevention and treatment are part of technological proceedings in all types of aquacultured species. In practice, the health state examination is more frequently required in fish, which is followed by the recommendation of treatment. Parasitic infections are frequently diagnosed. However, the recommended treatment for these parasitic diseases can be supported only by “off label” procedures. There are no authorized antiparasitic products for their commercial use in fish culture in Czech Republic.

The authors show some real cases of treatment and prevention in Czech aquaculture: Ichthyophthiriosis (*Ichthyophthirius multifiliis*) in catfish fingerlings, monogenean infection (*Dactylogyrus* sp., *Gyrodactylus* sp.) and in carp fingerling and eye fluke (metacercariae of *Diplostomum* sp.) in grass carp fingerlings.

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects „CENAKVA“(No. CZ.1.05/2.1.00/01.0024) and “CENAKVA II“ (No. LO1205 under the NPU I program).

P-005

SEX-BIASED GENE EXPRESSION REVEALS MECHANISMS RESPONSIBLE FOR SEXUAL DIMORPHISM IN THE SALMON LOUSE (*LEPEOPHTHEIRUS SALMONIS*)

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Sexual dimorphism is ubiquitous across the animal kingdom providing a comparative platform to study physiology, ontogeny, behaviour, and evolution. Males and females of the same species are nearly identical genetically, with the exception of sex chromosomes, and therefore sexual dimorphism is largely controlled by differences in gene expression (sex-biased gene expression). The salmon louse (*Lepeophtheirus salmonis*) has become an important ectoparasitic model due to it's detrimental impacts on the salmonid aquaculture industry. Salmon lice display many sexually dimorphic traits including differences in size, development rate, feeding patterns, drug tolerance, and behaviour, to name a few. Here, we apply a 38K oligonucleotide microarray to analyze sex-biased gene expression in *L. salmonis* from Atlantic and Pacific Canada. Principal component analysis indicated basal gene expression differences were greatest between gender, regardless of population or life stage. A total of 771 probes showed female-biased expression while 629 probes showed male-biased expression across all populations. Despite less genes being overexpressed in males compared to females, more orphan transcripts (non-annotated probes) showed differential expression in males suggesting increased rates of gene-specific functional divergence. Males also showed overexpression of several peptidase inhibitors containing kunitz domains, which function in sperm protection and/or possibly in inhibiting host blood coagulation. Female-biased genes were enriched for *in utero* embryonic development (8 genes) providing insight on egg production and mating. This study presents gene expression profiles of *L. salmonis* responsible for sexual dimorphism, offering a scaffold for future studies examining sex-determination, drug response, and lice behaviour.

P-006

DEVELOPING AN *IN VITRO* CULTURE SYSTEM FOR THE SALMON LOUSE, *LEPEOPHTHEIRUS SALMONIS*

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The current experimental method to test treatment efficacy on the *Lepeophtheirus salmonis* chalimus stages is to infect Atlantic salmon, *Salmo salar*, with infectious copepodites allow them to develop to the chalimus stage, treat the fish and then sacrifice the fish to remove the chalimus. This results in the use of high numbers of experimental fish, in addition to unpredictability of experimental conditions and difficulty to determine the effect that treatment may have on individual parasites. The aim of this study, which is ongoing, is to reduce the use of experimental fish by only requiring reservoir hosts to allow for production of egg strings and to develop a system allowing for the development of chalimus stages in vitro. We will discuss the process of developing a closed seawater system allowing for the tracking of individual parasites; the identification of a suitable substrate that encourages the copepodites to attach and extrude their frontal filament; and finally the possibility of studying the effect of treatments on the survival and moulting of individual parasites.

P-007

EFFECT OF HOST NUTRITION ON HOST-PARASITE INTERACTION OF FISH

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Diseases caused by parasites, bacteria and viruses represent a major threat to fish, both in natural and managed (aquaculture) populations, and the importance of diseases is likely to be exacerbated in the face of rapid global environmental change. Changes in the type and availability of food can arise as a consequence of changing husbandry practices or because of changes in prey availability, and understanding how this impact the biology of host-parasite interactions represents an important challenge. In the current study, we are examining the effects of host nutrition on interactions between three-spined sticklebacks and the parasite *Schistocephalus solidus*. Here we present the results of a diet manipulation study in which we fed infected and non-infected sticklebacks on a diet of either *Artemia* spp., *Daphnia* or bloodworms (*Chironomus* larvae) at a rate 10% fish body mass per day for 8 weeks under controlled lab conditions. We report the consequences of diet for the growth rate, energetic condition (body condition, hepatosomatic index) and health status (splenosomatic index, haematocrit) and sexual development (ornamentation, kidney somatic index, gonadosomatic index). Our results indicate that diet type had a significant effect on the performance of both infected and non-infected fish in the study, and suggest that host diet plays an important role in determining the emergent phenotypes of infected fish.

P-008

THE COMPLEXITY OF STUDYING MESOZOOPLANKTON PARASITES AND SOME GUIDELINES UNDER CLIMATE CHANGE SCENARIO

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Climate change is addressed as a cause of potential effects on parasitism in marine ecosystems. From an ecological perspective, habitat loss or fragmentation, overfishing, pollution, invasive alien species, hypoxia, acidification, altered hydrology, etc. are some examples of these other stressors that play an additive role in the climate change effect. These stressors interact at different levels with both the mesozooplankton community and their parasites, eventually interacting with human ecosystems. Mesozooplankton has been considered a good indicator of climate change in the marine environment as well as their parasites, which in fact represents an extended phenotype of their hosts. Marine parasites, as component of the marine ecosystems, are important, but very often neglected at the mesozooplankton level in the pelagic realm. This is mainly due to the difficulty of finding them in or on the little components of the mesozooplankton in a three-dimensional and very diluted realm. Their study implies lot of material and personal resources as well as the development of an interdisciplinary work in which taxonomists, genetics, statisticians and oceanographers work together. The aim in this work was (i) to provide some important impacts under the climate change scenario that affect both mesozooplankton community and human activities, (ii) to provide the guidelines to address the study of parasitism at the mesozooplankton level, (iii) to give some solutions with could help in this huge task.

P-009

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF INFECTIVE STAGES OF *SCHISTOCEPHALUS SOLIDUS* IN THE FIRST INTERMEDIATE HOST

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Changes to thermal regimes resulting from global environmental changes have the potential to alter the growth and development of the life cycle stages of parasites, with consequences for their infectivity to fish hosts. Such changes have the potential to increase, decrease, or alter the level of infection faced by fish, or alter the timing of infections. Here, we describe the results of experimental studies examining how water temperature affects the survival, growth and development of life stages of the diphyllobothriidean cestode *Schistocephalus solidus*, the plerocercoids of which are ecologically important parasites of stickleback fish. Following the *in vitro* culture of adult worms, individual copepods (*Cyclops strenuus abyssorum*) were exposed to a controlled dose of newly hatched *S. solidus* coracidia and held under controlled temperatures of 15C, 19C or 23C. Infectivity of the parasite under the different temperatures was quantified by screening copepods after 7d, and the subsequent growth rates of procercoids was tracked over 6 weeks post-infection, by microscopic examination and image analysis at 7d intervals. Our results provide detailed information on the role of temperature dependent development of key developmental life stages of this parasite life cycle, and give insight into how life cycle completion rates might be altered under environmental warming.

P-010

DEVELOPING *LIGULA INTESTINALIS* AS AN EXPERIMENTAL MODEL FOR EXAMINING THE IMPACTS OF ENVIRONMENTAL CHANGE ON HOST-PARASITE INTERACTIONS

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A wide range of both temperate and tropical cyprinid fish is the suitable 2nd intermediate hosts for the cestode parasite *Ligula intestinalis*. Infections have economic as well as ecological importance, because the parasite castrates host fish and hence reduces productivity in commercial or subsistence fisheries. Yet, despite being a well-studied ecological system, most studies are undertaken on naturally infected fish hosts, and the experimental model is not well developed, precluding experimental studies that could otherwise shed light on important questions such as how altering climates affect the relative performance of hosts and parasites. In this presentation we outline the work we are doing to develop experimental infection systems to utilise the model cyprinds *Pimephales promelas* – which is a natural host of *Ligula* in North America - and *Danio rerio* as experimental hosts fish.

P-011

FISH SAMPLING METHODS MAY AFFECT OBSERVED PARASITE COMMUNITY

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Parasite communities observed in host fishes may be biased by the fish sampling method used. First, habitat-specific sampling methods may miss target species (or sex, age, size-class) if the parasite(s) affect host habitat utilisation (use of shelter, bottom or upper water layer, etc.). Second, a fish species' reaction to particular sampling gear (e.g. escape response) may be affected where the parasite(s) affect host behaviour. Third, certain sampling methods may affect the parasite community (especially ectoparasites) due to physical contact (manipulation) with the fish host (e.g. gill nets). Here, we compare the parasite community of a freshwater fish species sampled from the same site using three common lentic-water sampling methods: electrofishing, beach seine and gill-nets.

Our results indicate that level of physical manipulation appears to be associated with an absence of some ectoparasite species, while host fish 'personality', e.g. inquisitiveness, may not only expose individuals to higher numbers of endoparasites but also increase their likelihood of capture. In order to obtain a representative assessment of a fish species' parasite community, therefore, two important factors need to be taken into account during sampling, i.e. level of physical manipulation and 'personality' of the host fish.

This study was supported by a grant of the Czech Science Foundation (project P505/12/G112).

P-012

COMPARATIVE PHYLOGEOGRAPHY BETWEEN A MARINE FISH WITH LOW VAGILITY AND ITS DIGENEAN PARASITE

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Phylogeography or the study of genetic lineages and their association with the space has advanced the understanding of biogeographic patterns and their relationship with the history of the species and the historical and contemporary events that influence. Incorporating phylogeographic information of a complex association such as a parasite and its host, can help us to improve the understanding of processes driving the evolution of both members of the association, as well for predicting the potential dispersion of a parasite over a broad spatial range. We expect that the pattern of genetic diversity for both the host and parasite can be because the different host's populations are interconnected. We test the congruence between population genetic structure of a host species with little displacement ability (*Sicyases sanguineus*) and its digenean parasite (*P. cf. lintoni*) over a wide geographic range. Using the COI gene, we assess whether the population genetic structure of this parasite is determined by the vagility of its host or alternatively respond to macro-scale processes that have established the biogeographic characteristics of the area. Our results shows that host's species do not show population genetic structure along their geographic range, unlike what is observed in the pattern of genetic diversity of *P.* cf. *lintoni*.

Acknowledgements: Postdoctoral FONDECYT Project 3130659 to IMV and Regular FONDECYT Project 1140173.

P-013

MICROHABITAT DISTRIBUTION OF A DIPLOZOID PARASITE (PLATYHELMINTHES: MONOGENOIDEA) ON GILLS OF *BARILIUS BENDELISIS* (TELEOSTEI) FROM INDIA

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Microhabitat distribution (among and within the gill arches) of a parasitic monogenoid, tentatively identified as *Paradiplozoon* sp., was studied on gills of *Barilius bendelisis* from the River Dikrong (state of Arunachal Pradesh, India) between January 2013 and December 2014. Of the 340 fish examined, 154 were infected by 190 individuals of *Paradiplozoon* sp. (prevalence 45.29%; mean intensity 1.31). Results were analysed by nonparametric statistical tests: Kruskal-Wallis ANOVA test and Mann-Whitney U test in relation to the general occurrence of the parasite in all examined fish. *Paradiplozoon* sp. appeared to have a clear statistical preference for the posterior face of proximal-ventral segment of the fourth gill arches. *Barilius bendelisis* is a new host record for diplozoid parasites.

P-014

DOES HOST MOBILITY PREDICT PARASITE DISTRIBUTION PATTERNS?

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Parasite distribution patterns in lotic environments may be influenced by the combined influences of unidirectional water flow and the mobility of the most agile host. However, the relative importance of these processes to parasite distribution patterns in limnic environments is unclear. We investigate these processes as part of a larger study assessing the re-establishment of fish parasites in a Norwegian catchment where all fish (and many intermediate hosts) were completely eradicated by the use of the indiscriminate poison rotenone. We assessed the pre-rotenone distribution patterns of parasites of two salmonids with differing dispersal tendencies; non-anadromous/resident Arctic charr *Salvelinus alpinus* (genetically distinct populations per lake) and migratory brown trout *Salmo trutta* (mixed populations between lakes). We hypothesised that prior to rotenone application, parasite abundance and prevalence would increase in a downstream direction for species using fish as definitive hosts, whereas parasite distributions would be mixed for species using birds as definitive hosts. Here, we present preliminary findings with particular focus on the distribution patterns of *Diplostomum* sp. and *Phyllodistomum umblae*, which utilise bird and fish as definitive hosts, respectively.

P-015

PATTERNS OF THE DYNAMICS OF PARASITE COMMUNITY SPECIES RICHNESS IN AGE SERIES OF FOUR SPECIES OF FISH IN THE LAKE BAIKAL BASIN

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Four species of fish from reservoirs of the Lake Baikal basin divided into 2 clusters depending on the dynamics of parasite community species richness in the age groups of host age series. Species richness of parasites in the age groups of fishes from the first cluster increases with host age. The one of parasites in the age groups of fishes from the second cluster has a non-linear relationship with host age. The first cluster includes the Baikal whitefish and roach and the second cluster includes the Baikal omul and European perch. Species richness of parasite communities of age groups of the Baikal omul first increases and then decreases in older groups. Species richness of parasite communities of age groups of European perch or first increases and then decreases (Lake Gusinoye), or decreases in age series (Chivyrkuisky Bay of the Lake Baikal). This division of the four fish species into two clusters depending on dynamics of parasite communities of host age groups is connected, first of all, with the difference in the changes of the food spectra of these fish species during ontogeny. The similarity of the dynamics of species richness of parasite communities in the age series of Baikal whitefish and roach are caused by the resemblance of the food type (both hosts being benthophagic). The change in food specialisation in the Baikal omul and European perch in the middle of their life cycles is the cause of the similarity of the dynamics of species richness of parasite communities in the age series of these fish species.

P-016

GOUSSIA CRUCIATA AND *G. CLUPEARUM* (APICOMPLEXA) IN CARANGID AND SCOMBRID FISHES FROM SOUTHEASTERN PACIFIC COAST

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The liver coccidian *Goussia cruciata* is considered as a specific parasite of species of the genus *Trachurus* from the Atlantic (*T. picturatus, T. lathami, T. trachurus and T. mediterraneus*) and Pacific (*T. murphyi*) oceans. *Goussia clupearum*, has been recorded in fish species of different families (e.g. *T. trachurus* and *Scomber scombrus*). In this study, fish species of the Carangidae (*T. murphyi* and *Seriola lalandi*) and Scombridae (*Scomber japonicus* and *Sarda chilensis*) captured along the Southeastern Pacific coast were examined for liver coccidians. The relationships of the parasites with host variables including total length, condition factor and hepatosomatic index were evaluated for *T. murphyi* and *S. japonicus*. The coccidian species were determined and quantified via observation of liver smears, using light microscopy. Parasitological descriptors such as prevalence (P%) and abundance (A) and the distribution of parasites were calculated using the coefficient of distribution (CD) and index of aggregation (K). Correlations between fish length, condition factor, hepatosomatic index and abundance of parasites were analyzed with Spearman's correlations. *Goussia cruciata* was recorded only in *T. murphyi* (P=100%, A= range 2-1500) and *G. clupearum* was recorded in livers of *S. lalandi* (P=33%, A= range 2–35), *S. japonicus* (P=50%, A= range 11–130) and one specimen of *T. murphyi* (P= 0.07%, A= 10). No coccidian infections were recorded in *S. chilensis. Goussia cruciata* and *G. clupearum* showed an aggregate distribution. The abundance of the coccidia was correlated with host length in *T. murphy*, but not in *S. japonicus*. The infection levels apparently do not produce negative effects on fish condition.

P-017

ECOLOGY AND SEASONIAL DISRIBUTION OF TAPEWORM *LIGULA INTESTINALIS* (LINNAEUS, 1758) (CESTODE) INFECTING *BARBUS CALLENSIS* (VALENCIENNES, 1842) (CYPRINIDAE) FROM FOUM EL KHANGA DAM (SOUK AHRAS, EASTERN ALGERIA)

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In this research, intestinal parasite (plerocercoid larvae of the tapeworm *Ligula intestinalis)* of Cyprinid fish (*Barbus callensis) from* lake of Foum El Khanga dam (Souk-Ahras, Algeria) was studied. Fish sample were obtained seasonally (90 fish individuals per season) during 2014. However, *L. intestinalis* was recorded in several fish hosts, the ecological studies of this parasite are limited and there are none pointed on *B. callensis*. During the seasonal study of this Cestode, many individuals of *B. callensis* were found infested with the tapeworm *Ligula intestinalis* (Linne´, 1758), which led us to study the effect of this parasite on *Barbus callensis* using the ecological parasites' index (prevalence, abundance and parasite intensity). The prevalence of *L. intestinalis* was 68.33% and the average intensity was 3.92 parasites per infected fish. We note, moreover, that the infection rates and parasite loads vary from one season to another. The parasite infection indeces were high in the autumn period and low during the spring season, which coincides with the fish breeding period.

P-018

SEASONAL VARIABILITY OF PARASITE COMMUNITIES OF *SCOMBER COLIAS* (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN

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The Atlantic chub mackerel (*Scomber colias* Gmelin, 1789) is a pelagic fish inhabiting temperate and warm waters of the Atlantic Ocean and associated seas. In the Mediterranean, the parasite fauna of *S. colias* is poorly known and parasite communities in this host have not been studied yet. This study describes the parasite fauna and assesses the seasonal variability of the parasite communities of *S. colias* from the Gulf of Alicante throughout the year 2011. Twenty-four metazoan parasite species were found in 102 fish examined; of these two were new host records. There were significant differences in infracommunity parameters between seasons, the richness and total abundance being greater in summer, Brillouin's diversity index significantly lower in winter and Berger-Parker's dominance index in spring. PERMANCOVA performed with total fish length as a covariate provided strong support for the differentiation of parasite infracommunities associated with the factor season (pseudo-$F_{(1,99)}$=10.82, P(perm)<0.0001). The overall dissimilarity between parasite communities of different seasons was relatively high (78.13–79.60%) with nine species contributing most to this dissimilarity: the digeneans *Allonematobothrioides* sp., *Lecithocladium excisum*, *Nematobothrioides filiforme*, *Neonematobothrioides faciale*, *Opechona bacillaris*, *Prodistomum orientalis*, the monogenean *Pseudokuhnia minor*, the larval nematode *Anisakis* sp. type I and the acanthocephalan *Rhadinorhynchus lintoni*. GLMs built on abundance and presence/absence data of these key species also revealed seasonal variability. The seasonal changes observed in parasite communities of *S. colias* may be associated with the seasonal variability of water conditions and unequal exploitation of trophic resources or with differences in the occurrence of intermediate hosts.

P-019

SEASONAL DIVERSITY OF METAZOAN PARASITES OF *PSEUDOCRENILABRUS PHILANDER* (WEBER, 1897) FROM THE MIDDLE LIMPOPO RIVER BASIN

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The importance of *Pseudocrenilabrus philander* (Weber, 1897) as an ornamental fish, has received attention due to its opalescence blue and pale yellow colour. This fish species is already utilized by subsistence fishermen in the Limpopo Province as an additional source of protein.

Eighty-six specimens of *P. philander* (total length = 5.46 ± 0.99 cm) were collected during summer period in November 2013 and February 2014 and during winter in July and August 2014 from Nwanedi River (South Africa) and Bubi River (Zimbabwe), respectively. Fish were sacrificed by severing the spinal cord. All parasites found were isolated from different organs and fixed by appropriate methods. Parasite identification studying important structures and documentation were done using an Olympus BX50 microscope. Morphometric evaluations revealed the presence of six species of monogeneans, *Enterogyrus coronatus*, *Cichlidogyrus tilapiae*, *Cichlidogyrus* spp. (three different species) and *Gyrodactylogyrus* sp.; three species of digenean metacercariae, *Petasiger* sp., *Clinostomum* sp. and *Neodiplostomum* sp.; eight species of cestode larvae *Neogryporhynchus* spp., *Paradilepis scolecina*, *Paradilepis* sp., *Parvitaenia macropeos*, *Parvitaenia* spp. and *Valipora minuta*; and L3 stage larvae of five nematode species, *Procamallanus* sp., *Camallanus* spp. and *Contracaecum* sp. NMDS was used to visualize the differences in the multivariate parasite species. PERMDISP and PERMANOVA pairwise tests showed significant difference in dispersion for parasite species in Bubi River ($p=0.05$, $p=0.001$) and a non-significant difference in Nwanedi River ($p>0.05$, $p=0.004$) for summer and winter. Many of the observations are first geographical and host records for both localities.

P-020

SEASONAL CHANGES IN PARASITE COMMUNITIES OF THREE-SPINED STICKLEBACK *GASTEROSTEUS ACULEATUS*

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Three-spined sticklebacks *Gasterosteus aculeatus* occupy a wide variety of aquatic ecosystems across the Northern hemisphere, and are often present at high population densities. Sticklebacks occupy central positions in food webs, and so play an important role in ecosystem processes, as well as acting as a host for wide variety of parasites. Hence sticklebacks frequently act as hosts to a wide range of parasite organisms, and make ideal subjects for the study of parasite communities. In our study, samples of between 30-60 young-of-the-year sticklebacks were collected from the River Soar, Leicesterhire (N 52°37'42.7", W 1°08'33.0") at approximately monthly intervals over an annual cycle. The prevalence and intensity of a range of parasite infections were quantified, alongside data on host growth rates and energetic indices. Here we present the findings of this study, which identify seasonality in the acquisition of infections, the development of parasite communities, and associations between infection levels and a range of host fitness correlates, including energetic condition and sexual development.

A NEW SPECIES OF *HEMIPERA* NICOLL, 1913 (DIGENEA: DEROGENIDAE) FROM THE INTERTIDAL FISH *SCARTICHTHYS VIRIDIS* (PISCES: BLENNIIDAE) FROM OFF CENTRAL CHILE

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A new digenean species from the genus *Hemipera* Nicoll, 1913 is described from the intertidal fish *Scartichthys viridis* (Valenciennes) off the central coast of Chile. A total of 1,514 specimens of *S. viridis* was examined, 24 of which hosted this parasite at an intensity of 1–12 worms. The new species is distinguishable from the five known congeneric species in the body size, being the smallest and narrowest species, reaching five times longer than wide. This is the first species of the genus described from the South Pacific Ocean off South America. The ITS2 rDNA sequence data for the new species of *Hemipera* were deposited in the GenBank.

NEW SPECIES OF *PARADISCOGASTER* (DIGENEA: FAUSTULIDAE) FROM BATFISHES ON LIZARD ISLAND, GREAT BARRIER REEF: FIRST REPORT OF FAUSTULIDAE IN EPHIPPIDAE WITH MORPHOLOGICAL AND MOLECULAR DATA

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Combined morphological and molecular analyses allow us to describe two new *Paradiscogaster* species from six Ephippidae specimens collected from Lizard Island, Great Barrier Reef (GBR): *Paradiscogaster* new species 1 from *Platax pinnatus* and *Paradiscogaster* new species 2 from *Platax orbicularis*. Molecular analysis of the ITS2 region of rDNA from the two species confirm the distinction between the two morphotypes proposed. Each *Platax* species was infected by only one *Paradiscogaster* species despite similar diet and distribution. This is the first study to identify faustulids from the genus *Paradiscogaster* in Ephippidae. A Neighbour Joining Tree was generated to evidence the comparison of these two new species with published sequences from other congeneric *Paradiscogaster*.

CRYPTIC DIVERSITY OF *DIPLOSTOMUM* SPP. IN LYMNAEID SNAILS FROM THE RUHR AREA, GERMANY, WITH A FOCUS ON THE ‘*DIPLOSTOMUM MERGI*’ SPECIES COMPLEX P-023

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Trematodes of the genus *Diplostomum*, important pathogens with serious impacts on fish populations utilise freshwater lymnaeid snails, fish and fish-eating birds as hosts. Recent molecular studies indicate greater species diversity than previously estimated, including three species complexes in North America and Europe. However, data on the diversity and distribution in snail hosts are scarce. This study applies morphological and molecular approaches to the species diversity of *Diplostomum* spp. in lymnaeid snail populations in a reservoir system of the Ruhr River, Germany with a focus on the ‘*D. mergi*’ species complex. Detailed morphological and molecular data based on *cox*1 and ITS1-5.8S-ITS2 sequences from 52 isolates gathered in an extensive sampling of four snail species (1,909 *Radix auricularia*, 349 *R. peregra,* 668 *Stagnicola palustris* and 245 *Lymnaea stagnalis*) provided evidence for three named species: *Diplostomum spathaceum*, *D. pseudospathaceum* and *D. parviventosum*, and four distinct lineages: ‘*D. mergi* Lineages 2–4’ and ‘*Diplostomum* sp. Clade Q’. The clarification that ‘*D. mergi* Lineage 1’ in fact represents *D. parviventosum* and evidence of the first link between ‘*D. mergi* Lineage 3’ from isolates of metacercariae in fish (Georgieva et al. 2013) and cercariae in snails substantially contribute to our knowledge of the ‘*D. mergi*’ species complex. These results suggest a diverse fauna of *Diplostomum* spp. in snail populations in the Ruhr reservoirs and represent an important step to the knowledge on species identification of these important parasites of snails and fish in Europe.

This study was supported by the Czech Science Foundation (project No. 15-14198S).

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF HIMASTHLINAE (DIGENEA: ECHINOSTOMATIDAE) METACERCARIAE, PARASITES OF *CERASTODERMA EDULE* (BIVALVIA) IN AVEIRO ESTUARY, PORTUGAL P-024

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Most adult trematodes of Himasthlinae (Digenea: Echinostomatidae) are intestinal parasites found in birds as adults, and occasionally in humans. In Aveiro estuary (northwestern coast of Portugal), three congeneric trematode species, namely *Himasthla quissetensis*, *H. elongata*, *H. interrupta* and another species, *Curtuteria arguinae,* often co-occur as encysted metacercariae in bivalves, and, in particularly, in edible cockles, as *Cerastoderma edule*. These four species utilize prosobranch snails and bivalves as their first and second intermediate hosts, respectively, and various seabirds, mostly seagulls (predators of second intermediate host) as definitive hosts. In the performed survey, metacercariae were found infecting the foot, digestive gland, gonads and the mantle of the cockles. The isolated cysts presented distinct diameters between the different species of Himasthlinae. However, cyst dimension is not sufficient to identify species. Thus, chemically excysted metacercariae were studied by scanning electron microscopy. A specific and distinctive character that was recorded was the number of oral sucker collar spines. Moreover, when this feature is constant, their differentiation could be made by differences in the morphology of the tegument, namely the number and shape of spines that cover the body, and also the distribution of the sensory papillae. This study also aims to present preliminary results of the development of molecular markers, based on ITS1 and ITS2 rDNA sequences, which would allow us to easily differentiate Himasthlinae metacercariae, parasites of *C. edule*. In addition, a comprehensive phylogenetic analysis was performed within Echinostomatidae, including new Himasthlinae sequences.

P-025

MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *DIDYMOSULCUS* SPP. (DIGENEA: DIDYMOZOIDAE), PARASITES OF THE ATLANTIC BLUEFIN TUNA *THUNNUS THYNNUS* (SCOMBRIDAE)

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The genus *Didymosulcus* Pozdnyakov, 1990 comprises hermaphroditic species of the family Didymozoidae (Trematoda: Digenea) in which the body is divided into two distinct regions: an elongate, narrow forebody and a greatly enlarged comma shaped hindbody, with an anterior dorsal median furrow. They occur encapsulated in pairs in the tissues of the head of scombrid fishes, especially *Thunnus* spp. The aim of this study is the morphological and molecular characterisation of *Didymosulcus* spp. of the Atlantic bluefin tuna *Thunnus thynnus* from the North East Atlantic and the Western Mediterranean. Heads of 779 *T. thynnus* were examined for parasites between 2008 and 2014. A total of 23,606 specimens of *Didymosulcus* sp. was collected and assigned based on morphology to four types: *Didymosulcus* cf. *soleiformis* (prevalence 19%), *Didymosulcus wedli* (53%); *Didymosulcus* sp. 1 (9%), *Didymosulcus* sp. 2 (3%). Sequence analysis using partial sequences from the large subunit of the nuclear ribosomal DNA confirmed the morphological results for the distinctness of the morphotypes. Results of the phylogenetic analysis including didymozoid sequences available on GenBank will be discussed; these indicate the existence of a putative new species of *Didymosulcus.*

This research was supported by the Spanish MICIN project PARATUN (AGL2010-20 892) and the Regione Autonoma della Sardegna grant (S.M., PRR-MAB-A2013-17 605).

P-026

ACANTHOSTOMUM SP. (DIGENEA: CRYPTOGONIMIDAE) PARASITE OF SMOOTH STARGAZER *KATHETOSTOMA AVERRUNCUS* (PERCIFORMES: URONOSCOPIDAE) OF THE NORTH COAST OF PERU

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Kathetostoma averruncus (Uronoscopidae) is a bentoneritic species buried on sandy and muddy bottoms at depths up to 300 m and distributed from California (USA) to Lobos de Tierra Islands (Peru). According to the International Union for Conservation of Nature and Natural Resources, *K. averruncus* is listed as a species of least concern. The aim of this study was to identify the flukes that parasitize *K. averruncus* on the north coast of Peru. Specimens of *K. averruncus* were acquired in December 2014 from Puerto Pizarro, Tumbes, Peru. The flukes were collected, fixed, preserved in 70% ethanol, stained with Semichon acetic carmine and mounted in Canada balsam. Sampling throughout a total of 740 trematode parasites was collected. The flukes were identified as *Acanthostomum* sp. (Cryptogonimidae) due to the following characteristics: (1) spiny tegument; (2) funnel-shaped oral sucker, surrounded by a circular row of spines; (3) subspherical tandem and contiguous testes; (4) bipartite and sinuous seminal vesicle; (5) preacetabular genital pore; (6) spherical and pretesticular ovary, and (7) vitelline follicles in two lateral fields. *Acanthostomum* sp. is a new record for Peru and the South Pacific. *Kathetostoma averruncus* is a new host for this species.

P-027

IN VITRO EVALUATION OF GINGER (*ZINGIBER OFFICINALE*) EXTRACT AS A TREATMENT AGAINST *NEOBENEDENIA* SP.

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Capsalid monogeneans *Benedenia seriolae* and *Neobenedenia* sp. cause severe problems in Yellowtail Kingfish, *Seriola lalandi*, aquaculture. We investigated the use of natural extracts from ginger, *Zingiber officinale*, as an alternative to synthetic chemicals for capsalid monogenean treatment in aquaculture. Toxicity of three dilutions of methanolic ginger extract (1:10, 1:50 and 1:100 mL, applied in continuous immersion), were evaluated *in vitro* against *Neobenedenia* sp. life stages (i.e. eggs, oncomiracidia and adults). Temperature was maintained at 25±1°C in natural light. Adult parasites and oncomiracidia were monitored following immersion every hour over an 8 h period. Eggs were monitored every 24 h over nine days. The effect of the treatments was measured as percentage survival (adults and oncomiracidia), egg production by adults parasites, and egg hatching success. The 1:10 mL ginger extract was lethal for *Neobenedenia* sp. All adult *Neobenedenia* sp. died and no eggs were laid following 2h of continuous immersion. Furthermore, no oncomiracidia survived following 1h immersion in 1:10mL and no eggs hatched. The concentration of 1:100 mL had no measurable effect on any life stage. The 1:50 mL treatment did not affect survival of adults, but it reduced egg production to 2% and hatching success to 6% compared to seawater controls where adults yielded 405 eggs (100%) and 72% hatching success. Similarly oncomiracidia survival was reduced to 3% after 5h immersion in 1:50 mL ginger extract compared to 97% obtained in seawater controls. This research indicates that ginger extracts may be an effective natural treatment against *c*apsalid monogenean infections.

P-028

A SURVEY OF ACTINOSPORES FROM ANNELIDS IN A PORTUGUESE FISH FARM

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The Myxosporea Bütschli, 1881 are obligate microscopic endoparasites of vertebrate and invertebrates species. They infect fishes, birds, amphibians, reptiles and mammals, and alternate their life cycle in annelids and sipunculids. A fish farm is the ideal environment for the dissemination of these parasites since it settles advantageous conditions in terms of the presence of possibly both the vertebrate and the invertebrate hosts. Studying the extrapiscine development of Myxosporea in annelids is important for the understanding of their full life cycle, which in turn allows the development of better tools for the control of myxosporidioses in culture environments. For this purpose, a parasitological survey in annelids was conducted in a fish farm located in the Algarve. The results of the survey, which included the microscopic analysis of 1900 oligochaetes and 4275 polychaetes, revealed the presence of actinospores belonging to 5 different collective groups. In oligochaetes, 2 triactinomyxon morphotypes (with a prevalence of infection of 1.8 to 1.9%), and about 3 sphaeractinomyxon morphotypes (2.8% in total) were registered. In polychaetes, 1 tetractinomyxon morphotype (0.1%), 1 echinactinomyxon morphotype (0.2%) and 1 unicapsulactinomyxon morphotype (4.2%) were registered.

Acknowledgements: The Portuguese Foundation for Science and Technology (FCT), within the aim of the Project DIRDAMyx (Reference: FCOMP-01-0124-FEDER-020726 / FCT- PTDC/MAR/116838/2010) for the grants attributed to F. Cavaleiro and R. Castro, and the Ph.D. fellowship grants attributed to L. Rangel (SFRH/BD/82237/2011) and to S. Rocha (SFRH/BD/92661/2013) through the programme QREN-POPH/FSE; and the project EUCVOA (NORTE-07-0162-FEDER-000116).

P-029

ZSCHOKKELLA AURATIS (MYXOZOA) INFECTING THE GALLBLADDER OF THE GILTHEAD SEA BREAM, *SPARUS AURATA* (SPARIDAE): PARASITE-HOST-ENVIRONMENT RELATIONSHIPS

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The infection with myxozoans has generally been recognized among the most important causes of economic losses in fish farm facilities. *Zschokkella auratis*, a parasite recently described by Rocha *et al.* 2013, is found infecting the gallbladder of the gilthead seabream, *Sparus aurata* (Sparidae). In this work, the relationships between that parasite, its host and the environment in which it lives is investigated, based on the parasitological records for 288 fish reared in an aquaculture facility located in Southern Portugal. The occurrence of *Z. auratis* was related with host (total weight and length, condition factor and sex) and environmental (season and water temperature and salinity) characteristics. *Z. auratis* recorded a prevalence of 11.3% for the gallbladder. Concerning the host characteristics, the statistical analyses (Mann-Whitney's U test) suggested a significant negative impact of *Z. auratis* in the host's total weight and length and condition factor ($P < 0.007$). As for the environment, a significant difference was found for water salinity ($P = 0.000$), with higher salinities favoring the occurrence of the infection. These results suggest that myxozoan parasites infecting the gallbladder, usually reported as innocuous for their hosts, may have been overlooked and could actually affect their hosts.

Acknowledgements: The Portuguese Foundation for Science and Technology (FCT), within the aim of the Project DIRDAMyx (Reference: FCOMP-01-0124-FEDER-020726 / FCT- PTDC/MAR/116838/2010) for the grants attributed to F. Cavaleiro and R. Castro, and the Ph.D. fellowship grants attributed to S. Rocha (SFRH/BD/92661/2013) and to L. Rangel (SFRH/BD/82237/2011) through the programme QREN-POPH/FSE; and the project EUCVOA (NORTE-07-0162-FEDER-000116).

P-030

PRESENCE OF *NEOBENEDENIA MELLENI* ON SPANISH ATLANTIC CULTURES OF *SERIOLA DUMERILI*

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This study documents recent outbreaks of *Neobenedia melleni* on *Seriola dumerili* cultured in Canary Islands. Monogenan capsalids of the genus *Neobenedenia* are common skin and gill parasites of marine fishes, frequently highly pathogenic. Moreover, the species of this genus usually show low host specificity, what hampers their control and epidemiological follow-up. *Neobenedenia* spp. have been described parasitising some commercial important species as *Seriola dumerili* and *S. quinquerata,* being related to high mortalities in marine aquaculture facilities in the western Pacific (Japan and Australia), dealing in important economic loses.

The aim of present study is to identify the species of *Neobenedenia* sp. parasitising the cultures of *S. dumerili* in Canary Islands (Eastern Atlantic). The species of *Neobenedenia* was identified through their morphology and confirmed with molecular analyses due to the puzzling taxonomy of the group. Specimens isolated were compared with morphologic and morphometric descriptions and available sequences of *N. melleni* and *N. girellae.*

We conclude that the monogeneans herein analysed correspond to the species *N. melleni*. Until now, *N. melleni* has not been reported from wild or cultured fish in the Atlantic waters. The presence of *N. melleni* can deal in health fish problems and in economic loses in the Atlantic cultures of *S. dumerili*, showing the importance to develop a proper monitoring and management planning in the aquaculture installations.

Funded by ISIC/2012/003 & PROMETEO 795/2015/018 projects.

P-031

DISTRIBUTION OF *CARDICOLA* SPP. IN THE GILL FILAMENTS OF THE ATLANTIC BLUEFIN TUNA *THUNNUS THYNNUS*: DESIGN OF USER-FRIENDLY DIAGNOSIS METHODS

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Blood-flukes of the genus *Cardicola* (Trematoda: Aporocotylidae) are highly pathogenic parasites for bluefin tuna farming worldwide. The aim of this study is to describe the distribution of blood-flukes in the gills of farmed Atlantic bluefin tuna *Thunnus thynnus* and, based on this information, to provide an efficient and user-friendly diagnostic method. The gills of 100 Mediterranean farmed tuna were analysed: holobranchs were divided into five transversal areas, a pair of gill filaments was cut from each area and processed by gill clarification and observation under stereomicroscope. The results from each area and the whole gills were compared. No adults of *Cardicola* spp. were found in the gill filaments, while eggs were detected in 92% of the samples. They were uniformly distributed among holobranchs (prevalences from 45% to 57%), whereas they were aggregated among transversal areas: the dorso-distal ones had the highest prevalence (80%). Caution must be taken to extrapolate the results to other bluefin tuna species, as the observed distribution differs to those reported in other studies. Based on the observations, accurate, cheap and low time-consuming diagnostic method, also utilizable by fish producers, is proposed.

JPA and JRL benefit from a PhD fellowship from the University of Valencia and the Spanish Government respectively. Funded by project AGL2010-20892 of the Spanish Government.

P-032

FOUR SPECIES OF *CARDICOLA* (TREMATODA: APOROCOTYLIDAE) INFECTING THE ATLANTIC BLUEFIN TUNA *THUNNUS THYNNUS* (L.) IN THE MEDITERRANEAN SEA

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Blood flukes (Trematoda, Aporocotylidae) are the one of the most pathogenic parasites infecting pelagic fish in aquaculture. Pathologies associated to these aporocotylids of the genus *Cardicola* Short, 1953 have been reported in tunas (*Thunnus* spp.) from Australia and Japan. Two *Cardicola* species have been reported to date in the Atlantic Bluefin Tuna (*Thunnus thynnus*) from the Mediterranean, although no significant damages have been described. Morphological and genetic analyses of ribosomal ITS-2 sequences revealed the occurrence of four aporocotylid species in 274 tuna fished from the wild and 147 tuna from sea-cages. Adults of three species were recovered: *C. forsteri*, *C. opisthorchis* and *C. orientalis.* A fourth undescribed species was found, based on molecular analyses of eggs apparently indistinguishable in size and shape from the eggs of *C. orientalis*. The findings provided evidence that infections with *Cardicola* spp. differed in relation to locality, host origin (wild *vs* cage-reared) and site of infection. This is the first time that four species of *Cardicola* (and to our knowledge, of any aporocotylid species) are cited together in the same species and area, and the first record of *C. orientalis* and *Cardicola* sp. in *T. thynnus*. It is necessary to estimate the possible different pathogenic effects of each species of *Cardicola* in order to take appropriate control measures.

JPA and JRL benefit from a PhD fellowship from the University of Valencia and the Spanish Government respectively. Funded by project AGL2010-20892 of the Spanish Government.

P-033

PARASITE FAUNA AND RISK ASSESSMENT FOR AQUACULTURE OF *SERIOLA DUMERILI* IN THE WESTERN MEDITERRANEAN SEA

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The greater amberjack *Seriola dumerili* is a fish species with high economical value and very appreciated by consumers, mainly in Japan but also in the Mediterranean region. Whereas this species is intensively cultured in Japan, incipient attempts of culture in the Mediterranean have been truncated by illnesses associated to parasites among other causes. Therefore management of parasite infections is critical to enhance the culture of this fish species. The aim of this work is to describe the parasite fauna of greater amberjack in the western Mediterranean Sea and to assess the potential risks of the recorded parasite species for cultures. To attain this objective 165 greater amberjacks from Majorca were periodically sampled and analysed. Parasites were collected, mounted and identified and thereafter, different species were arranged into groups representing gradual risk levels for cultured greater amberjacks. As a result of this study, a total number of 36,824 specimens from 26 species belonging to three different phyla (Arthropoda, Nematoda and Platyhelminthes) were recorded: 1 isopod, 3 copepods, 4 nematodes, 15 trematodes, 2 cestodes and 1 monogenean. Monogeneans and aporocotylid trematodes were considered the most hazardous species, with fast proliferation and transmission and causing severe pathological effects on host. Copepods, isopods and nematodes represent thus a moderate hazard for greater amberjack in culture conditions. Finally, most of the trematodes, as well as accidental parasite species with low prevalences and without severe associated pathologies were considered harmless.

Funded by AGL2010-20892, ISIC/2012/003 & PROMETEO 795/2015/018 projects

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P-034

ECTOPARASITES *VERSUS* SHARK SQUAMATION: IMPLICATIONS OF GROUPING BEHAVIOUR ON SHARKS PARASITISM

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Grouping behavior is widespread among sharks being present in almost all lifestyles or ecological groups. It is well known that there are some costs associated with schooling or shoaling such as decrease in foraging efficiency or the increase of the risk of pathogen transmission. In this sense, this work aims to analyze the abundance of ectoparasites with direct life cycles in schooling and non-schooling sharks and to detect adaptative responses of their squamation pattern to this selection pressure. For this purpose, we have reviewed 195 shark species and their parasite richness have been compared between schooling and non-schooling species using analysis of covariance (ANCOVA). Furthermore, the squamation pattern and the scales morphology of 10 specimens of each ecological group of sharks have been studied in the Museum für Naturkunde (Berlin, Germany). Our results suggest that ectoparasites with direct life cycles are more abundant in schooling species. Interestingly, squamation seems to respond to a trade-off where hydrodynamic and protective functions prevail over defense against ectoparasites. As a consequence, only schooling species which are not strong swimmers or benthic species living on hard substrates have optimized some aspects of their squamations in order to avoid the settlement of ectoparasites (reducing the scale density and increasing the angle of crown insertion). The location of the ectoparasites on these species provides some evidence in this regard, being found mainly on gill bars and areas without scales or with smooth scales, such as the snout, eyes and fin leading edges, where the attachment is easier.

P-035

WATER LEVEL FLUCTUATION INFLUENCES FISH PARASITE ABUNDANCE IN SEMI-LOTIC ENVIRONMENTS IN A LONGITUDINAL GRADIENT OF A TROPICAL RIVER

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The structure and dynamics of populations and communities in floodplains can be explained by physical and chemical features of these ecosystems. Although the effects of these properties on free-living communities are relatively well studied in Upper Paraná River floodplain (UPRF), this prediction has not been effectively tested for parasites. Multivariate analyses were performed to determine which physical environmental descriptors (distance among environments, location on the island “right and left side”, and water level) that influence the abundance of fish parasite species in five semi-lotic environments within fluvial islands in a longitudinal gradient from Paraná River, southern Brazil. Six hundred individuals of *Moenkhausia forestii* were collected between June 2011 and April 2012 at periods of low and high water levels. A total of 5939 parasites belonging to 17 species were found parasitizing the fishes. Due to the intimate relationship of parasites with their hosts, the parasite community also reports a similar pattern in free-living communities from the UPRF. The decrease in water level reduces the connectivity among environments (low similarity) with different hydrological features, and local processes act in different intensities on host populations. On the other hand, the increase in water level permits connectivity (high similarity) among environments with different hydrological features and as a result, host populations tend to be more similar among environments. Our results show that water level variation is the main environmental descriptor that affects the parasite abundance due to influence on abiotic and biotic variables from each environment along the longitudinal gradient.

P-036

DOES THE SPATIAL DISTRIBUTION OF *SPHAEROSPORA DICENTRARCHI* (MYXOZOA: SPHAEROSPORIDAE) VARIES ACCORDING TO HOST’S AGE?

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Sphaerospora dicentrarchi (Myxozoa: Sphaerosporidae) is a histozoic parasite, commonly found infecting *Dicentrarchus labrax* (Teleostei: Moronidae). It is usually found in the connective tissue of different organs and both in wild and cultured fish. However, despite the numerous studies conducted so far, only little information is available on the parasite’s spatial distribution within the host’s body. This study aimed at evaluating whether such distribution varies during the course of the host’s life. Parasite records respecting a total of 409 specimens of *D. labrax* – 78.9±38.2 (21.1-257) g; $N_{0\ years}$=7, $N_{1\ year}$=152, $N_{2\ years}$=188, $N_{3\ years}$=54 and $N_{\geq 4\ years}$=8 –, collected in the Ria de Aveiro (40º38.1’N, 8º43.9’W) between July 1993 and March 1996, were used in the analysis. *S. dicentrarchi* was isolated from different sites, including: the posterior kidney (overall prevalence: 38.1%); the gall (39.9%), swimming (44.0%) and urinary (36.7%) bladders; the gonads (45.7%); the stomach (24.4%); the pyloric caeca (36.4%); and the anterior (38.6%), medium (39.4%) and posterior (38.9%) intestine. A correspondence analysis was conducted to evaluate whether the infection spreads within the body as the host grows older. According to the results found, older fish tended to present a higher number of sites infected with *S. dicentrarchi*. Furthermore, in older fish, the prevalence of infection was maximum for the gall and urinary bladders, the pyloric caeca and the intestine. In the posterior kidney, gonads and swimming bladder, the prevalence reached the maximum level in the third year, decreasing thereafter. This result is of interest, in the perspective of infection control.

P-037

SEASONAL VARIABILITY IN THE PARASITE FAUNA OF *SOLEA SENEGALENSIS* (KAUP, 1858) FROM EBRO DELTA (NW MEDITERRANEAN) RELATED TO HOST DIET AND HISTOPATHOLOGY: A PRELIMINARY STUDY

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Solea senegalensis Kaup, 1858 (Teleostei: Pleuronectiforme) is a commercially important benthic species frequently found in Ebro Delta platform. Despite some studies dealing with metal concentrations and enzymatic activity levels have been done for this species, its parasite fauna is poorly known and, as far as we are aware, no data on its parasite communities from the Mediterranean Sea exist. The aim of this study is to assess the seasonal variability on the parasite fauna of *S. senegalensis* and to evaluate its relationship with host diet and histopathology. A total of 120 specimens of *S. senegalensis* was collected in Ebro Delta (NW Mediterranean) in autumn 2014 and winter and spring 2015 at a depth of 10-12m. Half of the specimens of each season were fixed in 10% buffered formalin for histopathological study and the rest were frozen for parasitological study. Parasitological, dietary and histological analyses were performed according to standardized protocols. A total of nine parasite taxa was found: two digeneans, one cestode, two acantocephalans, three nematodes and one copepod. The most prevalent and abundant parasites were the copepod *Bomolochus* cf. *bellones,* the digenean *Galactosomum* sp., and the acanthocephalan *Pseudorhadinorhynchus* sp. Total mean abundance and total mean species richness values were relatively uniform throughout the year. However, differences in prevalence and abundance among seasons were observed at the component population level for some taxa, probably related to seasonal dietary changes of the hosts.

P-038

PATTERNS ON ABUNDANCE OF METAZOAN FISH PARASITES ON THE MEDITERRANEAN PLATFORM OFF CATALONIA: HOST- AND ENVIRONMENT-RELATED FACTORS

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A total of 683 fish representing ten species (*Scyliorhinus canicula, Trisopterus minutes, Merluccius merluccius, Pagellus acarne, Pagellus erythrinus, Boops boops, Spicara maena, Mullus barbatus, Trachinus draco, Citharus linguatula*) was collected in 2007 during an extensive survey of macroparasites from off Barcelona in the Western Mediterranean at depths ranging from 53 to 68 m. A total of 99 parasite taxa were identified. Relationships between infracommunity abundance and biotic factors characterising host-species (condition factor, fish density, swimming capacity, diet and trophic level) and environmental factors (temperature, turbidity, etc.) were analysed. The abundance of parasites sharply contrasted among host species, *Citharus linguatula* exhibiting the lowest total abundance (TA) (2.53±0.40 parasites/fish) and *Scyliorhinus canicula* the greatest (46.94±6.46). Abundance of *Proleptus obtusus* and *Anisakis physeteris* was associated with polychaetes and shrimps, in turn linked as preferential prey with *S. canicula*. High near-bottom temperature was linked to the abundance of *Lernaeocera lusci, Helicometra fasciata* and *Ascarophis mullusi*, coinciding with the hauls performed in summer and autumn. Infracommunity abundance of endoparasites showed a positive relationship with host size and swimming capacity and a negative relationship with condition factor and trophic level, but these positive relationships were not observed for TA of ectoparasites. Species richness and Brillouin's diversity index showed a positive relationship with fish density. We provide strong evidence that several factors determine parasite abundance, with a prominent role of host species, individual fish size, condition factor, swimming capacity and trophic level of host.

The study was supported by the MICINN projects BIOMARE (CTM2006-13508-C02-01) and ANTROMARE (CTM2009-12214-C02-02).

P-039

PARASITE COMMUNITIES OF *ALEPOCEPHALUS ROSTRATUS* (RISSO, 1820) IN THE MEDITERRANEAN SEA BETWEEN 1000 AND 2000 M DEPTH

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Alepocephalus rostratus Risso, 1820 is the second most important fish species, in terms of biomass, inhabiting the deep slope of the Catalan Sea. A total of 152 individuals of *A. rostratus* were collected on board the R/V García del Cid in the Catalan Sea (Western Mediterranean) during July 2010 and October 2011 with a semi-balloon otter-trawl (OTSB-14). Fish were captured between 1000 and 2000 m depth at two different localities (Barcelona coast and Balearic Islands). Fish were dissected according to a standardized parasitological protocol. The most abundant and prevalent parasites were the cestode *Scolex pleuronectis* (Müller, 1788), the nematode Cucullaninae gen. sp. and the monogenean *Paracyclocotyla cherbonnieri* (Dollfus, 1970). *Scolex pleuronectis* showed the highest prevalence along the slope, mainly in summer when this parasite achieved a 100% prevalence. Cucullaninae gen. sp. was the parasite with the highest total abundance at 1000-1400 m depth, whereas at 1400-2000 m its abundance decreased. This could be explained by a diet shift of the hosts at increasing depths. This tendency has also been reported in previous studies. The digenean *Paraccacladium* sp. exhibited seasonal and bathymetric variability in terms of prevalence and abundance, achieving highest values in autumn and at 1400-2000 m depth. The bathymetric effect is probably related to higher water temperatures at 1400-2000 m as it has been observed in the same area in summer.

This study was supported by the Spanish Science and Innovation Ministry project ANTROMARE (CTM2009-12214-C02-02).

P-040

DESCRIPTION OF THE PARASITE COMMUNITY OF *PHYCIS BLENNOIDES* (BRÜNNICH, 1768) FROM DEEP WATERS OF THE MEDITERRANEAN SEA

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The greater forkbeard, *Phycis blennoides* (Brünnich, 1768) (Gadiformes), an important constituent of the western Mediterranean demersal fisheries, is distributed in the central and eastern Atlantic Ocean as well as in the Mediterranean Sea. A total of 80 specimens of *P. blennoides* were collected in the Balearic Sea (W Mediterranean). Samples were obtained from the continental slope of two different areas: the coast of Catalonia (off Barcelona) and the Balearic Islands, at depths between 400 and 1400 m in summer (July 2010, June 2011) and autumn (October 2011). Environmental variables were recorded at 5 m above the sea-bottom. The fishes were dissected and examined in the laboratory for parasitological study. Parasitological data were analyzed by comparing areas, depth strata, and seasons. The parasite community of *P. blennoides* was dominated by nematodes, which constituted more than half of the total number of taxa, followed in importance by digeneans. Most parasites were adult stages, except some nematodes such us *Anisakis* type 1 and 2, which were found as larvae. The most prevalent parasites were the nematodes *Collarinema* cf. *collaris, Hysterothylacium* cf. *aduncum*, *Capillaria* cf. *gracilis* and *Cucullanus* spp. and the digeneans *Bathycreadium brayi* and *Lepidapedon* spp. Other remarkable taxa were the copepod *Clavella* sp. and the acantocephalan *Echinorhynchus* sp. Parasite infracommunities showed differences between depth strata, areas and seasons, probably related to changes in host diet.

The study was supported by the MICINN project ANTROMARE (CTM2009-12214-C02-02).

P-041

NATURAL VARIABILITY OF PARASITE COMMUNITIES OF MACROURID FISHES IN MEDITERRANEAN LOWER SLOPE

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The parasite communities of the three macrourids with their bathymetric centre of gravity in the deep lower slope (below 1400 m depth) of the Catalan Sea (Western Mediterranean) were analysed for the first time. Specimens of *Coryphaenoides guentheri* (n=126), *Coryphaenoides mediterraneus* (n=45) and *Coelorinchus mediterraneus* (n=102) were collected on board the R/V García del Cid in July 2010, September 2011 and May 2012. The parasite fauna infecting macrourids was relatively poor, being lower for the fishes of the genus *Coryphaenoides* (5 species in each host) than for *Coelorinchus mediterraneus* (9). These three hosts shared only two parasite species: the nematode *Hysterothylacium aduncum* and the copepod *Hamaticolax* sp. *Hysterothylacium aduncum* was the most abundant and prevalent parasite for the genus *Coryphaenoides*, reaching 100% prevalence at the lowest depth in *Coryphaenoides guentheri*. Likewise, the abundance of this nematode increased with depth in all hosts. This pattern has also been observed in other fishes of the same area (i.e. *Alepocephalus rostratus* and *Bathypterois mediterraneus*). The copepod *Hamaticolax* sp. was mostly present in both *Coryphaenoides mediterraneus* and *Coelorinchus mediterraneus*, and its abundance tends to increase at lower depths. The digenean *Lepidapedon desclersae* was the most abundant and most prevalent parasite in *Coelorinchus mediterraneus* and it exhibited its higher abundance and prevalence in summer. Overall, nematodes seemed to be the main group parasitizing the Mediterranean Macrouridae since they represented almost half of the parasite fauna in these fishes.

The study was supported by the MICINN project ANTROMARE (CTM2009-12214-C02-02).

P-042

PARASITE FAUNA OF *PAGRUS PAGRUS* (L.) AND SESONAL VARIATION OF ITS PARASITE COMMUNITIES ALONG THE WESTERN MEDITERRANEAN COASTS OF ALGERIA

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The red porgy, *Pagrus pagrus*, is a benthopelagic sparid fish with a high commercial value, which occurs in warm temperate and subtropical areas of the Atlantic including the Mediterranean. It closely resembles to *Pagrus major*, one of the most valuable and widely farmed marine fishes, and thus, there is an increase of interest in its culture in the Mediterranean. However, the parasite fauna of *P. pagrus* is relatively unknown in this area. Our study describes for the first time the parasite fauna of *P. pagrus* and assesses the seasonal variability of its parasite communities in the Mediterranean. Sixty *P. pagrus* collected in summer and autumn of 2013 were examined and a total of 926 parasites of 22 species was collected: four monogeneans (*Choricotyle chrysophryi*, *Encotyllabe* sp., *Lamellodiscus* cf. *baeri* and *Microchotyle erythrini*), nine digeneans (*Allopodocotyle jaffensis*, *Aphallus tubarium*, *Cardicola* sp., *Derogenes latus*, *Diphterostomum brusinae*, *Holorchis pycnoporus*, *Macvicaria crassigula, Pachycreadium carnosum* and *Stephanostomum euzeti* met.), one larval cestode (*Scolex pleuronectis*), four nematodes (*Anisakis* sp. larva), *Ascarophis* sp., Capillariidae gen. sp. and *Hysterothylacium* sp. larva), one acanthocephalan (*Neoechinorhyncus* sp.) and three crustaceans (*Clavellopsis* sp., *Gnathia* sp. and *Caligus* sp.). Analyses of the composition and structure of parasite communities in *P. pagrus* showed a seasonal variation associated with the higher abundance in autumn than in summer of the ectoparasites *Gnathia* sp., *Lamellodiscus* cf. *baeri* and *Microcotye erythrini*.

P-043

SPATIAL-TEMPORAL DISTRIBUTION OF EPIDEMIOLOGICAL INDEX OF HATSHEKIIDAE PARASITES IN THIRTEEN TELEOST FISH SPECIES FROM EAST OF ALGERIAN COAST

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This work focuses on the study of the infestation by Hatshekeidae parasites collected on 2060 fish belonging to 13 species known for their economic interest. Six species were used for the spatial study (*Diplodus vulgaris, D. sargus, D. annularis, Pagellus erythrinus, P. bogaraveo, P. acarne*) collected in 2004 from off the Algerian East coast (Annaba, El Kala and Skikda Gulf and El Mellah lagoon), and three were used for a seasonal study (*D. annularis, Lithognathus mormyrus, Mullus barbatus*) sampled in 2007 from gulf of Annaba. Additionally, *Scorpaena scrofa, D. cervinus, D. puntazzo, D. marrocanus, M. surmuletus* were collected from Annaba gulf in 2008 for the Inventory Study. A total of 2519 Hatschekeidae parasites was found belonging to three species (*Hatschekia pagellibogneravei* (Hesse 1879); *H. mulli* (Van Benden, 1851); *Prohatschekia mediterranea (*Hamza et al, 2007). The infection levels of *H. pagellibogneravei* (Hesse, 1879) were significantly higher *in D. vulgaris* from Skikda gulf. We note, in fact, the absence of the other two copepods species on the three species of the genus *Diplodus* which have been the subject of a spatial study. Seasonal samples of the three host species collected revealed higher infection levels of *Hastchekia pagellibogneravei* (Hesse, 1879) and *Hatschekiamulli* (Van Benden, 1851) during the summer. The results of the application of χ^2test of independence by using the software Statistica 8 show that the site presents an influence on the fish infestation rates by Hatschekeidae.

P-044

INFECTIONS WITH *DIPLOSTOMUM SPATHACEUM* IN THE EYES OF FISHES FROM THE ARAS RESERVOIR LAKE IN IRAN

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In this survey infections with *Diplostomum spathaceum* in 240 fish specimens of seven species (*Sander lucioperca, Siluris glanis, Cyprinus carpio, Hypophthalmichthys molitrix, H. nobilis, Ctenopharyngodon idella* and *Abramis brama*) samples in the Aras Reservoir Lake was evaluated from spring to winter in 2012. The results indicate that 40% of fish (96 fish) harboured this parasite thus indicating a high rate of infection in the fish in this water resource. The mean abundance was 6.07±1.34 and the range for intensity was between 1–52 parasites. Statistically significant differences were observed in the intensity of infection with *D. spathaceum* between different seasons ($P > 0.05$) with the highest infestation rate of 25% in summer and the lowest rate of 5% in winter. Infections with metacercariae of *D. spathaceum* were observed in all host species studied, but *H. molitrix* was most heavily infected.

P-045

SEASONAL DISTRIBUTION OF PARASITES OF *OREOCHROMIS NILOTICUS* FROM TWO RESERVOIRS IN BURKINA FASO

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The study aimed to investigate the diversity, abundance, intensity and seasonal distribution of parasites of *Oreochromis niloticus*. A total of 254 specimens of *Oreochromis niloticus* was sampled in Loumbila and Ziga reservoirs, in both rainy and dry season and examined for parasites. The total prevalence was 55.90% and the highest seasonal prevalence, abundance and intensity were observed during the rainy season. Parasites recovered were the myxozoan *Myxobollus tilapiae*, the copepode *Lamproglena monodi*, the monogeneans *Cichlidogyrus tilapiae* and *Cichlidogyrus* sp., the digenetic trematode *Clinostomum* sp., the nematode *Paracamalanus cyathopharynx* and the acanthocephalan *Acanthogyrus tilapiae*. The latter species had higher prevalence (45.67%) and high abundance. *Lamproglena monodi, Cichlidogyrus* sp., *P. cyathopharynx* were only observed in Loumbila reservoir. *Acanthogyrus tilapiae, Clinostomum* sp. and *Myxobolus tilapiae* were found in both reservoirs with a high abundance.

P-046

PARASITES OF *JENYNSIA MULTIDENTATA* (CYPRINODONTIFORMES: ANABLEPIDAE) FROM TWO ESTUARIAL ENVIROMENTS OF BUENOS AIRES PROVINCE, ARGENTINA

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The aim of this work was to describe and compare the parasites of *Jenynsia multidentata* collected in two estuarial environments of the Buenos Aires Province, Argentina. The Salado River Channel (Samborombón Bay) and the Sauce Chico River near the Bahía Blanca estuary. Fishes were grouped together according to their sex and age. Parasites were studied alive and fixed with heat without pressure under slides and cover slips, then conserved in 10% formalin, stained and mounted in Canada balsam. We calculated the prevalence, median intensity and abundance. Sixteen species of parasites were found: one adult digeneans (*Lecithaster confusus*); eigth metacercariae (*Ascocotyle (P.) diminuta*, *Ascocotyle (P.) angeloi*, *Ascocotyle (P.) felippei*, *Ascocotyle (P.) hadra*, *Pygidiopsis macrostomum*, Hemiuridae gen. sp., *Thylodelphys* sp., Echinostomatidae gen. sp.); one metacestod (Cyclophyllidea) , two larval nematods (L4 Order Spirurida and *Contracecum* sp.), one monogenean (*Gyrodactylus* sp), two copepods (*Ergasilus sieboldii* and *Lernaea cyprinacea*) and one acanthocephalan (*Wolffhugelia matercula*). The high number of larval stages is in relation with the role of this fish as a link with the plankton species and upper stages in the food chain. The species number and abundance of parasites was bigger in Sauce Chico, this was probably for an increase in the ratio of encounter between parasite and fish due to the smaller size of this water body. We observed niche differentiation (or differential susceptibility) between males and females depending on the environment and the parasite considered.

P-047

STRUCTURE OF PARASITIE FAUNA IN TWO NATIVE FISH SPECIES FROM THE AGUAPEÍ RIVER, UPPER PARANÁ RIVER, BRAZIL

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The Neotropical region is known for the high diversity of freshwater fishes, with approximately 6,025 species. The Aguapeí river is an aquatic ecosystems with a fish fauna comprising over 310 species, including the two native species, *Auchenipterus osteomystax* and *Parauchenipterus galeatus* (Siluriformes: Auchenipteridae), which exhibit biological and ecological similarities. This study aims to survey the parasite fauna of these hosts and the influence of the flood pulse imposed by the environment on the structure and composition of their parasite faunas. The results were compared with previous studies carried out in the same hosts from the Upper Paraná River floodplain. Fish specimens (n = 112) of *A. osteomyxtax* (n = 59) and *P. galeatus* (n = 53) were analyzed. We collected 7,282 parasite specimens, of which 1,556 in *A. osteomyxtax* and 5,726 in *P. galeatus*. The higher quantity of parasites in *P. galeatus* was related to the presence of digenetic metacercariae in the eyes of these hosts, in which 3,201 individuals were recovered. More than 70% of the parasites (5,217) were present in the hosts collected during January 2014, indicating a likely effect of the rainy season on the parasites. *Auchenipterus osteomystax* and *P. galeatus* shared the digenetic *Microrchis oligovitelum* and eye metacercariae. The host populations from the Aguapeí River and from the Upper Paraná River floodplain present parasitic similarity. All parasites collected are new location records, and some these are new species, increasing the knowledge about the diversity of Neotropical fish parasites.

Financial Support: FAPESP (2013/25786-7), CNPq (307808/2014-9).

P-048

PARASITES OF *SATANOPERCA PAPPATERRA* (CICHLIDAE) IN A BIOLOGICAL RESERVE IN SOUTHEASTERN BRAZIL

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Parasites are extremely diverse and have key roles in ecological processes in several ecosystems. Although many studies on fish parasites have been realized in the last years in Brazil, there are still many freshwater fish species unexploited, especially in relation to forage species. We therefore conducted a study to investigate the parasite fauna of an important forage fish species, *Satanoperca pappaterra*, in a biological reserve in restoration process. Fish were collected on the rainy (January 2014) and dry (June 2014) seasons in ponds in the Biological Reserve of the Foz do Rio Aguapeí, Southeastern Brazil. Once collected, fish were weighed (g), measured (cm) and dissected in the field to obtain the parasites. We tested whether differences between seasons had an effect on parasite abundances but no significant statistical association was found, according to an analysis of covariance (ANCOVA, $F_{4,55} = 1.73$; $p = 0.15$). In contrast, we observed a positive correlation between the standard length of *S. pappaterra* and abundance of metacercariae of *Clinostomum* sp. ($r = 0.397$; $p = 0.0014$). A potential explanation for the association between length and this digenean larval stage is that the increase of the surface area of the body influenced the increment in the abundance of this parasite with the advance of the host's age. Our result suggests that *S. pappaterra* has an important role in the transmission of *Clinostomum* sp. in ponds and lotic rivers in Brazilian freshwater ecosystems

Financial Support: FAPESP 2012/09771-7.

A NEW SPECIES OF *ECHINOBOTHRIUM* VAN BENEDEN, 1849 (CESTODA: DIPHYLLIDEA), PARASITE OF *RAJA MIRALETUS* (ELASMOBRANCHII: RAJIDAE) OFF ALGERIAN COASTS

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A new species of diphyllidean cestode, *Echinobothrium* n. sp., is described from the spiral intestine of *Raja miraletus* Linnaeus collected off the Algerian coasts near Algiers. The four closest species of *Echinobothrium* Van Beneden, 1849 are *E. longicolle* Southwell, 1925 from *Dasyatis kuhlii*, *E. pigmentatum* Ostrowski de Nurfez, 1971 from *Zapteryx brevirostris*, *E. elegans* Tyler, 2001 from *Taeniura lymma*, and *E. hoffmanorum* Tyler, 2001 from *Urobatis maculatus*. *E. algeriensis* n. sp. is similar to these four species in possessing 19 large rostellar hooks but differs from them by the hook arrangement, number of lateral hooklets, morphology and number of cephalic peduncle spines, and number of testes per proglottids.

BARCODING OLD PARASITES – HIGH LEVEL OF VARIATION WITHIN THE GYROCOTYLIDS

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The Gyrocotylidea are parasites in the spiral valve intestine of cartilaginous fish (order Chimaeriformes). They may be the earliest divergent lineage among the tapeworms, and their association with the Chimaeriformes may go as far back as 350 - 420 Ma. Three taxa of gyrocotylids have been described from the North East Atlantic *Chimaera monstrosa*; *Gyrocotyle urna*, *G. confusa* and *Gyrocotyloides nybelini*.

Colin, Williams, and Halvorsen (1986) examined Gyrocotylid material from *C. monstrosa* caught off the Norwegian coast to evaluate characters previously applied by other authors when describing the three species from *C. monstrosa* and six other species of *Gyrocotyle*. The material included living worms, non-relaxed and relaxed (using various anaesthetics), worms killed in different fixatives, and deep frozen worms. They concluded that the morphological characters applied to gyrocotylid systematics were of little value for identifying *G. urna* or to distinguish it from the other species. No clear and undisputed morphological differences were identified, and they therefore synonymised *G. confusa* and *Gyrocotyloides nybelini* with *G .urna*. Later, Berland, Bristow, and Grahl-Nielsen (1990) analysed the chemometry of the fatty acids of *G. urna*, *G. confusa* and *G. nybelini* and four gyrocotylids from the Pacific ratfish *Hydrolagus colliei*. Based on the results they suggested that all species could be completely separated.

To elucidate the taxonomic status of the gyrocotylids infecting *C. monstrosa* we sequenced mitochondrial (CO1) and ribosomal markers (18S and 28S) from a large number of specimens and recovered several phylogenetically supported groups/species and a high level of molecular variation.

P-051

REVISION OF *ANISAKIS* SPP. OF INDONESIAN WATERS

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The nematode genus *Anisakis* parasitizes marine mammals, fishes and squids. Some species cause zoonotic diseases, triggering severe pathological effects such as gastro-intestinal inflammation or life-threatening anaphylactic shocks. DNA analyses showed that the genus consist of two “clades” and different “complexes”: clade one includes *A. typica* and *A. ziphidarum*, and three siblings forming the *A. simplex* (*sensu lato*) complex (*A. simplex* (*sensu stricto*), *A. pegreffii*, *A. simplex* C). The second clade consists of three species forming the *A. physteris* (*s.l.*) complex (*A. paggiae*, *A. brevispiculata*, *A. physeteris* (*s.s.*)). Additionally, siblings such as “*Anisakis* HC-2005” are reported, indicating taxonomic complexity. Another sibling was reported from Indonesia in 2008, distinguishable from *A. typica* by 4 base pairs in the ITS-1 region, similar to the distance between *A. simplex* (*s.s.*) and *A. simplex* C (4 bp). Until today, this Indonesian *A. typica* was recorded as *A*. sp. 1/I/2/II in four further publications, all from Indonesian and surrounding waters. We analyzed all genetically identified *Anisakis* spp. (ITS region) from Indonesia (233 individuals, mostly sampled in the present study) and GenBank, and present an overview of their identities (81 % are the Indonesian *A. typica*), host distributions and records from 53 teleosts. *A. simplex* C and *A. pegreffii* are documented for the first time between Tropic of Cancer and Capricorn.

P-052

PROTEOMIC ANALYSES OF SOFT TUNIC IN ASCIDIAN *HALOCYNTHIA RORETZI* INFECTED WITH A KINETOPLASTID, *AZUMIOBODO HOYAMUSHI*

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Soft tunic syndrome, an infectious disease of the ascidian *Halocynthia roretzi*, causes mass mortality in the aquaculture in Japan and Korea. We isolated a kinetoplastid from diseased ascidian, and identified it as a new species, *Azumiobodo hoyamushi* belonging to the order Neobodonida (Euglenozoa: Kinetoplastea).

In this study, we carried out proteomic analysis for tunic protein to identify the protein(s) involved in tunic softening. Healthy and soft tunics were employed for the proteomic analyses. Trypsin digested tunic peptides were analyzed by LC-MS/MS. The data was subjected to MASCOT program and BLASTX.

From the results of proteomic analysis, some decreased proteins were detected in the softening tunic. Among top-seven decreased proteins, five proteins were annotated to be myofibril-related proteins such as actin, myosin, calponin and HR-29 by BLASTX. Since protein function in tunic is less well understood, it is difficult to explain the relationship between tunic softening and decrease of these proteins. Here, we focused on the most decreased protein, HR-29 because the protein is suspected to stabilize the fibrillar structure. The degradation of synthetic HR-29 by extracellular proteases of the parasite, and localization and changes of the protein in the softening tunic will be shown in this presentation.

P-053

STRAIN IDENTIFICATION OF *SAPROLEGNIA PARASITICA* IN DISEASED FISH POPULATION

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Oomycetes represent a ubiquitous group of eukaryotic organisms. They are common inhabitants of terrestrial and aquatic environments and include saprophytes as well as pathogens living mainly in wet soils or freshwater environments. Oomycetes have been traditionally considered as fungi because of their morphology (mycelial growth), ecology and they also share the same nutritional mode. Several species have developed parasitic lifestyles and belong to the most devastating organisms on farmed and wildlife population of plant and aquatic animals. One representatives of parasitic water mold with the impact on aquaculture and animal health is *Saprolegnia parasitica.* At the beginning of our research we had diseased fish with unknown reason because abnormally high mortality was observed among fish in rivers Doubs and Loue in 2009. The most affected fish were trouts (*Salmo trutta*) and graylings (*Thymallus thymallus*). All affected individuals have shown similar symptoms: whitish spots or patches (mycoses) on the different parts of their bodies. Initial findings isolated a species of the genus Saprolegnia from lesions of diseased fishes mainly *Thymallus thymallus*. And also in 2011 fish with the same symptoms were observed in Sorne, totally disconnected from the river Doubs. Molecular studies using Internal Transcribed Spacer region sequencing (ITS-rRNA) show that this species is closely related to the complex *S. diclina/S. parasitica* and *S. salmonis* (hereafter *Saprolegnia parasitica s.l.*). These results indicate the extension of the disease that warrants immediate large scale environmental survey of Swiss rivers and aquatic ecosystems to prevent the destruction of these fragile ecosystems.

P-054

MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF AN AFRICAN FRESHWATER FISH TRYPANOSOME, INCLUDING ITS DEVELOPMENT IN A LEECH VECTOR

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Trypanosomes are ubiquitous blood parasites of fishes and at least 13 species were originally described infecting African freshwater fishes. This number was later reduced to three and in the late 1990’s it was proposed that most records of freshwater fish trypanosomes across Africa might be *Trypanosoma mukasai* Hoare, 1932. Recently, results from a molecular analysis of fish trypanosomes from the Okavango Delta, Botswana reported the presence of at least two genotypic groups and concluded that the identification of *T. mukasai* remains problematic. The aims of the present study were thus to elucidate the life cycle of the freshwater fish trypanosome from southern Africa and to do a morphological and molecular characterisation of the parasites from both the fish host and leech vector. To locate trypanosome stages, leeches were removed from fishes captured in the Phongolo River, South Africa, and fish blood films and leech squashes were Giemsa-stained and screened. To determine whether trypanosome stages in fishes and leeches were of the same genotype, DNA was extracted and fragments of the 18S rRNA gene were amplified and sequenced. Trypanosomes were detected in the fish families Cichlidae, Clariidae, Mochokidae and Schilbeidae. Sequence data showed that trypanosomes from leeches, identified as *Batracobdelloides tricarinata* (Blanchard, 1897), were identical to those obtained from fish. This paper presents the first completely life cycle of a freshwater fish trypanosome from southern Africa as well as the first study to link the vertebrate hosts and vector of an African freshwater fish trypanosome by molecular means.

P-055

MOLECULAR CHARACTERIZATION OF THE CILIATE *TRICHODINA RECTUNCINATA* (CILIOPHORA: TRICHODINIDAE), PARASITE OF MARINE FISHES IN SEVERAL LOCALITIES IN MEXICO

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Among the fish parasites, the ciliated protozoans are considered as one of the most common. The family of ciliates with the highest number of parasitic species is Trichodinidae family. The species *Trichodina rectuncinata* is one of the most widely distributed among trichodinids, and has been frequently recorded parasitizing gills of numerous marine fish species. The morphological description is an important part in the characterization of a species, however, the use of molecular tools integrated to the taxonomy of parasites have evidenced the presence of cryptic species in diverse groups of parasites. Since the species *T. rectuncinata* has been recorded from numerous localities around the world, showing very low host specificity, we consider that the different populations could be recognized as cryptic species. The aim of this study is to evidence the genetic diversity in some populations of *T. rectuncinata* geographically distant, based on the analysis of18s rRNA genes. Specimens of some host fish species were collected and examined for trichodinid parasites. A few individuals were isolated in a PCR tube. DNA was extracted using REDExtract-N-AmpTM tissue PCR Kit. The genes were amplified by PCR with the Universal Eukaryotic Primers. The nucleotide sequences were analysed by Maximum likelihood and Bayesian Inference in RaxML and Mr. Bayes respectively. Our data suggest the presence of cryptic species based on the genetic divergence found. Further analysis integrating molecular and morphological approaches are suggested to confirm the specific status of each analysed population.

P-056

GENETIC DIFFERENCES AMONG GEOGRAPHICALLY DISTINCT *ICHTHYOPHTHIRIUS MULTIFILIIS* ISOLATES

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Ichthyophthirius multifiliis Fouquet 1876 (Ciliophora), commonly known as Ich, is the highly pathogenic fish endoparasite responsible for white spot disease which causes severe declines among natural fish populations and significant economic losses in freshwater aquaculture. The cosmopolitan parasite has an extremely broad host range, which involves exotic fish species as well. A severe infection, particularly of the gills of fish fry, often results in asphyxiation and death. Fish that recover from infection usually develop acquired immunity for a certain period. Previous studies identified a family of abundant glycosylphosphatidylinositol-anchored membrane proteins (immobilization antigens, or i-antigens, IAG) that are important targets of the host immune response. Genomic studies detected high variations in the expression of IAGs, and indicated that intra- and intergenic recombination might play a role in the evolution of these genes. In the present study, we genetically characterized and compared *I. multifiliis* isolates originating from natural fish populations, from various farmed fish species and from common ornamental fish species. The partial DNA sequences of the mitochondrial cytochrome c oxidase subunit 1 (COI) and the IAG genes (among others IAG48 and IAG52) encoded in the genome of the somatic macronucleus were amplified. The DNA sequence comparison revealed notable differences among geographically distinct isolates, while the most common genotype was found both in isolates from ornamental and farmed fish (with 100% sequence identity for COI). The relatively high variability found among IAG sequences seems to confirm the theory that a rapid genetic drift influences the evolution of IAGs.

Financial support: AQUAFUTURE project (VKSZ_12-1-2013-0078).

P-057

SHOCKING HOST REVEALS NEW GENUS AND SPECIES OF HETEROCOTYLINAE (MONOGENEA)

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A new genus and species of Heterocotylinae (Monogenea: Monocotylidae) is proposed from off the gills of a Onefin electric ray (*Narke capensis*) collected for public exhibition for Two Oceans Aquarium in Cape Town, South Africa. All representatives of Heterocotylinae are gill parasites of stingrays, guitarfishes or sawfishes. The majority of these monogeneans are found in marine waters and at least one known representative is considered pathogenic on hosts kept in captivity. During a mandatory quarantine period the ray exhibited laboured breathing and refused all food offered. A monogenean infestation of the gills was suspected and confirmed using a non-invasive sampling technique. An unknown heterocotyline was observed that could not be placed into any of the known genera representing the Heterocotylinae, or Decacotylinae. The new taxon is the first monogenean reported from *Narke capensis* and is morphologically similar to members of *Heterocotyle* and *Potamotrygonocotyle*, but differs from them by the absence of any sclerotisation of the dorsal haptoral accessory structures, the presence of sclerotised walls of the vagina, and the hamulus and egg morphology. The absence of sclerotisation of the dorsal haptoral accessory structures is significant because this feature was previously only reserved for *Decacotyle* species. The new taxon differs from *Decacotyle* species in the number of haptoral loculi and the presence of deep longitudinal grooves on the sclerotised male copulatory organ. These grooves were previously only recorded from representatives of *Dasybatotrema* (Dasybatotreminae). A phylogenetic analysis showing the placement of the new genus will be presented.

P-058

AFRODIPLOZOON KHOTENOVSKY, 1981 (MONOGENEA: DIPLOZOIDAE): REVISION OF THE GENUS USING MULTIDISCIPLIONARY APPROACH

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The genus *Afrodiplozoon* was proposed by Khotenovsky in 1981 when *Afrodiplozoon polycotyleus* (Paperna, 1963) has been exluded from the genus *Neodiplozoon* Tripathi, 1959 based on the number of clamps on the attachment apparatus. Since then, the inconsistency in the use of the name for this parasite can be seen in the literature.

Specimens of *A. polycotyleus* were collected during a fish survey carried out in April and July 2014 in the Venda region, Limpopo Province, South Africa, from *Labeobarbus maraquensis* (n=36; mean total length=7.9 cm) and *Barbus paludinosus* (n=1; total length=6.6 cm). A prevalences of 64% and 59% were recorded for *A. polycotyleus* during April and July, respectively. Morphological analysis of the composition of the internal organs and attachment clamps using different microscopic methods (light microscopy of stained/unstained specimens and scanning electron microscopy, SEM) provided details for the redescription of the genus. The type material was also studied. Molecular characterization based on the variabily of the second internal transcribed spacer (ITS2) rDNA showed the taxonomic relationship to other representatives of Diplozoidae.

Parasites can bear asymetrically from 7 up to 10 clamps in one row on each side of the attachment apparatus, with the first clamp is significantly smaller. The connection sclerite of posterior end of the central plate is wide in all its length. The anterior end of the central plate has short processes. The SEM observations showed the presence of numerous papilae around the mouth of the worm.

P-059

A NEW SPECIES OF *TRILOCULOTREMA* KEARN, 1993 (MONOGENEA: MONOCOTYLIDAE) FROM THE NASAL FOSSAE OF *MUSTELUS PUNCTULATUS* FROM OFF TUNISIA

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Examination of the nasal fossae of the Smooth hound *Mustelus punctulatus* Risso,1826 from the gulf of Tunis (Central Mediterranean) revealed a new Merizocotylinae (Monogenea : Monocotylidae) belonging the genus *Triloculotrema* Kearn, 1993. Until now there are two described species in this genus: *T. japanicae* Kearn, 1993 from *Hemitriakis japanica* (Muller & Henle, 1839) and *T. chisholmae* Justine, 2009 from *Squalus melanurus* Fourmanoir & Rivaton, 1979. We describe here the third species. *Triloculotrema* n. sp. is easily distinguished by the morphology of the sclerotised male copulatory organ with longitudinal ridges and slightly curved (*versus* without any ridges and straight in *T. chisholmae* and curved in *T. japanicae*). The body size, ((3700-5700 µm) long and (600-1450 µm) wide), is distinctively bigger than those of *T. chisholmae* ((990-1260 µm) long and (350-675 µm) wide) and *T. japanicae* ((2830-3110 µm) long and (350-675 µm) wide). The morphology of the hamuli is variable and the presence of the three loculi in the haptor is questionable. *Triloculotrema euzeti* is the first species of the genus reported from the Mediterranean.

P-060

MOLECULAR AND MORPHOLOGICAL EVIDENCE FOR THE DESCRIPTION OF THREE NEW *GYRODACTYLUS* SPECIES (MONOGENEA:GYRODACTYLIDAE) PARASITIZING CICHLIDS IN SOUTHERN AFRICA

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Viviparous monogeneans belonging to family Gyrodactylidae Cobbold, 1864 are microscopic ectoparasitic flatworms with direct and extremely short life cycle. In Africa, 48 species from five genera parasitizing hosts belonging in 14 families, including freshwater fishes and frogs, have been recorded. The highest number of *Gyrodactylus* species has been described from hosts from Clariidae Bonaparte, 1846 and Cichlidae Heckel, 1840. Fishes from Cichlidae are a highly diverse group within the African bony fishes and have noteworthy economic importance especially on local scale. The present study deals with the parasites of the genus *Gyrodactylus* parasitizing cichlids collected in Zimbabwe and South Africa. Fishes were sampled from Nwanedi River in South Africa and at three localities in Zimbabwe; Zambezi River, Lake Kariba and Lake Chivero. Results from analyses of metrical parameters and shape of hard parts of attachement organ supported by molecular sequences of rDNA ITS fragment were compared with those available in the nucleotide database. The comparison revealed the presence of three new *Gyrodactylus* species. *Gyrodactylus* sp.1 was observed on gills of *Coptodon rendalli* and *Pseudocrenilabrus philander*. *Gyrodactylus* sp. 2 was found on fins of *Oreochromis niloticus*, *Pharyngochromis acuticeps* and *P. philander*, and *Gyrodactylus* sp. 3 was identified from *P. philander*, *O. niloticus* and *Tilapia* sp. Space visualization of individuals based on the combination of 12 metric parameters using principal components analyses shown differences between species. Phylogenetic positions based on molecular sequences applying Bayesian Interference and Neighbour Joining evaluated positions of new species within the clusters of *Gyrodactylus* species of African cichlids.

P-061

A NEW SPECIES OF *ANACANTHORUS* (DACTYLOGYRIDAE) PARASITIC ON *MARKIANA NIGRIPINNIS* (CHARACIDAE) FROM THE PANTANAL, BRAZIL

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Anacanthorus was proposed to accommodate three species that shared absence of dorsal and ventral anchors and bars, and actually comprises 70 species parasitic on Characiformes fishes from the Neotropical region. *Markiana nigripinnis* is a small benthopelagic fish with known distribution in Paraguay and Paraná River basins, and thus far, there is no record of parasites from this fish species. Between December 2013 and September 2014, 57 specimens of *M. nigripinnis* were caught in a perennial lagoon (19°34,576' S, 057°00,823' W) from the Pantanal floodplain, Sate of Mato Grosso do Sul, Brazil. Gills were removed and observed under a stereomicroscope. Monogeneans were fixed in 5% hot formalin, and some specimens were mounted in Grey and Wess's medium whereas others were stained with Gomori's trichrome and mounted in Canada balsam. Measurements, in micrometers, are followed by the range within parentheses. Brief description: body 545.21 (314.65-1034) long, fusiform, maximum width 130.85 (87-155); haptor 54.4 (37.6-84.7) long, 80,2 (46-114.9) wide; hooks 25.9 (25-27) long; dorsal and ventral pairs of 4A hooks 10.2 (8-12.3); MCO 73.6 (57.7-106.6) long, slight curved; accessory piece 65.4 (47-106.6) long, proximally articulated to MCO, bifurcated, one branch longer and another smaller slightly sigmoidal branch, both distally pointed and presenting a hatchet-like projection on the medial portion. This species is most similar to *Anacanthorus dipelecinus* mainly because it shares a bifurcated accessory piece with hatchet-like processes in the branches. However, the *Anacanthorus* species from *M. nigripinnis* do not present terminal spines in each branch of its accessory piece like *A. dipelecinus*.

P-062

A NEW SPECIES OF MEXICANA FROM *ORTHOPRISTIS RUBER* (CUVIER, 1930) FROM THE BRAZILAN COAST OFF RIO DE JANEIRO

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The genus *Mexicana* was described by Caballero & Bravo-Hollis, 1959 from a Sciaenid fish from Mexico. The genus is mainly characterized by having 3 head organs, bi-lobed haptor with 2 pairs of anchors, 2 pairs of sclerotized plates, 2 dissimilar bars (the ventral one with wing-like projections). During studies on the monogeneans of corocoro (*Orthopristis* ruber) captured off Urca area, Brazilian coast of Rio de Janeiro, specimens of a different *Mexicana* species were collected from the gills. The worms were fixed and preserved in 70% ethyl alcohol, cleared in Gray & Wess and alternatively stained in Gomori's Trichrome or Mayer's paracarmine. The illustrations were made with the aid of a drawing apparatus and finalized with Adobe Illustrator. The morphological study by light microscopy revealed it to be a new species that can be differentiated from its congeners by the size and shape of the sclerotized male copulatory organ, shape of pharynx and the sclerotized structures of the haptor.

P-063

NEW SPECIES OF PARASITE FAUNA OF SANTER SEABREAM *CHEIMERIUS NUFAR* (VALENCIENNES, 1830) (SPARIDAE)

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The Sultanate of Oman is set to establish an aquaculture industry which initiates an intensive research project in Oman in 2012 aiming to study the parasites fauna of several fishes which are considered as promising targets for aquaculture. *Cheimerius nufar* being among selected fish species. The parasite fauna of *C.nufar* is one of the least studied among other species of seabreams (Bray,1986). Our investigation resulted in recording of 23 parasites. Some of them were already described as new species to science. This paper describes a new monogenea species from gills of *C.nufar*.
Aim: Detection parasites that are potential harmful to *C.nufar* farming.
Methods: 15 specimens of *C.nufar* sampled from Shuwaymiyyah (Dhofar Governorate, Oman) were examined.
Results: In one fish a new monogenean was found, 27 morphological parameters were obtained to describe this new spp. The structure of the atrium revealed is consistent with monogenea of the genus *Lutianicola* Lebedev, 1970 (Microcotylidae Taschenberg, 1870). Mamaev (1990) described two new microcotylid species of *Lutianicola vittae* Mamaev, 1990 and *Lutianicotyle indica* Mamaev, 1990 from the *Lutjanus vitta*, *L. fulviflammus, L. malabaricus* and *L. sanguineus,* respectively*.* Investigation of the descriptions of three detected specimens showed the similarity of the monogenean species in *C. nufar* with the atrium structure and shape, number of hooks to *Lutianicola haifonensis*. However, some morphological and anatomical differences were also observed suggesting that monogenea of the *C. nufar* could be a new species.
Conclusion: Genus *Lutianicola* are exclusively found in Snappers (Lutjanidae), however newly discovered in fish of another family.

P-064

THE TAXONOMY OF *MICROCOTYLE ERYTHRINI*: NEW CONSIDERATIONS FOR MICROCOTYLID MONOGENEANS

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Microcotylidae is a large monogenean family recurrently revised in the past century. Many species from the type-genus *Microcotyle* were transferred to new genera; however, the validity of many species of this genus is also often doubtful. One of the main problems that taxonomists must face is that many species of this group are often characterized by quantitative traits such as the number of clamps or testes. Ranges of numeric traits are often close, especially when ranges are increased in descriptions in new host records. Moreover, descriptions are based on the traditional two-dimensional representations of mounted specimens that impede to appreciate differences only visible in 3D (SEM, confocal, *in vivo* studies). *Microcotyle erythrini* is reported in several sparid fishes in the Mediterranean. We have performed a detailed morphological and molecular analysis on specimens collected from *Boops boops* and *Pagellus erythrinus* in order to assess the validity of the species *M. erythrini*. SEM observations revealed that the body is perpendicularly connected to the haptor, a previously undescribed morphological characteristic and most likely typical of the genus. This feature together with a number of other morphological features differs among the specimens found in the two hosts, suggesting the presence of two species of *Microcotyle*. The molecular analyses based on cytochrome *c* oxidase subunit 1 (*cox*1) mitochondrial gene confirmed the existence of two different species.

Partially funded by projects UV-INV-PRECOMP14-206687, Fundación Biodiversidad, PROMETEO 795 2015-018 and ISIC/2012/003.

P-065

DACTYLOGYRIDS (MONOGENEA: DACTYLOGYRIDAE) OF CATFISHES (SILURIFORMES) FROM SUDAN

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Survey on dactylogyrids parasitizing catfishes was carried out in White Nile and Blue Nile, Sudan, in 2014. Except this study, no records about monogeneans from this country exist. Dactylogyrids were isolated from 12 siluriform species, belonging to six families (Bagridae, Clariidae, Claroteidae, Malapteruridae, Mochokidae and Schilbeidae). Dactylogyrid species of the following genera were found: *Bagrobdella, Protoancylodiscoides*, *Quadriacanthus*, *Schilbetrema* and *Synodontella*. The taxonomic evaluation has been made on the basis of morphology, and also the partial 28S rDNA (LSU) sequences have been analysed for a purpose of species identification and evaluation of the phylogenetic positions of representatives of dactylogyrids infecting African catfishes wihin the family Dactylogyridae.

We acknowledge the financial support from ECIP – Centre of excellence, Grant Agency of the Czech Republic, No. P505/12/G112.

P-066

SCLERITE COMPARISON OF DIPLOZOIDAE SPECIES USING SEM

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As with most of the Monogenea, the sclerite structures of Diplozoidae are essential for the study of their taxonomy. However, these sclerites are situated within the tissue of the haptor and visibility with light microscopy can be problematic. In a recent study, the tissue was digested away from the sclerites of *Paradiplozoon vaalense* to expose the sclerites for scanning electron microscopy (SEM). To expand on the application of this technique, a comparison of the sclerites of *P. vaalense* and two other diplozoid species was performed. The two species were *Paradiplozoon ichthyoxanthon*, a recently described African species, and *Diplozoon paradoxum*, the type species for the Diplozoinae. A haptor from each individual parasite pair was removed, halved and digested on separate concavity slides. The digested tissue was used for molecular analysis, while the remainder of the parasite body was prepared for light microscopy. Much of the clamp ultrastructure for these species was very similar, with only certain features showing variation. For example, the trapezoid projection of the anterior end of the median sclerite in *P. vaalense* was shallowly forked to truncate, in *D. paradoxum* the projection was more sharply forked while in *P. ichthyoxanthon* there are fragile lobed ends to the projection. The results obtained match the morphology seen in light microscopy, but bring a new dimension to the study and understanding of these structures.

Funding from National Research Foundation and University of Johannesburg is acknowledged.

P-067

MICROSATELLITE MARKERS FOR IMPROVED RESOLUTION OF STRAINS OF *GYRODACTYLUS SALARIS*

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Previous studies on the Norwegian epidemic of *Gyrodactylus salaris* using mitochondrial cytochrome oxidase 1 markers found three haplotypes (A, B and F), each unique to each historically known introduction route. Later, a fourth route of introduction was also found. Here, the Norwegian epidemic of *G. salaris* on susceptible Atlantic salmon was revisited by investigation of 10 nuclear microsatellite loci in order to refine the division of the mitochondrial matrilines.

The previous mtDNA analyses found that all parasites from populations/infected rivers originating from the main introduction to Norway in 1975 (Norwegian main epidemic, NME) carried only one invariable haplotype, the haplotype A. The microsatellite loci of all specimens with this haplotype from several infection regions were almost invariable and confirmed that this lineage was indeed a clone of single origin, imported to Norway via stocking.

The introduced parasites in two northern rivers were characterized as haplotype B. However, the nuclear genome consisted of alleles of two different origins, recognized as haplotypes B and A, suggesting that this strain originated from a hybridization between these two haplotypes on Atlantic salmon.

The third introduction to Atlantic salmon in Norway carries the mtDNA-F, a strain common for *G. salaris* on rainbow trout in Northern Europe. The rainbow trout is no longer stocked in Norway, but our analyses show the impacts of historical farming. Based on the msat analyses, the non-pathogenic strain on rainbow trout seems to have hybridized with NME and created a pathogenic mtDNA-F strain. Thus, rainbow trout was acting as a vector.

P-068

GENETIC CO-STRUCTURE IN THE PARASITE-HOST SYSTEM *ZEUXAPTA SERIOLAE* AND *SERIOLA LALANDI* FROM THE SOUTHEASTERN PACIFIC OCEAN

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Information about the parasite population genetic structures is important for an understanding of parasite dispersion and ultimately the co-evolution with their hosts. The genetic structure of parasite populations depends on the intrinsic host-parasite relationship and environmental conditions. However, parasites often have shorter generation times and, in some cases, faster mutation rates than their hosts, which can lead to greater population differentiation in the parasite relative to the host. The monogenean, *Zeuxapta seriolae* is a host-specific parasite with extensive geographical distribution on its host, *Seriola lalandi*, which is a highly migratory pelagic fish. In this study, the population genetic structure of *Z. seriolae* and *S. lalandi* are compared to evaluate factors that affect parasite gene flow. Wild fish and their parasites were sampled from two geographical fishery areas: northern Chilean coast (NCC: 20°S to 30°S) were *S. lalandi* arrives annually in the summer; and oceanic island (JFA: 80°W) distant about 700 km from continental Chile where fish are present permanently. Fragments between 700 and 816 bp of the cytochrome *c* oxidase subunit I (COI) were sequenced for 212 fish and 175 parasites between 2012-2014 and compared using AMOVA. Genetic diversity, demographic history and mismatch distribution were calculated for the host and parasite. Geographical genetic structuring was detected in *Z. seriolae* but not in the host *S. lalandi*. However, temporal genetic structuring was detected in the host. These different genetic patterns for this host-parasite system suggest that host behavior can play a role in the geographic structure detected by this parasite.

Financial-support: 1130629.

P-069

EUDIPLOZOON NIPPONICUM (MONOGENEA): CHALLENGE FOR NEXT-GENERATION SEQUENCING

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Eudiplozoon nipponicum (Polyopisthocotylea: Diplozoidae) is a haematophagous ectoparasite from the gills of the cyprinid fish *Cyprinus carpio*. The properties of genes (e.g. identity, structure, evolution) and proteins (e.g. function) of monogeneans are among the less investigated in the phylum Platyhelminthes. Up to now only two genomes and one transcriptome of monogenean species have been sequenced: the genome of *Protopolystoma xenopodis* (Polyopisthocotylea) and *Gyrodactylus salaris* (Monopisthocotylea) and the transcriptome of *Neobenedenia melleni*. Therefore we adopted the Illumina MiSeq/HiSeq techniques for sequencing the genomic DNA and RNA isolated from adult worms of *E. nipponicum.* This was followed by comprehensive bioinformatic analysis of sequence data.

Acknowledgements: Czech Science Foundation (P506/12/1258, GBP505/12/G112), grants of the Masaryk University (MUNI/A/1484/2014) and grants of the Charles University in Prague (UNCE 204017, PRVOUK P41, SVV260074/2014, GAUK 502313).

P-070

METAZOAN PARASITES OF *BRAMA AUSTRALIS* AS BIOLOGICAL TAGS FOR STOCK IDENTIFICATION IN SOUTHERN CHILE

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The "Southern rays bream" *Brama australis* is an important pelagic fish with landing of ca 12.000 ton (2013). Despite this importance, Chilean fishery regulations only assume that there is a single stock along the Chilean coast. Knowledge of stock structure and the degree of transfer among populations is crucial for the sustainable management of marine resources. Parasites as biological tags for stock identification purposes have recently been used successfully in both, pelagic and demersal Chilean fish. We studied metazoan parasites from 380 specimens obtained from three fishing ground in southern Chile (From Lebu: 37°40'S to Punta Arenas 53°S). We found a total of 12 species of metazoan parasites, being the copepod *Hatschekia conifera* the most prevalent parasite, accounting for more than 95% of the total metazoan parasite abundance. The second most important parasite was the larval cestode *Hepatoxylon trichiuri* (< 3.0%). Univariate and multivariate analysis (Correspondence analysis and Discriminant analysis) were unable to detect significant differences between metazoan parasites from the three fishing ground suggesting the existence of a unique stock of *B. australis* in the studied zone, but migratory movements from the southern area northward are not discarded.

P-071

PARASITES OF JUVENILE MEDITERRANEAN BLUEFIN TUNA *THUNNUS THYNNUS* L. AS TAGS TO IDENTIFY NURSERY AREAS

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The stock management of the Atlantic bluefin tuna (ABFT), *Thunnus thynnus*, is particularly difficult due to the complex biology of this migratory fish with a wide geographic distribution. Moreover this species is under a high fishing pressure as it is one of the most valued fish worldwide. During the first year of life ABFT are non-migrant, living in nursery areas that in the case of the Eastern Atlantic population is restricted to the Mediterranean. This is one of the most vulnerable and critical periods of tuna life, therefore it is crucial to obtain tools to characterize young-of-the-year (YOY) fish of each nursery area in order to develop adequate management strategies. The heads of 102 YOY specimens of ABFT from four nursery areas of the Mediterranean (Balearic, Ionian, Ligurian and Tyrrhenian Seas) were analysed for parasites during 2009–2013. A total of 11 parasite species was found: the monogeneans *Capsala magronum, Capsala onchidiocotyle, Capsala paucispinosa, Nasicola klawei, Hexostoma thynni* and the digeneans *Didymocystis* sp. 2 (*sensu* Rodríguez-Marín *et al*., 2008), *Didymosulcus* sp. 2 (*sensu* Rodríguez-Marín *et al*. 2008), *Didymosulcus wedli*, *Didymozoon pretiosus*, Nematobothriinae gen. sp. and *Wedlia* sp. The significant differences observed on prevalence and abundance of some food-borne parasites among localities suggests that parasites of ABFT could be used as biological tags to identify the different populations from the corresponding nursery areas.

JRL and JPA benefit of doctoral fellowships of the Spanish Government and the University of Valencia, respectively. Funded by projects AGL2010-20892, PROMETEO 795 2015-018 and ISIC/2012/003.

P-072

ARE FISH PARASITES USEFUL BIOINDICATORS OF THE "RESERVE EFFECT" IN THE MARINE PROTECTED AREA OF MEDES ISLANDS?

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Parasites are useful bioindicators of anthropogenic impacts such as pollution or overfishing, but also to assess the role of marine protected areas (MPAs) and effects of fisheries management on ecosystems. This study evaluates potential differences in metazoan parasite communities of *Diplodus sargus* (Sparidae) inside and outside the marine reserve of Medes Islands (NW Mediterranean), in order to explore the use of parasites as indicators of the “reserve effect”. In the framework of the project MAPUCHE, 54 specimens of *D. sargus* were captured outside (fished area), within the partially protected zone (buffer area) and inside the fully protected zone (no-take area) of the marine reserve. Fish specimens were captured with long line, gillnet and fishing rod by artisanal fishermen. Samples were gathered in winter, spring and summer in the fished and buffer areas and in spring in the no-take area (2008–2009). PERMANCOVA and GLM analyses of data among seasons revealed non-significant differences between fished and buffer area and an effect of season on parasite communities. Thus, pooled abundance data for spring of fished and buffer areas was compared with data from the no-take area. Multivariate analyses revealed a good differentiation of the parasite communities between the fished-buffer area and the no-take area. The higher abundance of the monoxenous parasites (*Lamellodiscus* spp.) agrees with previous studies indicating a higher density of *D. sargus* in the no-take area. Moreover, the homogeneous distribution of heteroxenous parasites in the no-take area reflects the already described site fidelity of this species.

P-073

ANISAKIS SIMPLEX COMPLEX (NEMATODA: ANISAKIDAE) IN ZOOPLANKTON COMMUNITIES FROM TEMPERATE NE ATLANTIC WATERS

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The euphausiid *Nyctiphanes couchii* and an unidentified mysid have been found, for the first time, with third-stage larvae (L_3) of the *Anisakis simplex* complex in the mesozooplanktonic community of the coastal upwelling system in Galicia (NW Spain). Parasite larvae were molecularly identified using the internal transcribed spacer (ITS) region. The prevalence of these parasites in the euphausiid population was 0.0019%. The existence of parasites in a variety of mesozooplankton organisms suggests that the transmission routes of *A. simplex* sensu stricto and *A. pegrefii* are wider than expected. The results suggest that these two *Anisakis* species are not specific to their intermediate hosts. Finally, the recruitment of *A. simplex* complex may be affected by oceanography, differing under upwelling or downwelling conditions.

P-074

RISK ANALYSIS OF HUMAN ANISAKIDOSIS THROUGH THE CONSUMPTION OF FRESH FISH SOLD AT SPANISH SUPERMARKETS – AN OVERVIEW

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Human anisakidosis is a serious health problem in Spain owing to the frequent consumption of fresh raw fish and some modes of preparation, which do not inactivate parasites. The aim of this study was to analyse the presence of anisakid larvae in fresh fish and to evaluate the risk for consumers. A total of 1786 fish specimens belonging to nine species frequently consumed in Spain (*Micromesistius poutassou*, *Merluccius merluccius*, *M. bilinearis*, *Mullus barbatus*, *Engraulis encrasicolus*, *Sardina pilchardus*, *Trachurus trachurus*, *Scomber scombrus* and *S. japonicus*), acquired at various supermarket chains across greater Valencia, were examined for anisakid larvae, both by visual microscopic inspection of the viscera and artificial digestion of the flesh. Prevalence and abundance of anisakids were registered, and the influence of origin, season of capture, body size and days of storage was analysed. All species were infected with anisakid larvae, which were morphologically classified as *Anisakis* spp., in all host species, while *Contracaecum* spp. occurred only in *M. bilinearis*. *M. poutassou*, *Merlucius* spp., *T. trachurus* and *S. scombrus* were the heaviest infected while *S. pilchardus* and *M. barbatus* presented the lowest infection level. Atlantic fish with a large size posed the highest risk; and the number of days of storage seemed to influence larval migration into the flesh in most fish species. The risk of infection can be minimized following the measures established by the European Commission Regulation 1276/2011 concerning the inactivation of viable parasites in fishery products.

ANALYSIS OF PARASITES AND MICROBIOLOGICAL ASPECTS OF FISH FROM OUAGADOUGOU MARKETS

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Fish is a source of digestible proteins, essential amino acids, polyunsaturated fat acids and vitamins. However, it is a food very perishable, which becomes rapidly inappropriate to consumption after the capture. The purpose of this study is to analyze the parasitological and microbiological quality of most currently consumed fish in Ouagadougou.

A total of 40 samples of fish have been analyzed from May to November 2013. The study method based on 3 points: an investigation conducted with fish sellers, aiming to evaluate handling hygienic conditions of fish; microbiological analysis, to determinate the microbiological quality of fish in total and faecal coliforms, *Staphylococcus aureus* , fungic flora, and to isolate *Salmonella sp*., *Shigella sp*. and *E. coli*; parasitological analysis, to research and identify fish helminthes.

Fish sellers are in majority illiterate, without any training in good hygienic practices. The consequences on microbiological level, is an abundance of *Staphylococcus aureus* mainly on smoked fish. 57,5% , 55% and 7,5 of samples have been found unsatisfactory in relation to *Staphylococcus aureus*, faecal coliforms and fungus. Besides, 1%, 15% and 25% of samples were positive in *Shigella sp*., *E. coli*, and *Salmonella sp*. Fried and braised fish were not contaminated in parasites, but, we noticed a massive presence of *Mucidae* larva on smoked fish, trematoda and some nematoda on fresh fish. Those results must draw the attention of responsible of medical structures, to bend over seriously on the questions of aliments sanitary monitoring, in order to guarantee the microbiological a parasitological quality of fish.

ANISAKIS SP. MIGRATION PROCESS AFTER DEATH IN *TRACHURUS TRACHURUS*

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The aim of this study is to describe the post-mortem migration of *Anisakis* sp. larvae in *Trachurus trachurus*, in order to establish the best hygienic procedure and prevent consumer health. We have analysed 50 individuals of the horse mackerel, *T. trachurus*, coming from Tarragona fishing port. During 7 days, each fish was examined in detail and all nematodes were localised. Fishes were kept in refrigerator and covered with ice until the next day. Each day we tried to localize excapsulated parasites.

We never found parasites encapsulated in the muscle in fresh fishes, but occasionally, some parasites seemed to encapsulate in this tissue after the death of the fish. We already observed penetration of some parasites in the flesh. Although encapsulation could not be completed after the fish death, this penetration of the parasite in the muscle must be considered as a possible transmission focus. The last day, after repeating the same process, parasites were removed and classified. All parasites were *Anisakis* sp. type I. For this reason, even if not encapsulated parasites were found in flesh, either fresh or death fishes, we recommend to eviscerate this fishes as soon as possible, in order to prevent this post-mortem *Anisakis* migrations.

P-077

BROAD FISH TAPEWORMS: DIFFERENT FISH HOST – DIFFERENT CAUSATIVE AGENT OF HUMAN DIPHYLLOBOTHRIOSIS

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Fourteen species of diphyllobothriid tapeworms have been reported to cause human diphyllobothriosis. Marine as well freshwater fishes of several orders represent source of human infections if eaten raw or undercooked. Cestode larvae named plerocercoids may be located in the body cavity, internal organs or in the musculature. Complete life cycles are known for three human-infecting species only, but the spectrum of the second intermediate, i.e. fish hosts is known also for the fourth most common causative agent of diphyllobothriosis, *Adenocephalus pacificus* (syn. *Diphyllobothrium pacificum*). This species matures in otariid seals and different groups of marine fish serve as its second intermediate hosts. Two freshwater species, *Diphyllobothrium dendriticum* and *D. latum*, use freshwater fishes as their intermediate hosts, namely coregonids, salmonids and sticklebacks in the case of the former species and pike, percids and burbot in the case of *D. latum*, which was also found in trouts, but exclusively in Chile. Pacific salmons of the genus *Oncorhynchus* are exclusive intermediate hosts for the marine *D. nihonkaiense*. The plerocercoids in fish are devoid of developed genital organs and thus provide only a limited number of morphological characteristics suitable for species differentiation. Despite this limitation, identification of plerocercoids of the above-mentioned four human-infecting species is possible based on their gross morphology (size and shape of the body, presence/absence of transverse wrinkles on the body, and degree of retraction of the scolex), the number of the longitudinal muscles (seen in histological sections), and the length and density of microtriches.

P-078

ANISAKIS INFECTION IN ALLIS SHAD, *ALOSA ALOSA* (LINNAEUS, 1758), AND TWAITE SHAD, *ALOSA FALLAX* (LACÉPÈDE, 1803) FROM WESTERN IBERIAN PENINSULA RIVERS: ZOONOTIC AND ECOLOGICAL IMPLICATIONS

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Spawning individuals of allis shad, *Alosa alosa* (Linnaeus, 1758), and twaite shad, *Alosa fallax* (Lacépède, 1803) were sampled from three rivers on the Atlantic coast of the Iberian Peninsula (Ulla, Minho, Mondego) during 2008 to 2013 to assess the presence of potentially zoonotic marine parasite *Anisakis* spp. larvae. The results revealed that both shad species were infected by third-larval stage *Anisakis simplex* s.s. and *Anisakis pegreffii*. The latter is reported in mixed infections in both shad species from the Western Iberian Peninsula for the first time. In *Alosa alosa* the prevalence of *Anisakis* infection can reach 100%, while in *Alosa fallax* prevalence was up to 83%. Infected individuals of the former species also show higher parasite intensities in the internal organs and flesh, ranging1 - 1138 *Anisakis* spp. larvae, as compared to 1 - 121 larvae in *A. fallax*. In general, numbers of *A. pegreffii* were higher than those of *A. simplex* s.s. Our results suggest that in the marine environment of the Western Iberian Peninsula both anadromous shad species act as paratenic hosts for *A. simplex* s.s. and *A. pegreffii*, thus widening the distribution of the infective nematode larvae from the marine to the freshwater ecosystem. This finding is of great epidemiological relevance for wildlife managers and consumers, considering the zoonotic and gastro-allergic threats posed of these parasites.

P-079

RISK ASSESSMENT OF HUMAN INFECTION WITH *ANISAKIS* SPP. IN THE EUROPEAN ANCHOVY AND SARDINE FROM THE EASTERN ADRIATIC SEA

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The consumption of thermally unprocessed or lightly processed traditional seafood represents a risk of anisakiasis, considered one of the most significant fish-borne parasitic infections in humans today. Thermally unprocessed or lightly processed anchovies (*Engraulis encrasicolus*) and sardines (*Sardina pilchardus*) are basic ingredients of numerous traditional Mediterranean dishes. Therefore, our objective was to genetically identify *Anisakis* spp. and determine its prevalence and intensity in the European anchovies (N= 785) and sardines (N=789) collected during 2 years from the eastern Adriatic Sea. The UV-Press method was used for visual inspection of flattened, deep-frozen fillets and viscera as it conveniently utilizes fluorescence of frozen anisakids. A subsample of isolated anisakid larvae was identified to species level using mitochondrial marker cytochrome oxidase 2 (CO2). Larvae isolated from anchovy and sardine confirmed their clustering within *Anisakis pegreffii* sister group, as usually recorded in the Adriatic Sea. The overall prevalence in the European anchovy was 29.70% (95% CI 26.56–32.98) and mean intensity 2.41 (bootstrap 95% CI 2.13–2.73) in contrast to 2.50% (95% CI 1.63–3.90) overall prevalence and 1.30 (bootstrap 95% CI 1.05–1.60) mean intensity in sardine. Prevalence in fillets was 3.2% (95% CI 2.15–4.69) in anchovy and 0.5% (95% CI 0.18–1.3) in sardine. Subsequently, collected epidemiological data were used to perform risk assessment of human *Anisakis* spp. infection, indicating a greater risk of consumption of unprocessed European anchovies than sardines.

P-080

EXPERIMENTAL RESEARCH OF *KUDOA NOVA* (MYXOSPOREA: KUDOIDAE) SPORES IN SALINE AND FRESH WATER

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On June 20, 2014 marine myxosporean *Kudoa nova* Najdenova, 1975 was found in the muscle of 12 out of 25 round goby *Neogobius melanostomus* (Pallas, 1814) specimens, caught in the Sea of Azov (Zolotoye, Crimea). Two percent of the myxospores appeared to be abnormal (deformed). 30 isolated parasite cysts were kept in twice-filtered sea (17.6 ‰) or fresh (0.22 ‰) water at a constant temperature of 16°C (volume 60 ml). The remaining infected muscle tissues were placed in sea or fresh water and put on the sill of a deep niche behind the window (water temperature at day time in shadow 18°C, volume 1.5 l) Penicillin (1g) and streptomycin (1 g) were added to 1 l of water as anti-microbial and anti-fungal agents. It was found that placing of *K. nova* spores into fresh water influences more negatively their structure and preservation when spores are in cysts isolated from muscles. The percentage of anomalous spores in these cysts at day 34 reached 87 %, while deformation of 48% of spores was observed already at the third day. By day 20, the majority of spores and polar capsules darkened, polar filaments did not eject when placed to 8% solution of KOH. When cysts of *K. nova* were placed into sea water and kept at 16 ºC the percentage of deformed spores was only 2-6% and spores were not dark. If the spores of *K. nova* were contained in muscle tissue and kept in waters of different salinity under natural weather conditions, the maximum proportion of deformed spores was 17 % in fresh water and 20 %in the sea water.

P-081

MYXOSPOREAN (MYXOZOA) PARASITES OF FRESHWATER FISH FROM DIFFERENT IRANIAN REGIONS

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Iranian freshwater fish habitats can be categorized according to three different faunal regions: the Ponto-Caspian Territory of northern Iran, as a part of the Palaearctic zone; south-eastern Iran that is greatly influenced by the Indian faunal region and south-western Iran that falls within the Mesopotamian intermediate faunal region. The composition of the fish fauna distribution in Iran reflects these territorial differences. Fifty-two species of myxosporean parasites were found from the studied Iranian freshwater fish. The parasites found were recorded including data from host species, parasitized organs, province, faunal region and reference number. Several aspects of parasite infections will be presented and discussed: records of the most predominant parasites, the richest regions of parasite fauna, records of parasites according to province and the most severely infected fish species and finally host specificity in terms of exotic or endemic fish.

P-082

BIODIVERSITY OF MYXOSPOREAN (MYXOZOA) PARASITES OF FRESHWATER FISH IN CAMEROON (CENTRAL AFRICA)

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This study shows the result of research conducted from 1985 to date, on the Myxosporean parasites of freshwater fish from Cameroon rivers.The methodology used is a combination of classical methods that involve fishing, necropsy of fish, and the coloration, mounting and study of parasites using a light microscope. The examination of these fish revealed that the Myxosporean fauna was abundant and diversified. A total of seventy-three myxosporean species were recorded. They belong to the genera *Myxobolus* (38 species), *Henneguya* (12), *Myxidium* (9), *Thelohanellus* (9), *Sphaerospora* (4) and *Chloromyxum* (1). Among the recorded parasites species, fifty were new and belong to the genera *Myxobolus* (20 species), *Henneguya* (11), *Myxidium* (6), *Thelohanellus* (6), *Sphaerospora* (4) and *Chloromyxum* (1). Twenty-three species were previously described in other geographical areas: some widened their host range, whereas others widened their target organs relative to their original descriptions. The use of myxospore morphology as the major criterion for the identification and description of new myxosporean species was not feasible in several cases, because of the high degree of similarity with other species and the variability within a given species. Therefore, the main task for the future is a rigorous, detailed morphological description combined with molecular techniques in the establishment of new species and revision of the existing ones. The classification of Myxozoa acceptable from morphological, biological and phylogenetical points of view is necessary in Cameroon.

P-083

THREE NEW *MYXOBOLUS* SPP. (MYXOZOA: MYXOBOLIDAE) INFECTING THE COMMON NASE *CHONDROSTOMA NASUS* (L.) IN THE RIVER DANUBE

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The common nase *Chondrostoma nasus* (L.) is a frequent cyprinid fish of the river Danube. In a survey on its infection with myxosporeans, eight different *Myxobolus* spore types were found in the gills, swimbladder, fins and intestinal wall. Of them, spore types representing three species were studied in detail by morphological and molecular biological methods. Based on differences in 18S rDNA sequences, two new *Myxobolus* species from the gills and one from the swim bladder will be described. In their spore size and location in the host the new species resembled *M. muelleri* Bütschli, 1882*, M. intimus* Zaika, 1965 and *M. cycloides* Gurley, 1893, respectively, the parasites of leuciscine cyprinids, but showed the following differences from these latter species in partial 18S rDNA sequences: *Myxobolus* sp. 1. – *M. muelleri* 1.4 %, *Myxobolus* sp. 2. – *M. intimus* 2.8 %, *Myxobolus* sp. 3. – *M. cycloides* 2.4 %. The authors conclude that based on the significant differences in DNA sequences, the three species represent new, hitherto undescribed species despite their morphological similarities to some *Myxobolus* spp. forming spores in identical locations in genetically closely related cyprinids of the Leuciscinae subfamily.

This study was supported by KTIA-AIK-12-1-2013-0017 and OTKA 100132 grants.

P-084

DESCRIPTION OF A NEW *MYXOBOLUS* SP. (MYXOZOA: MYXOBOLIDAE) FROM THE COMMON CARP *CYPRINUS CARPIO* L. IN LAKE BALATON, HUNGARY

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The common carp *Cyprinus carpio* L. is the most important species of freshwater fish culture. Its parasite fauna is well studied all over the world. To date, 21 species of the genus *Myxobolus* Bütschli, 1882 from Asia and ten species from other parts of the world have been described (Eiras et al., 2005, 2014). In a long-term parasitological survey conducted on fishes from Lake Balaton in Hungary, a new species of the genus *Myxobolus* was found. This poster provides a description of a new *Myxobolus* sp. in the gills of the common carp and differentiates the new form from the known *Myxobolus* spp. based on its morphology, site selection and 18S rDNA sequences. The new *Myxobolus* sp. was detected in the gill filaments of the common carp *Cyprinus carpio* L. collected in Lake Balaton, Hungary. Its oval plasmodia measuring 600–800 × 300–400 µm were located intravasally in the filamental arteries. The spores measured 11.2 ± 0.92 × 9.5 ± 0.41 × 7.4 ± 0.33 µm and had two equal polar capsules with six polar filament coils. Both morphology and DNA sequence analysis revealed that the new *Myxobolus* sp. is distinct from all of the *Myxobolus* spp. described from the European common carp and from the Asian common carp subspecies. Phylogenetic analysis placed the new *Myxobolus* sp. in a clade of gill-infecting myxobolids.

Acknowledgements: This study was supported by KTIA-AIK-12-1-2013-0017 and OTKA 100132 grants.

P-085

IN VITRO INACTIVATION OF *KUDOA SEPTEMPUNCTATA* SPORES, THE CAUSATIVE MYXOSPOREAN OF A FOOD POISONING IN HUMANS

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Kudoa septempunctata, a myxosporean parasite infecting the trunk muscles of olive flounder (*Paralichthys olivaceus*), has been recently reported to be the causative agent of a type of food poisoning in humans. Patients showed acute diarrhea and vomiting after ingestion of the raw flesh of infected flounder. Recent increase in number of food poisoning cases has urged us to develop a control strategy of this parasite. To prevent food poisoning caused by *K. septempunctata*, freezing at –15°C to –20°C for > 4 h or heating at 75°C for > 5 min have been recommended by the Ministry of Health, Labor, and Welfare of Japan. However, such treatments are impractical because olive flounder are commonly eaten raw. Thus, an alternative treatment method to inactivate *K. septempunctata* is still required. In this study, we applied the vital staining assay with the fluorescent dyes Hoechst 33342 and propidium iodide to determine the viability of *K. septempunctata* spores. Screening tests of treatment methods against *K. septempunctata* spores suggested that 25%-ethanol for 5 min, 80ºC for 10 sec, limonene at 10 µl/ml for 5 min, and salinities at 0‰ and 160‰ for 5 min were effective for killing spores. To verify toxicity loss in treated spores, tight junction barrier assays with Caco-2 cells were conducted. The results of the Caco-2 assays corresponded well with those of the Hoechst-PI staining assay. Further studies are required to determine a practical treatment procedure for inactivating spores considering the treatment application in the production process of cultured olive flounder.

P-086

PROLIFERATIVE KIDNEY DISEASE (PKD) – THE CAUSE FOR DECLINING STOCKS OF ARCTIC CHARR, *SALVELINUS ALPINUS*, IN ICELAND?

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Proliferative kidney disease (PKD), a serious disease of salmonids caused by *Tetracapsuloides bryosalmonae* (*T.b*), was first identified in Iceland in 2008. The disease is temperature dependant and only emerges when water temperature exceeds 12-14°C for several weeks. Paralleled with increasing water temperature over the last decades, populations of Arctic charr have severely declined in many lowland lakes in Iceland.

In 2008 – 2014, Arctic charr were regularly sampled from two lowland lakes in SW-Iceland experiencing severe declines in Arctic charr populations. Clinical signs of PKD were determined and kidney samples examined by histology and PCR. Furthermore, samples from fish caught in theses lakes in the 1990s were examined by PCR for the presence of *T.b.*

The prevalence of *T.b.* was high in all years 2008-2014, irrespective of fish age. Clinical signs of PKD were also common in younger fish (1-3 years) but varied between years of sampling. Similarly, *T.b.* infections were prevalent in Arctic charr in the lakes in the 1990s, but no data on clinical signs of PKD exist for those fish.

T.b. existed in Icelandic freshwater in the 1990s and hence is not new in the ecosystem. In the early 1990s the first indications of the decline in Arctic charr became evident. At the same time the water temperature reached the critical level necessary for PKD to emerge. The results of this study indicate that PKD has played a significant role in the decline of populations of Arctic charr in Iceland.

P-087

MYXOBOLUS ANATOLICUS (MYXOZOA) INFECTING THE GILL OF ANATOLIAN KHRAMULYA *CAPOETA TINCA* (CYPRINIDAE) IN TURKEY

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This work is part of an ongoing investigation into the characteristics of myxozoan parasites of freshwater fish in Turkey and was carried out using morphology, histopathology and molecular analysis. *Myxobolus anatolicus* was found infecting the gills of 3 of 34 specimens (8.8%) of Anatolian khramulya *Capoeta tinca* from the Samsun Province, Northern Turkey. Both morphology and 18S rDNA sequence data revealed that *M. anatolicus* was distinct from other *Myxobolus* species found in the gills of cyprinid fishes. The small, white and round-shaped plasmodia, measuring 0.2 to 1.4 mm in diameter, were observed macroscopically in the gills. Histological analysis revealed that the cyst-like plasmodia have an intralamellar-vascular type development. Mature spores of *M. anatolicus* were oval in both frontal and sutural views, and tapered at the anterior poles. The spores were 10.1 ± 0.41 (9.4 to 10.7) µm long, 6.9 ± 0.28 (6.6 to 7.2) µm wide, and 4.5 ± 0.36 (4.4 to 4.6) µm thick. The 2 polar capsules were pyriform, equal in size, 4.6 ± 0.45 (4.4 to 4.8) µm long and 2.1 ± 0.12 (2 to 2.3) µm wide. Polar filaments within the polar capsules were coiled with 5 or 6 turns. Pathological changes or deformities were not observed, and no other myxozoans were found in other organs of the *C. tinca*. Phylogenetic analysis placed *M. anatolicus* in a clade of gill-infecting myxobolids. This is the first record of a *Myxobolus* species infecting Anatolian khramulya *Capoeta tinca,* and the first record of this species from Eurasia.

P-088

MORPHOMETRY AND MOLECULAR CHARACTERIZATION OF TWO *CERATOMYXA* SPECIES FROM THE GALL BLADDER OF THE BOGUE, *BOOPS BOOPS*, IN THE GULF OF GABES

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During serial campaigns extended from July 2013 to August 2014 in the Gulf of Gabes (South Eastern of Tunisia), 180 specimens of the bogue *Boops boops* (Linnaeus) were examined for Myxozoan parasites. Two morphologically distinct Ceratomyxid mature myxospores were found floating in the bile. Morphometric and molecular analyses pointed out as new species of the genus *Ceratomyxa*

Myxospores of *Ceratomyxa-1* were crescent-shaped with two symmetrical valves and are formed in mono- , di- or polysporous spherical plasmodia. Mature spores measured 31. 5 ± 0.63 (30-33) µm in length and 5.5 ± 0.21 (5-6) µm in thickness. The polar capsules were spherical, equal in size and measured 2.75 ± 0.03 (2.5-3) µm in diameter.

Mature myxospores of *Ceratomyxa-2* were stubby-shaped with convex anterior and almost straight to slightly concave posterior. They measured 6 ± 0.39 (5-7) µm in length and 12.5 ± 0.42 (11-14) µm in thickness. Valves were unequal. The polar capsules were equal, spherical and measured 2 µm in diameter.

The PCR products of the SSU rDNA sequence obtained from the two species showed a difference in size of about 100 bp in gel electrophoresis. Phylogenetic analysis confirms the membership of these two parasites to the *Ceratomyxa* genus and the absence of any identical previously reported species.

P-089

CHARACTERIZATION OF THREE MYXOZOAN PARASITES INFECTING THE GALL BLADDER OF EUROPEAN PILCHARD *SARDINA PILCHARDUS* FROM TUNISIAN COAST

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During a biodiversity survey on the occurrence of myxozoan parasites in the European pilchard, *Sardina pilchardus* in Gulf of Gabes, hundreds of specimens were sampled and examined. Three new species belonging to three different genera *Ceratomyxa, Trilospora* and *Thelohanellus* were identified infecting the gall bladder in 46% of examined fish. Characterization of these parasites was based on the mature spores. The first species belonging to the genus *Ceratomyxa*, is characterized by elongated mature spores having two symmetrical shell valves and two small spherical polar capsules. Spores (n = 30) measured 23.5 (18-25) × 7 (5-9) µm in size and a diameter of polar capsules around 2.75 µm. Spores occurs in disporous or polysporous trophozoites. The spores of *Thelohanellus* are identified by their unique polar capsules measuring (n = 30), 12.5 (10-15) × 5.5 (5-6) µm and 6.5 (6-7) × 3.5 (3-4) µm for the polar capsule. The myxospores of *Trilospora* sp. in apical view show three equal shell valves and three spherical polar capsules arranged in the center. Spore measurements (n = 25) were 22 (20-24) × 15 (14-16) µm. The polar capsules have 3.5 µm of diameter. Infections with these three myxozoan parasites could be single or mixed. Hosts having less than 15 cm in length are the most infected ones. Currently, characterization based on the small subunit rDNA sequence for the three species is under process to finalize their specific identification.

P-090

VIABILITY OF *CRYPTOCOTYLE LINGUA* METACERCARIAE FROM ATLANTIC COD (*GADUS MORHUA*) AFTER EXPOSURE TO FREEZING AND HEATING IN THE TEMPERATURE RANGE FROM -80 °C TO 100 °C

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The presence of parasites in fish products is a problem that concerns consumers and authorities due to the potential hazards it may cause. Few studies on the viability of parasites in marine fish products are concerned with trematodes. In this study the trematodes *Cryptocotyle lingua* were identified by morphometric and molecular techniques and isolated metacercariae from Atlantic cod (*Gadus morhua*) were incubated in cod muscle tissue at different temperatures ranging from -80 °C to 100 °C for subsequent test on their viability. SEM images were made to assess the physical damage caused to parasites exposed to different temperatures. Temperatures between 50°C and 100°C and between -80°C and -20°C killed the metacercariae present in fish flesh in less than two hours. Controls kept at 5°C survived for nine days. Extreme freezing temperatures caused minimal visual physical damage to cysts, but the tegument of metacercariae was severely affected at all temperatures when incubated for long periods. The current legislation requires freezing of fish at a temperature not higher than -20 °C for at least 24 hours. Although this treatment is sufficient to inactivate *C. lingua* metacercariae, cod is not present in the list of fish species that should go through freezing before smoking process. Based on this investigation we recommend the industry to freeze cod before cold smoking procedures are performed.

P-091

ZOONOTIC HELMINTHS IN LACUSTRINE FISH FROM NORTHERN ITALY

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According to EFSA Scientific Opinion on risk assessment of parasites in fishery products issued in 2010, all wild fish should be considered at risk of containing any viable zoonotic parasites if eaten raw or almost raw, pointing out the need to carry out epidemiological surveys on presence/diffusion of zoonotic parasites in all fishery grounds. With this regard, in the framework of a research project (RF-2010-2311360) financed by the Italian Ministry of Health, a parasitological survey aimed at assessing qualitative and quantitative data on zoonotic helminths in commercially important fish caught in lakes (Maggiore, Como, Iseo, Garda) from northern Italy has been carried out from March 2013 to December 2014. Concerning Diphyllobothriid plerocercoids, abdominal cavity and fillets of 2216 fish (1820 perch, 172 shad, 104 whitefish, 92 burbot and 28 pike) were examined by visual inspection. *Diphyllobothrium latum* plerocercoids were found in perch (Maggiore 6.6%; Como 25.4%; Iseo 7.8%), pike (Maggiore 100%; Como 84.2%; Iseo 71.4%) and burbot (Como 3.6%; Iseo 3.8%), while shad and whitefish were negative. All the fish from Garda Lake were negative. Moreover, 239 cyprinids were analyzed for *Opisthorchis felineus* metacercariae by microscopical examination of compressed/digested muscular tissue. All the cyprinids were negative for *O. felineus*, while metacercariae of the Opisthorchiid Pseudamphistomum truncatum were detected in 2 tench and 1 rud from Como Lake. These data confirm *D. latum* as the most important fish-borne zoonotic helminth in the areas under study and identify the consumption of raw perch (“perch *carpaccio*”) as the main source of infection.

P-092

REAL-TIME PCR ASSAY BASED ON MITOCHONDRIAL DNA COX2 GENE FOR THE DETECTION OF ANISAKID SPECIES OF THE GENERA *ANISAKIS* AND *PSEUDOTERRANOVA* IN FISH

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Larval stages of anisakid nematodes of the genera *Anisakis* and *Pseudoterranova* have been reported as responsible of human infections (anisakiasis) caused by the consumption of raw, undercooked seafood, harbouring zoonotic anisakid larvae. Aim of the present work was to develop primers/probes based on mitochondrial *cox2* gene, to be used in RT-PCR, for the DNA detection of the most frequent species of the genera *Anisakis* and *Pseudoterranova* occurring in European fish. Starting from sequences of mtDNA *cox2* previously carried out on those anisakid species, new primers were designed for six anisakid species of the genera *Anisakis (i.e. A. pegreffii*, A. *simplex* (s. s.), *A. physeteris)* and *Pseudoterranova* (i.e. *P. decipiens (s. s*.), *P. bulbosa* and *P. krabbei*). Shorter DNA fragments were obtained using the new internal primers. Further, species-specific probes for each species were designed and labeled with different fluorescent colors, able to detect and identify those anisakid species. DNA was extracted using DNAeasy blood and tissue kit (Qiagen).The primers/probe combinations were tested using different serial quantity of parasite larvae, taking fixed the amount of fish fillet. The real-time PCR assay was capable of detecting 1/2 of a single L3 in 10 gr of fish tissue, it exhibited a high level of specificity. The limit of DNA detection, based on hybridation probes in Real Time PCR, was 0,02 ng/µl. RT-PCR based on hybridation probes represents a rapid, sensitive and inexpensive method to detect anisakids in fish fillets.

The research received funding from the EU7FP grant agreement n°312068 PARASITE.

P-093

REEVALUATION OF THE IMPORTANCE OF ATLANTIC ANCHOVIES (*ENGRAULIS ENCRASICOLUS*) AS A MAIN RISK FACTOR FOR ANISAKIOSIS AND/OR SENSITIZATION TO *ANISAKIS* IN SPAIN

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Clinical studies pointed to the ingestion of boquerones (raw *Engraulis encrasicolus* in vinegar) as the main potential risk for anisakiasis and IgE sensitization to *A. simplex* in Spain. Conversely, most epidemiological surveys showed parasite-free fish lots or with prevalences around 10%, with only a few exceptions. In the surveillance program for PARASITE Project (http://parasite-project.eu/) we analyzed 178 anchovies from NE Atlantic waters. Upon capture, fish were kept in ice until landing, later transported to the lab and immediately frozen to prevent nematode migration. After thawing, fish were dissected, pressed, examined under UV light and digested enzymatically. Nematode samples were then stored frozen. Data for every fish and nematode sample were saved and coded in a BioBanking platform for further molecular species identification. Anchovy prevalence was 87% (viscera 83%; flesh 29%), representing larger numbers than any other reported for this species in EU fish production value chains. The nearest data in bibliography was reported for the Adriatic Sea (76%) but with much lower flesh prevalence (2%). Reasons for this dissimilarity may be attributable to the different geographical fish origin but also to complex interactions of different host-related and environmental conditions. More remarkably, probably also reflects differences between detection methods. The high prevalence of *Anisakis* in this species is of public health concern, considering that flesh prevalence may reach 29% in pre-harvest condition, besides the traditional raw consumption of this fish in Spain. An enhanced risk communication focus on control measures may clearly contribute to avoid this biohazard in seafood.

P-094

ANISAKIS SPP. AND VALUABLE LARGE PELAGIC FISH OFF EASTERN ADRIATIC – EPIDEMIOLOGY, MOLECULAR IDENTIFICATION AND RISK ANALYSIS

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Nematodes of the genus *Anisakis* are considered an emerging public health issue due to anisakiasis, a zoonotic disease caused by consumption of raw or lightly processed infected seafood. Previous study conducted in Dalmatia, Croatia, showed high anti-*Anisakis* IgE seroprevalence among island population.

In order to assess occurrence of *Anisakis* larvae in fish consumed among Dalmatian island population and possible health risks, large and valuable pelagic fish species caught off the coast of Dalmatian islands (chub macekerel, *Scomber japonicus*, horse mackerel, *Trachurus trachurus*, little tunny, *Euthynnus alletteratus* and atlantic bluefin tuna, *Thunnus thynnus*) were examined. UV-Press method was used to reveal the presence of larvae in visceral mass and fillets. For molecular identification a ~650 bp fragment of mitochondrial cytochrome oxidase 2 (COX2) was sequenced and phylogenetic tree has been inferred using Bayesian Inference.

In total 349 *Anisakis* larvae were isolated, while total prevalence was 64.4% (Sterne's exact 95% CI 0,48 to 0,77) and mean intensity 12,03 (bootstrap 95% CI 7,03 to 20,55). Both values differ between species with highest prevalence observed in little tunny, while highest mean intensity was observed in chub mackerel. Molecular identification revealed *A. pegreffii* to be the predominant species, while only few *A. simplex* were identified. Although both *A. pegreffii* and *A. simplex* are capable of penetrating into fish muscle, the later has up to 12 times higher ability to migrate *postmortem* into fillets. Given this fact and epidemiological data collected, the risk of infection by consumption of targeted fish among Dalmatian island population is moderate.

OCCURRENCE OF *ANISAKIS SIMPLEX* SENSU STRICTO IN IMPORTED ATLANTIC MACKEREL (*SCOMBER SCOMBRUS*) REPRESENTS A RISK FOR TURKISH CONSUMERS

P-095

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Anisakid larvae are prevalent fish-borne potential pathogens that are found in numerous fish species destined for human consumption. The presence of *A. simplex* (s.s.) in seafood products may present a health risk for consumers. In Turkey, Atlantic mackerel (*Scomber scombrus*) is marketed frozen, mainly imported from Norway. The aim of this study was to identify the *Anisakis* species found in deep-frozen whole Atlantic mackerel destined for human consumption, obtained at fish markets that imported fish from Norway to Turkey. All *Anisakis* larvae isolated from imported Atlantic mackerel were identified by morphology as third larvae of *Anisakis* Type I. The ITS region was amplified and digested with the restriction enzymes Hinf I and Hha I. The larvae were identified via PCR-RFLP as belonging to *A. simplex* (s.s.), and this was confirmed by sequencing the cox2 gene. The overall prevalence of *Anisakis* larvae was 25% (95% confidence limits: 13–41%), and the mean intensity was 19.1 (bootstrap 95% confidence limits: 15.3–25.5). *A. simplex* (s.s.) larvae found in imported Atlantic mackerel could represent a risk since consumers could acquire parasitic allergies. The results will have an important impact on public health risk assessment in that they suggest reviewing critical control points at the Hazard Analysis Critical Control Point (HACCP) programs to reduce the risk of anisakid-induced allergies among consumers. Consequently, the present study provides the first data regarding the occurrence of *A. simplex* (s.s.) larvae in imported Atlantic mackerel in Turkish markets.

MYXOZOAN PARASITE FAUNA OF SIGANIDS FROM OMANI WATERS: NINE SPECIES FROM A SINGLE HOST

P-096

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Myxosporea is an important group of aquatic parasites gaining increasing interest in the Arabian Peninsula region. Many new myxosporean species have been recorded from waters of the Kingdom of Saudi Arabia (Red Sea and Arabian Gulf). However, similar investigations are lacking from the waters of the Sultanate of Oman, with only a single record of *Kudoa amamiensis* from *Nemipterus peronii* to date (Al Jufaili, unpublished data). Parasitological examination of *Siganus canaliculatus* caught from several locations off the Omani coasts of the Arabian Gulf, the Sea of Oman and the Arabian Sea revealed several unknown coelozoic and histozoic myxosporean parasites. The parasites belong to five different genera and were detected from different host organs. Three Kudoid myxosporean were detected from cysts located at the basal region of the gill filaments. Cysts harbouring *Unicapsula* spores were found infecting the surface of the endothelium lining of the oesophagus and stomach. *Ortholinea* spores were recovered from the urine and urinary bladder of the host, whereas the gall bladder had one species of *Zschokkella* and three forms of Ceratomyxid myxosporeans. Spore morphometric data using light microscope, scanning and transmission electron microscope combined with molecular analysis of the 18s region of the SSU rDNA sequence data were used for species identification. In addition, histological investigations were carried out to analyse host-parasite relationship and evaluate possible pathological implications associated with these myxosporeans. Based on the obtained data, this study will provide new information on nine unknown species of myxosporean parasites from a single host caught off Omani waters.

P-097

UNEXPLORED MEXICAN MYXOZOANS: FIRST SCREENING ON DIVERSITY OF MYXOZOANS IN FRESHWATER FISHES IN MEXICO

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Myxozoans are cnidarian microparasites that are well known in fisheries and aquaculture for causing disease and mortality in their fish host. These parasites are widely spread affecting freshwater and marine habitats, with about 2500 species described worldwide. Despite its importance, myxozoan species reports in Mexico are practically inexistent and there is a need to explore the presence of this group of parasites in a country with growing aquaculture sector. This study explores for the first time the diversity of myxozoan species in freshwater fishes in some rivers from southeastern Mexico. Fish belonging to 9 families and 18 species were captured with electrofishing and gillnets from 8 localities: Río la Palma and Tlacotalpan (Veracruz State); Río Negro (Oaxaca State); Río San Juan, Pijijiapan, Río Xuixtla, El Triunfo and Nueva Francia (Chiapas State). Samples were taken of gall bladder, kidney and intestine to be screened molecularly by sequencing of the SSU rDNA with universal myxozoan primers. Preliminary results detected myxozoans in 30% of the fish sampled, with members belonging or closely related to different freshwater genera *e.g Zschokkella, Myxidium, Myxobolus* and *Henneguya*. PCR positive host species were screened microscopically (light microscopy, scanning electron microscopy, histological sections) to identify the new myxozoan species. This study provides the first details about the morphology, prevalence, habitat, distribution and diversity of myxozoans in rivers of the neotropical region of Mexico.

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OCCURRENCE OF *GLOBOSPORA* SP. IN EUROPEAN SEABASS, *DICENTRARCHUS LABRAX* L. REARED IN A PORTUGUESE FISH FARM

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Myxozoa are among the parasites that may cause growth delays in aquaculture fish. This motivated a survey for them, in European seabass (*Dicentrarchus labrax* (Linnaeus, 1758)) reared in a fish farm located at the Aveiro's Estuary (Portugal). In a sample of 20 fish, only one was found infected with *Globospora* sp. Free spores and disporic plasmodia (diameter 14-16 mm) were detected in the gall bladder and co-occurring with *Ceratomyxa diplodae* Lubat, Radujkovic, Marques & Bouix, 1989 infection. The spores were fixed in 5% glutaraldehyde buffered in 0.2 M sodium cacodylate for processing for transmission electron microscopy, differential interference contrast microphotographs were taken and morphometry was recorded. The spores were sub spherical to ovoid (9.6-9.5 x 6.5-6.4 mm) in lateral view, and circular in apical view, presenting one single pyriform polar capsule (2.6-2.4 x 2.3-2.0 mm); by its turn, inside this capsule is a filament with around 4 turns.

Globospora Lom, Noble et Laird, 1975 genus has, so far, one single species described, *G. sphaerica* which was reported from *Odontesthes incisa* (Actinopterygii: Atherinopsidae) off Argentina Atlantic coast, thus this is the second occurrence for this genus worldwide.

Acknowledgements: The Portuguese Foundation for Science and Technology (FCT), within the aim of the Project DIRDAMyx (Reference: FCOMP-01-0124-FEDER-020726 / FCT- PTDC/MAR/116838/2010) for the grants attributed to F. Cavaleiro and R. Castro, and the Ph.D. fellowship grants attributed to S. Rocha (SFRH/BD/92661/2013) and to L. Rangel (SFRH/BD/82237/2011) through the programme QREN-POPH/FSE; and the project EUCVOA (NORTE-07-0162-FEDER-000116).

P-099

A SURVEY OF ACTINOSPORES FROM ANNELIDS IN A PORTUGUESE ESTUARY

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The Myxosporea Bütschli, 1881 are obligate microscopic endoparasites of vertebrate and invertebrates species. They infect fishes, birds, amphibians, reptiles and mammals, and alternate their life cycle in annelids and sipunculids. Aveiro estuary is a vast enclosed estuary with an area of 11,000 hectares and is used by several species of fishes as home and nursery. Several fish farms grow fishes in this environment. The study of the myxosporean extrapiscine development in annelids is important to understand the biology and ecology of these parasites. The results of the survey, which included the microscopic analysis of 503 oligochaetes and 1621 polychaetes, revealed the presence of actinospores belonging to 3 different collective groups. In oligochaetes, 1 triactinomyxon morphotype (with a prevalence of infection of 0.6%), and more than 4 sphaeractinomyxon morphotypes (23.0% in total) were registered. In polychaetes we found 1 tetractinomyxon morphotype (6.0%).

Acknowledgements: The Portuguese Foundation for Science and Technology (FCT), within the aim of the Project DIRDAMyx (Reference: FCOMP-01-0124-FEDER-020726 / FCT- PTDC/MAR/116838/2010) for the grants attributed to F. Cavaleiro and R. Castro, and the Ph.D. fellowship grant attributed to L. Rangel (SFRH/BD/82237/2011) through the programme QREN-POPH/FSE.

P-100

A NEW SPECIES OF *HENNEGUYA* (MYXOBOLIDAE) PARASITIC ON *MARKIANA NIGRIPINNIS* (CHARACIDAE) FROM THE PANTANAL, BRAZIL

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Henneguya comprises approximately 200 species, of which 50 species have been reported to be parasitic on Brazilian fishes. *Markiana nigripinnis* is a small characid fish from South America and reports about its parasites are unknown. Between December 2013 and September 2014, 57 specimens of *M. nigripinnis* were caught in a perennial lagoon (19^{o}34,576’ S, 057^{o}00,823’W) from Pantanal floodplain, Sate of Mato Grosso do Sul, Brazil. Gills were removed and observed under a stereomicroscope. Cysts were removed from the gills, and some were stored in ethanol 70% for molecular analysis, whereas others were ruptured for the morphological study of the spores (fresh and Giemsa stained mounts). Measurements, in micrometers, are expressed by the range. Partial 18S rDNA sequence was obtained from spores and compared to the sequences available from GenBank. Mature spores: elongated, total length 25-34.6; spore body 8.9-12.6 long, 2.7-5.7 wide; caudal process 16-22.9; polar capsules 4.4-6.5 long, 1.0-1.9 wide; polar filaments 4-5 turns. Partial sequence of 678 base pairs did not match any with of the previous Myxozoa sequences. Phylogenetic analysis of the sequence using maximum likelihood showed that this species clustered with *H. rotunda*, *Myxobolus pantanalis*, *H. visibilis*, *H. pellucida* and *M. oliveriai*, all of which are parasites of Characiformes fishes from Brazil. *Henneguya pellucida* was the most closely related species. However, *H. pellucida* was found in the visceral cavity and organs of *Piaractus mesotamicus* and presented 6-7 turns of polar filaments, whereas the species found in the gills of *M. nigripinnis* presented 4-5 turns of polar filaments.

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THE REPRODUCTIVE STRATEGY OF *ACANTHOCHONDRIA CORNUTA* (COPEPODA: CHONDRACANTHIDAE)

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Acanthochondria cornuta (Müller O.F., 1776) is a chondracanthid copepod, commonly found in the branchial chambers of the European flounder, *Platichthys flesus* (Linnaeus, 1758) (Teleostei: Pleuronectidae). Information on this parasite is still scarce, namely as concerns its reproduction. In an attempt to characterize its reproductive strategy, the existence of seasonal variations in body size and measures of reproductive effort (egg sac size and egg number and size) was evaluated. A total of 120 ovigerous females, 30 from each season of the year, were included in the analysis. In winter, females tended to be larger (6.2±0.7 [5.0-8.0] mm [summer]; 6.4±0.5 [5.4-7.8] mm [autumn]; 7.2±0.6 [6.0-8.4] mm [winter]; and 6.7±0.6 [5.7-8.4] mm [spring]) and to produce (i) larger egg sacs (3.1±0.6 [2.2-5.5] mm [summer]; 3.7±0.4 [2.7-4.6] mm [autumn]; 5.0±0.6 [4.2-6.3] mm [winter]; and 4.7±0.5 [4.1-6.2] mm [spring]), (ii) a larger number of eggs (623.2±263.8 [268-1183] eggs [summer]; 812.5±283.3 [396-1620] eggs [autumn]; 1523.1±421.4 [759-2486] eggs [winter]; and 1360.9±452.7 [737-2439] eggs [spring]) and (iii) smaller eggs (0.001571±0.000306 [0.001073-0.002333] mm^3; [summer]; 0.001528±0.000256 [0.001017-0.002036] mm^3; [autumn]; 0.001237±0.000132 [0.000991-0.001621] mm^3; [winter]; and 0.001266±0.000137 [0.001040-0.001638] mm^3; [spring]), contrary to that found for the summer season. The results found are similar to those previously reported for a copepod parasitic on an invertebrate host, supporting the existence of a reproductive strategy common to all parasitic copepods.

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THE ROLE OF MYXOZOAN POLAR CAPSULES: INJECTING AS WELL AS ANCHORING

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The first crucial step in the infection process of myxozoans is the discharge of their polar capsules, whereby an evertable tubule is fired rapidly into the host cell. We set out to characterize the dynamics and function of this process, of which little is currently known. We used myxospores of *Myxobolus cerebralis* which had their polar capsules content labeled, to clearly visualize and investigate the discharge process. Capsule discharge was triggered under the microscope, and documented using a high-speed video camera.

We discovered several distinct steps in the discharge process: (i) tubule inversion to full outward extension, (ii) violent contraction of the tubule to about half its extended length, leading to (iii) rapid injection of a solution out through openings in the tubule. Additionally, spores were seen to be pulled strongly in the direction of the anchorage point, due to the fast contraction of the tubule.

The entire process lasts ~300 milliseconds. Our findings provide evidence that myxozoan polar capsules could function as more than anchor mechanisms: they can actively inject compounds into the host. The possible functions of such injection are intriguing, and include facilitating adherence and/or penetration to the host tissue (via adhesive or digestive substances), or rapidly decreasing the internal pressure inside the tubule, thereby leading to its rapid contraction - which then leads to the parasites pulling itself closer to the point of contact to aid sporoplasm penetration.

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MYXOSPOREAN INFECTIONS ON GILTHEAD SEA BREAM *SPARUS AURATA* L. IN TURKEY

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Aquaculture production has been expanding rapidly over recent years in Turkey. Gilthead seabream (*Sparus aurata* L.) culture have important proportion of aquaculture production. Diseases are most significant factor affecting to this industry such as infections of myxozoan parasites. The aim of this work was to state myxosporean parasite on seabream culture. total 120 seabream obtained from different four commercial enterprise in Izmir, were studied for myxosporean parasites. Parasites were identified and histopathological examinations were carried out for several tissues such as kidney, gall bladder, intestine and spleen. Prevalence and intensity of infection were determined.

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INFECTION DYNAMICS OF MYXOZOANS IN BLACK SCORPIONFISH *SCORPAENA PORCUS* L. FROM TUNISIAN COASTS

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The Black Scorpionfish, *Scorpaena porcus* (Linnaeus, 1758) is a venomous Scorpionfish and one of the most common species of Scorpaenidae family along the Tunisian coasts. The specimens of *Scorpaena porcus* were collected from three localities situated in North-East of Tunisia: Bay of Bizerte, Gulf of Tunis and Gulf of Hammamet.

During the parasitological survey, a total of six coelozoic myxosporean species belonging to five genera are found in the gallbladder of *Scorpaena porcus*. The presence of myxsospores in the bile is easily detectable under light microscope by the polar capsules which are highly refractive.

Morphological and morphometric characteristics of these species based on light microscopic observations of fresh and stained spores are presented. We have identify *Myxidium incurvatu*m Thélohan, 1892 which has been recorded in *Scorpaena scrofa* L. and other hosts from France (Eiras *et al.*, 2011).The five other species are *Ceratomyxa* sp. 1, *Ceratomyxa* sp. 2, *Pseudoalatospora* sp., *Ortholenia* sp. and *Zschokkella* sp. These species are different by their dimensions and shape from the species described worldwide.

The infection prevalence of described myxosporean species differs from one locality to other. Parasitism and its relationship with host sex and host size revealed that fish males are more infected than fish females. Furthermore, myxozoan infection increases with increased size of the host. Adults are more infected than juveniles.

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GILL MYXOSPOROSES AFFECTING AQUACULTURED FISH IN PUNJAB (INDIA)

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In Punjab (India), polyculture consist of Indian major carps - Catla (*Catla catla* Ham.), rohu (*Labeo rohita* Ham.) and mrigal (*Cirrhinus cirrhosus* Ham.), and exotic carps - silver carp (*Hypophthalmichthys molitrix* Valen.), grass carp (*Ctenopharyngodon idellus* Valen.) and common carp (*Cyprinus carpio* Linn.). In this system, carps are prone to several diseases like trichodiniosis, haemorrhagic septicemia and various parasitic infectious diseases especially those due to Myxozoa. The gills were examined under a trinocular stereo microscope for the presence of pseudocysts of myxosporeans and clinical symptomatology, if any. Intensity of the infection was measured with a gill pseudocyst index (GPI): countable number of pseudocysts present per gill. For identification, myxospores were studied fresh and in stained preparations with iron-haematoxylin and Ziehl-Neelsen. The histological study was made on paraffin sections (3-4 µm thick) of infected gills, stained with Luna's method. The pseudocysts were located in the gill lamellae, gill filament and also in the gill arch. A total of 19 species of myxosporean were found infecting various tissues in the gills. Among them, 14 species belong to the genus *Myxobolus*, 3 species to the genus *Thelohanellus* and 2 species to the genus *Henneguya*. Parasitism was of three type- bi, tri and polyparasitism. Clinical presentation and principal lesions caused by each species are described.

The financial grant by University Grants Commission (UGC), New Delhi, in the form of Major Research Project (MRP) is fully acknowledged.

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NEW COLONY DEVELOPMENT FROM MIGRATING AUTOZOOIDS OF *FREDERICELLA SULTANA* UNDER UNFAVOURABLE CONDITIONS

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The Bryozoa *Fredericella sultana* acts as an essential invertebrate host within the life cycle of the myxozoan parasite *Tetracapsuloides bryosalmonae*, the aetiological agent of Proliferative Kidney Disease (PKD) for salmonid fish. During the summer 2014, following the first PKD outbreak affecting brown trout (*Salmo trutta*) in Austria, several locations along the river Kamp were inspected and infected sites found several km upstream the infected farm. *Fredericella sultana* colonies were collected from these locations and attached to plastic petri dishes to be cultivated under laboratory conditions. Daily colony inspection revealed the dislocation of single zooids from larger branching colonies followed by its attachment in another place to start a new colony. Zooids kept under unfavourable conditions, e.g. hypertrophication or sudden temperature changes, retracted their funiculus, twitched to the top of the branch and actively separated from the remaining dying colony. Migrating individual autozooid, showed fully developed and contracting organs but no exoskeleton. Following attachment to a substrate they growth similarly as for those newly hatched from statoblasts, but without statoblast chitinous valves. Lateral budding is a common phenomenon occurring to mechanically damaged bryozoans; however this kind of zooid migration is not previously seen. Further study is required to better precisely assess this phenomenon and its relevance for *F. sultana* colonization of new habitats with the possibility of spreading *T. bryosalmonae* malacospores.

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PARASITOLOGICAL SURVEY OF SILURIDAE AND PERCIDAE SPECIES IN IRAN

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The parasitological survey of *Siluridae* (*Silurus glanis and Parasilurus triostegus*) and *Percidae* (*Sander lucioperca, Perca fluviatilis)* species was carried out during 2010 to 2014, with the aim of contribute to the knowledge of the fish parasites fauna from a less known or somewhat unstudied species in Iran. The only protozoan parasites recorded from *Sander lucioperca* were *Tripanosoma percae* in the blood and *Trichodina* sp and *Vorticella* sp on the skin. *Perca fluviatilis* was infected by *Anisakisschupakovi* and *Camallanuslacustris* (Nematoda) in intestine, and *Ancyrocephalusparadoxus* (Monogenea) and *Achtheres percarum* (Copepoda) in gills. *Silurus glanis* was found to be infected by two monogeneans, *Silurodiscoides siluri* and *S.vistulensis* in gills and one cestode *Protocephalus osculatus*;two digeneans *Aphanurus stossichi* and *Bunocotile cingulata* ; two nematodes,*Anisakis sp* and *Cuculanus sphaerocephalus* and two acanthocephalans: *Corynosoma strumosum* and *Pomphorhynchus perforator* all from intestine of examined fishes. The only parasite species found in *Parasilurus triostegus,* a native fish of Tigris region was the monogenean *Silurodiscoides vistulensis* typical *Ponto-Caspian* species recently introduced to the Tigris region. This species needs further studies due to longer dorso-apical length rather than *S.vistulenses* found on gills of the *Silurus glanis* of the Caspian region. In the present paper parasitic fauna of *Percidae* and *Siluridae* species are reported and morphological and biometrical similarity/differences of *Silurodiscoides vistulensis* from two hosts are stressed.

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FIRST REPORT OF THE NEMATODE *CONTRACAECUM* N. SP. AND THE ACANTHOCEPHALAN *TENUIOSENTIS NILOTICUS* IN THE JAPANESE THREADFIN BREAM (*NEMIPTERUS JAPONICUS*) IN THE PERSIAN GULF

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The threadfin bream *(Nemipterus japonicus)* is one of the most important commercial fishes in the Persian Gulf, however parasite infections of this fish cause economical losses. In this study 649 Japanese threadfin breams from the Persian Gulf were captured and necropsied. The parasites of each organ were separated and stained. Diagnosis of the parasites was done by identification keys. In total, 109 fish (17%) were infected by parasites, being the intestine the most infected microhabitat. In this study one nematode (*Contracaecum* n. sp.), two acanthocephala (*Serrasentis sagittifer, Tenuiosentis niloticus*) and their larval stages were diagnosed. The helminth infection levels were higher in male than in female fish. This is the first record of *Contracaecum* n. sp. and *T. niloticus* in Japanese threadfin breams of the Persian Gulf. *Serrasentis sagittifer* has been already recorded in this host at this area.

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INFESTATION OF PLANKTON CRUSTACEAN BY METACERCARIAE OF *BRACHYPHALLUS CRENATUS* (RUDOLPHI, 1802) (TREMATODA: HEMIURIDAE) IN THE PROSTOR GULF (ITURUP ISLAND, RUSSIA)

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Brachyphallus crenatus Rudolphi, 1802 is one of the most common parasite of fishes in the Arctic and subarctic. In the North Pacific it is often found in anadromous pacific salmon *Oncorhynchus* spp. and its life cycle is partly described in the North Atlantic (Reimer, 1971; Køie, 1992) and the Arctic Ocean (Grozdilova&Makrushin, 1985 etc). In May - July 2013, a plankton survey (44 samples) was performed in the Prostor Gulf (Iturup Island, Kuril Islands) in feeding area of wild salmon populations and young fishes from the near located hatcheries. Samples were obtained by Jedi plankton net (upper ring Ø 370 mm, gauze 168 µ) at depths between 10 and 100 m. Metacercariae of *B. crenatus* were found only in the samples collected in June and the beginning of July. Among all found plankton taxa only adults and 5 copepodid stages of *Pseudocalanus newmani* Frost, 1989 and adults of *Acartia longiremis* Lilljeborg, 1853 were infected. These copepods were caught mainly in the coastal zone at a depth up to 20 m, where 70% of twenty collected samples at this depth contained metacercariae. The metacercariae was located in haemocoel of the thoracic segments. Removed from crustaceans metacercariae (10 ind.) body length was 0.484—0.583 mm, maximal width - 0.137—0.174 mm. Thus, *P. newmani* and *A. longiremis* are recorded as second intermediate host of *B. crenatus* in the North Pacific for the first time.

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CHECKLIST OF PARASITES OF LABRID FISHES (PISCES: LABRIDAE)

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Labrid fishes, commonly known as wrasses, are distributed in tropical and subtropical seas globally. This study provides a list of the parasite species reported in labrids around the world based on published references from different sources. In addition, we record new data on parasites of 17 wrasses, one species from Robinson Crusoe Island in Chile, and the remaining 16 species from the GBR. The majority of biological knowledge of labrids, including parasitological studies, derives from the Tropical Indo Pacific, especially from the Great Barrier Reef (GBR), Australia. A total of 338 records of parasite taxa were found for 127 labrid species. From these records, 212 parasites were identified at the species level, 67 were identified at the genus level, and the remaining records were identified at a higher taxonomic level. Endoparasites were more diverse than ectoparasites. Trematodes were the richest endoparasites group (134 species) and crustaceans were the richest ectoparasites group (44 species). This checklist was based on 176 published articles, adding some unpublished parasitological records in 17 wrasse species.

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PARASITE PREVALENCE, INTENSITY AND ABUNDANCE COMPARISON IN PERUVIAN HAKE DURING SPRING SEASON 2013 AND 2014

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Merluccius gayi peruanus is a demersal fish, whose distribution in the Peruvian coast varies according to seasonal and interannual fluctuations of the Cromwell Flow. The purpose of this research was to compare the prevalence, intensity and parasite abundance in Peruvian hake during the spring season 2013 and 2014. Between October and November 2013 and 2014, 30 and 21 specimens of *M. gayi peruanus*, respectively, were acquired in the Fishing Terminal of Ventanilla-Callao, recording the length and weight of each individual. For parasitological examination outer surface, gills, arterial bulb, gut, visceral surface and muscle tissue were checked. Trematodes, cestodes and acanthocephalans were fixed using AFA 50% and nematodes were fixed in hot alcohol 70°. The coloration was made with acetic carmine and lactophenol was used for clarifying. The length and average weight of specimens of *M. gayi peruanus* examined in the spring of 2013, was 33.5 cm and 288.7 g, respectively; parasites belonging to the species *Aporocotyle wilhelmi* (20%), *Clestobothrium crassiceps* (16.7%), *Anisakis simplex* (6.7%) and a plerocercoid larva of the order Tetraphyllidea (6.7%) were found. In the spring of 2014, individuals examined had an average length and weight of 30.6 cm and 193.9 g, respectively; the parasite species found were *Aporocotyle wilhelmi* (14.3%), *Clestobothrium crassiceps* (4.8%) and *Corynosoma* sp. (4.8%). The comparative study of the parasitic fauna of *M. gayi peruanus*, between spring 2013 and 2014, shows a decrease in the prevalence of *A. wilhelmi* and *C. crassiceps*; however, the mean intensity and mean abundance of *A. wilhelmi* increased in 2014.

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HELMINTH PARASITES OF PERUVIAN HAKE *MERLUCCIUS GAYI PERUANUS*, ACQUIRED AT THE FISHING TERMINAL OF VILLA MARIA DEL TRIUNFO – LIMA, PERÚ

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Merluccius gayi peruanus is the most abundant and economically important species in the trawl fishing industry in Peru. The present study aims to contribute to the knowledge of the helminth fauna in the Peruvian hake.

30 individuals of *M. gayi peruanus* were acquired in the Fishing Terminal of Villa Maria del Triunfo, Lima, between October and December 2014. Morphometric data and sex of each specimen were recorded. Also, parasitological examination was performed on surface, gills, arterial bulb, gastrointestinal tract, muscle tissue and visceral surface of each fish. The parasites found were fixed in AFA and preserved with alcohol 70 °. Carmin acetic coloration was used for the flatworms identification, as was clarified with lactophenol for observation of internal structures in the case of nematodes.

Of the examined fishes, 16 were males and 14 females. The average total length was 37.1 cm and the average weight 364.1 g. The prevalence of infected fish with helminth parasites was 40%. We identified adults of *Clestobothrium crassiceps* (20%), *Aporocotyle wilhelmi* (16.6%), *Anthocotyle* sp. (10%), and third stage larvae of *Anisakis simplex* (13.3%). In the present study, the prevalence of parasitic *A. wilhelmi* and *Anisakis simplex* was higher than that recorded by Chero et al. (2014) in Peruvian hake acquired at the Fishing Terminal of Ventanilla, Callao. These findings represent an update of the helminth fauna in *Merluccius gayi peruanus* coming from the Peruvian sea and allow to make recommendations on how this fish should be consumed to prevent zoonotic parasitic infections.

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PARASITES IN SILURIFORMES FISHES FROM ECUADOR

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This contribution is part of a PhD study on parasites of Siluriformes fishes from rivers of the Pastaza Province in the Amazon region of Ecuador. In this area a rural and peri-urban population depends on fishing as an important source of food. The fish consumption levels are one of the highest in the world. The main objective is to make an initial contribution of parasites found from Loricariidae fishes (*Hypostomus oculeus*. *Hypostomus* sp, and *Ancystrus* sp). These fishes are usually caught by artisanal fishermen in Puyo and Bobonaza rivers, and their parasitological fauna is still unknown. Fishes were collected during two years along the rain and short rainy seasons, fixed in 10% formalin and then transferred to 70% until their study for external and internal parasites. For each fish, musculature was also revised according to the protocol for searching larval stages of *Gnathostoma* (zoonotic in Ecuador). At moment, eleven parasite species were identified. These included two Myxozoa morphotypes (*Myxobolus* sp. from gills and external surface of the gut.), four monogeneans from gills and skin surface (*Unilatus* sp.1, *Unilatus* sp.2, *Demidospermus* sp. and *Phanerothecioides* sp.), three trematodes from intestine belonging to the genera *Saccocoelioides* and *Crassicutis*, one hirudinean from the mouth (Glossiphonidae) and one isopod from body cavity (*Riggia* sp.). All these species are currently being studied to determine its correct systematic identification.

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LARVAL FISH SPECIES AS SOURCE OF DIVERSITY OF *TRIFUR* SPP. (COPEPODA: PENNELLIDAE) IN HUMBOLDT CURRENT SYSTEM, INFERRED BY MOLECULAR ANALYSES.

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Parasite diversity in fish species tends to increase with the use of molecular markers, which allow to identify morphologically similar species or species in larval stages that have no distinctive characters. The genus *Trifur* Wilson, 1917 is composed by five species: *T. tortuosus* Wilson, 1917; *T. puntaniger* Thomé, 1963; *T. chlorophthalmi* Yamaguti, 1939; *T. physiculi* Heegaard, 1962; and *Trifur lotellae* (Thomson, 1890). Two of these species have been recorded in the Humboldt Current System (HCS): *T. puntaniger* on *Merluccius australis* and *T. tortuosus* infesting fishes of different families (Bovichthidae; Labrisomidae; Merluccidae; Sebastidae; Moridae). In a recent study evaluating the presence of ectoparasites in ichthyoplankton from HCS, larval parasites belonging to genus *Trifur* were recorded on larval fish species (*Helcogrammoides* spp*., Gobiesox marmoratus, Myxodes viridis; Auchenionchus* spp.). Molecular analyses, based on gen CO-I, were performed to those larval parasites and two adults of *Trifur* spp. present in *Sebastes oculatus* and *Scartichthys viridis*. Sequences of 697 bp were compared and phylogenetic trees were constructed using NJ-K2P Model. Our results showed five species of *Trifur*, four were on larval fishes. The genetic distance among *Trifur* spp. varied between 11.9 and 13%. One of them, here called *Trifur* sp. 1, was present on larval fishes from different families (*H. cunninghami; G. marmoratus* and *M. viridis*) corresponding to *Trifur* sp. present in adult fish *S. viridis* (Blenniidae). The larval fish *G. marmoratus* was infested by three different species of *Trifur.* Morphological characters and importance of larval fish in the transmission of *Trifur* spp. are discussed. Financial support: FONDECYT 1120868.

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HELMINTH PARASITES OF *SCOMBER COLIAS* GMELIN, 1789 (SCOMBRIDAE) FROM RIO DE JANEIRO COAST

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Previous studies on the helminth fauna of scombrid fish from the Brazilian coast included reports on *Scomber japonicus* Houttuyn, 1782 and *Scomber colias* Gmelin, 1789. Nowadays it is considered that only *S. colias* occurs in the Atlantic Ocean, Mediterranean Sea and Black Sea, forming different populations. To assess and revise the parasite biodiversity of *S. colias* off the coast of Rio de Janeiro State, 69 fish were examined from March 2013 to November 2014. We report herein the presence of nine taxa: the monogeneans *Grubea cochlear* and *Kuhnia scombri*, the digeneans *Aponurus laguncula*, *Lecithocladium harpodontis* and *Allonematobothrioides scombri,* the larval cestode *Scolex pleuronectis* and the nematodes (*Raphidascaris* sp., *Hysterothylacium* sp., Anisakidae gen. sp. larvae). *Raphidascaris* sp. was the most prevalent (64%) parasites, followed by *K. scombri* (60%) and *A. laguncula* (38%). The most abundant species was *K. scombri* (2.30 ± 3.73). The mean infracommunity richness was 2.14 ± 1.09 species/fish and the total mean abundance was 7.08 ± 7.24 worms/fish. The mean Berger-Parker dominance index was 0.67 ± 0.27, *K. scombri*, *Raphidascaris* sp. and *A. laguncula* being the most dominant species. The Bray-Curtis similarity index between infracommunities was relatively low (32.78 ± 26.20) and *Raphidascaris* sp. and *Hysterothylacium* sp. were contributing most to the similarity between infracommunities of the *S. colias* studied.

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BIODIVERSITY OF HELMINTH PARASITES OF CHARACIFORMES FISHES FROM THE SAPUCAÍ-MIRIM RIVER, SOUTHEASTERN BRAZIL

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Parasites were recently recognized as important components of the global biodiversity because of the essential roles played by these organisms in natural ecosystems. Although knowledge about the diversity of parasites has increased in recent decades, there are still many species to be identified. The aim of this study is to survey the biodiversity of helminth parasites of fishes of Characiformes order from an area under influence of Small Hydro Power Plants (SHPP) in the Sapucaí-Mirim River, São Paulo State, Brazil, and to evaluate the structure of their parasitic communities. During 2012 and 2013, two annual samples (dry/rainy seasons) were carried out, in four sites situated among SHPP Palmeiras, Anhanguera, and Retiro. Specimens of *Astyanax altiparanae* (n=40), *Astyanax bockmanni* (n=40), *Astyanax fasciatus* (n=40), *Leporinus amblyrhynchus* (n=24), *Leporinus elongatus* (n=22), *Leporinus friderici* (n=20), *Leporinus octofasciatus* (n=17), *Prochilodus lineatus* (n=20), *Schizodon nasutus* (n=40), and *Steindachnerina insculpta* (n=34) were analyzed, totaling 297 fishes. Two hundred and sixty-three fishes (Prevalence = 88.6%) were parasitized by at least one *taxon* of helminth. It was found 68 helminth *taxa* belonging to Monogenea, Digenea, Cestoda, Nematoda, and Acanthocephala, totaling 2587 specimens. The Class Monogenea presented the highest richness (39 *taxa*), and 11 new species of this group were found: four species of *Urocleidoides* sp., five species of *Tereancistrum* sp., and one species of *Characithecium* sp. and Dactylogyridae gen. sp. This study provides data of new host records and geographic distribution, as well as is an important contribution to the knowledge about the helminthological fauna of Brazilian fishes.

Financial support: FAPESP (2011/23588-8), CNPq (307808/2014-9).

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BIODIVERSITY OF THE PARASITES OF *HYPOSTOMUS* SPP. (SILURIFORMES, LORICARIIDAE) FROM THE SAPUCAÍ-MIRIM RIVER, BRAZIL

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The limited knowledge about the biological aspects, biogeographic patterns of distribution and high morphological variability of Loricariidae fishes are factors that make difficult the studies on biodiversity of this group, including the parasitic diversity of these fishes. The aim of this study is to survey the biodiversity and composition at both the component community and infracommunity levels of *Hypostomus* species collected in an area under the influence of the construction of Small Hydroelectric Power Plants in the Sapucaí-Mirim River, São Paulo State, Brazil. Specimens of *Hypostomus regani* (n=50), *Hypostomus ancistroides* (n=50), *Hypostomus strigaticeps* (n=50), *Hypostomus heraldoi* (n=9), *Hypostomus margaritifer* (n=23), *Hypostomus* cf. *margaritifer* (n=18); *Hypostomus topavae* (n=10); *Hypostomus* aff. *topavae* (n=36) and *Hypostomus* n. sp. (n=23), were analyzed, totaling 269 fishes. It was found 16 parasite *taxa,* totaling 2,769 specimens, with the Monogenea Class showing the greatest richness (nine *taxa*). The parasites showed aggregated pattern of distribution, and were identified as follows: Dactylogyridae, Gyrodactylidae and Microcotylidae in the gills and skin (Monogenea); metacercariae in the vitreous humor, crystalline lens, liver and skin (Trematoda: Diplostomidae); third-stage larvae of *Contracaecum* (Anisakidae) and *Procamallanus* (*Spirocamallanus*) *inopinatus* (Camallanidae) in the intestine (Nematoda); hirudineans (Glossiphonidae) were found infesting all the fish species (overall Prevalence [P]=57.2%); and *Myxobolus* sp. (Myxozoa) were observed in *H. ancistroides* gills (P=10%). Monogenean new species were found, belonging to *Trinigyrus* and *Heteropriapulus* genus (Dactylogyridae). These new records increased the understanding about host-parasite relationships, their geographical distribution and patterns of composition of the parasite community.

Financial support: FAPESP 2012/07850-7; CNPq (307808/2014-9).

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TRICHODINIDS (CILIOPHORA: PERITRICHIDA) OF *CORYDORAS PALEATUS* (SILURIFORMES: CALLICHTHYIDAE) AND *JENYNSIA MULTIDENTATA* (CYPRINODONTIFORMES: ANABLEPIDAE) FROM ARGENTINA

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Trichodinid ciliophorans remain a poorly studied group in Argentina and still not formally described in freshwater fishes. In this study, 32 specimens of the pepper Cory *Corydoras paletus* Jenyns, 1842 and 25 specimens of the sided-livebearer *Jenynsia multidentata* Jenyns, 1842 were examined for gills and skin parasites. Fish were collected from Samborombón River (Buenos Aires, Argentina) (35°10'39.79"S and 58°15'29.70"W) between April 2013 and August 2013. Four trichodinids were found: *Trichodina cribbi*, Dove and O´Donoghue 2005, *Trichodina gobii*, Raabe, 1959, *Trichodina pediculus* Ehrenberg, 1838 and a new species of *Paratrichodina*. Taxonomic and morphometric data for these trichodinids based on dry silver nitrate-impregnated specimens are presented. The new species of *Paratrichodina* is characterized mainly by a prominent blade apophysis, the short section connecting the blade and central part, and by the adoral ciliary spiral that makes a turn of 370°–380°. This study is the first formal report of these trichodinid species from South America and from freshwater fishes in Argentina. Moreover, this is the first record of *Paratrichodina* sp. in Argentina.

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METAZOAN PARASITES FROM THE OAXACA KILLIFISH *PROFUNDULUS OAXACAE* (MEEK), FROM OAXACA STATE, MEXICO

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Oaxaca killifish *Profundulus oaxacae* (Meek, 1902) (Cyprinodontiformes, Profunduliade) are an indigenous freshwater fish species exclusively found at Oaxaca State (Southern Mexico). In March 2015 we undertook the first comprehensive assessment of parasite assemblages from this fish species, collecting 64 killifish from 3 localities (Arroyo los Sabinos, El Toronjo and Río Grande) from Oaxaca. A total of 10 parasite species was found. The following species had gravid individuals: the digeneans *Paracreptotrema blancoi* (prevalence (P) : 54.7% ; mean intensity (I) : 6.0 [4.0-9.4]), *Paracreptotrema profundulusi* (P= 17.2%; I= 2.6 [1.6-3.8]), and *Phyllodistomum spinopapillatum* (P= 1.6%; I= 1); the monogeneans *Gyrodactylus* sp. (4.7% [1.3-13.1]; 1) and *Urocleidoides* sp. (14.1% [7.3-24.9]; 12.7 [6.1-22.7]); and the nematodes *Rhabdochona salgadoi* (45.3% [33.5-57.9]; 4.5 [3.1-7.3]) and *Freitascapillaria* sp. (14.1% [7.3-24.6]; 6.4 [4.1-9.7]) . We also found metacercaries of *Uvulifer* sp. (7.8% [3.1-17.0]; 2.8 [1.0-5.6]) and a single juvenile specimen of *Bothriocephalus acheilognathi* (1.6% [0.9-8.3]; 1). Additionally, spores of the myxozoan *Myxobolus* sp. were observed in the gall bladder. Our results showed that the Oaxaca killifish harbors some of the metazoan parasites that were previously reported for other species of killifish (*i.e.,* species of the genera *Paracreptotrema*, *Phyllodistomum* and *Rhabdochona*). However, at least 3 parasite taxa (*Freitascapillaria* sp., *Myxobollus* sp. and *Uvulifer* sp.) represent new parasite records for species of *Profundulus*.

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METAZOAN PARASITE ASSEMBLAGES OF *LEPORINUS FRIDERICI* (CHARACIFORMES, ANOSTOMIDAE) FROM THE PARANAPANEMA RIVER, SOUTHEASTERN BRAZIL

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The Neotropical region is recognized by high diversity of fishes with more than 6,025 species, and approximately 4,000 species are found in Brazilian rivers. *Leporinus friderici* is a freshwater fish popularly known as “piava” or “piau-três-pintas” in Brazil, and is widely distributed along South American rivers. The main purpose of this study is to perform a survey of the metazoan parasites of *L. friderici* from three tributaries under the influence of the Jurumirim reservoir, Upper Paranapanema River, Southeastern Brazil. A total of 94 specimens of *L. friderici* were sampled in three tributaries from April/2012 to March/2013, with 34 in the Taquari River, 30 in the Paranapanema River and 30 in the Veados River. Sixty-six individuals (71.30%) were parasitized by at least one parasite species. The component community was composed of 15 *taxa*, seven belonging to Monogenea Class (*Jainus* sp.1, *Jainus* sp.2, *Urocleidoides* sp.1, *Urocleidoides* sp.2, *Tereancistrum* sp., Dactylogyridae sp.1, Dactylogyridae sp.2.), five Nematoda (*Contracaecum* sp., *Rabdochona acuminata*, *Spinitectus asperus, Procamallanus (Spirocamallanus) amarali* and *Procamallanus (Spirocamallanus) iheringi*), one Digenea (metacercaria), one Cestoda (plerocercoid) and one crustacean. The component community in the host population of the Paranapanema River showed highest diversity (Shannon-Wiener Index H´=1.99) despite the lowest richness with nine species. In some studies, the biodiversity of fish parasites from Brazil is underestimated and even neglected. *Leporinus friderici* showed high parasite species richness in the Upper Paranapanema River and some of these *taxa* are possible new species. This study contributes to the biogeographical knowledge of metazoan parasites in Neotropical fishes.

Financial support: FAPESP (2011/22603-3, 2014/14298-4), CNPq (307808/2014-9).

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METAZOAN PARASITES OF EASTERN PACIFIC BONITO *SARDA CHILIENSIS* CUVIER, 1832 (PERCIFORMES: SCOMBRIDAE) IN THE PERUVIAN COAST

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The aim of this study was to evaluate the metazoan parasites of *Sarda chiliensis*, endemic to the eastern Pacific, acquired by artisanal fishery in Chorrillos, Lima, Peru. Parasites were catalogued and evaluated using standard protocols. During all the sampling a total of 267 metazoan parasites were collected with a total mean abundance of 2.67. Of percentage of individuals collected, endoparasites dominated (97%) against ectoparasites (3%). 26.6% of the parasites collected were metazoan mature forms of gill and gastrointestinal location and 73.4% corresponds to larval forms. The prevalence, mean intensity and the total mean abundance of infection was 58%, 2.67 and 4.60, respectively. The three parasites with more specific importance were *Sphyriocephalus viridis*, *Anisakis physeteris* and *Unitubulotestis pelamydis*. The dispersion index for these three parasites showed the typical pattern of distribution aggregated. In ten parasites, distribution type was not determined due to present lower prevalence of 10% (*Caligus bonito*, *Tentacularia coryphaenae*, *Nybelinia* sp., *Diphyllobothrium pacificum*, *Anisakis simplex*, *Contracaecum* sp., Nematoda gen. sp, *Corynosoma obtuscens*, *Bolbosoma* sp. and *Rhadinorhynchus* sp.). The infective stages of *A. simplex*, *A. physeteris*, *Contracaecum* sp., *D. pacificum* and *C. obtuscens* have zoonotic importance in the Peruvian coast. The parasites *S. viridis* and *U. pelamydis* were considered new records for Peru and *S. chiliensis* was a new host for *T. coryphaenae*, *Nybelinia* sp., *A. simplex*, *Contracaecum* sp., Nematoda gen. sp., *C. obtuscens*, *Bolbosoma* sp. and *Rhadinorhynchus* sp.

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FISH PARASITES IN NORTH EUROPEAN FRESHWATER BODIES

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The parasite fauna and feeding ecology of 376 freshwater fishes belonging to ten different species mainly from northern Germany (Lake Malchin, Lake Hohen Sprenz, Mecklenburg-Western Pomerania) and Latvia (Lake Baltezers) were studied between 2011 to 2015. *Abramis brama*, *Alburnus alburnus*, *Anguilla anguilla*, *Blicca bjoerkna*, *Carassius gibelio*, *Gymnocephalus cernuus*, *Perca fluviatilis*, *Scardinius erythrophthalmus* and *Tinca tinca* were examined only from Lake Malchin for the presence of protozoan and metazoan parasites. Roach, *Rutilus rutilus,* was studied from all sites. So far, 63 different protozoan (11) and metazoan (52) parasite taxa could be identified, including new host and locality records also for German freshwaters bodies. The composition of the parasite fauna differed according to the fish species, based on their different feeding behaviour. Comparison of the northern German freshwater fish parasite fauna with more southern and eastern (Poland, Russia, Latvia, Lithuania) localities revealed a close similarity, predominated by generalists. The two helminths *Diplostomum spathaceum* and *Tylodelphys clavata* as well as the crustacean genus *Ergasilus* had a wide geographical range and lowest host specificity. Most new host records were recorded from *G. cernuus*. The Digenea *Azygia* could be detected in already well-studied *A. anguilla* and *P. fluviatilis*. The parasite fauna of Lake Malchin is as commonly described for medium sized lakes. Only few parasite species with relevance to fisheries and aquaculture could be detected in Lake Malchin. However, parasites with a high disease potential such as *Dactylogyrus* spp., *Trichodina* spp. and a typical pond or aquarium ciliate *Ichthyophthirius multifiliis* could be identified.

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SMALL BUT MIGHTY-TREMATODE DIVERSITY IN SMALL PLANORBID SNAILS FROM A RESERVOIR IN GERMANY

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Man-made reservoir systems have been shown to offer ideal conditions for species-rich and diverse trematode communities. The recent discovery of cryptic and unknown species from the Ruhr area in Germany highlight the importance of extensive screenings of snails that serve as first intermediate hosts for trematodes. In this study we assessed the diversity of trematodes in small planorbids in a reservoir to estimate their contribution to the ecosystem's biodiversity. Snails were collected from the reservoir Hennetalsperre in 2014. Each snail was examined for trematode infections; trematode species were identified morphologically and molecularly. Altogether, 1,516 snails belonging to two species were examined: *Gyraulus albus* (n = 1,289) and *Segmentina nitida* (n = 226). The sampling revealed a diverse trematode fauna and seven trematode species were identified (*Australapatemon burti, Apharyngostrigea cornu, Cathaemasia hians, Hysteromorpha triloba, Petasiger* sp. 3*, Paryphostomum radiatum, Gigantobilharzia* sp.). *G. albus* showed a higher overall prevalence (7.8 %) and more diverse trematode fauna (seven species), whereas *S. nitida* was infected with only one species and showed a lower prevalence (0.9 %). The survey provides the first report of *C. hians* from Germany and the first report of both *C. hians* and *A. cornu* ex *G. albus*. Despite their small size, *G. albus* serves as an important first intermediate host for trematodes and contributes to the biodiversity in the studied reservoir. The findings of rare species that have not yet been reported from this region show the importance of extensive screening approaches to better understand the distribution of trematode species.

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MOLECULAR IDENTIFICATION OF METACERCARIAE OF *DIPLOSTOMUM* SPP. IN FISHES FROM THE RIVER DANUBE

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Species of *Diplostomum* are important parasites of fish but reliable data on their diversity in natural populations are virtually lacking due to the simple morphology of the metacercariae and the lack of reliable differentiating features. This study is the first to apply a barcoding approach to the diversity of *Diplostomum* in fishes from a large river in Europe. A total of 18 species of fish (81 specimens) was sampled opportunistically in the River Danube near Nyergesújfalu in Hungary. Specimens of *Diplostomum* spp. were recovered only from the lenses of nine fish species (*Alburnus alburnus*, *Ballerus sapa*, *Blicca bjoerkna*, *Chondrostoma nasus*, *Gymnocephalus schraetser*, *Leuciscus aspius*, *Lota lota*, *Rutilus rutilus* and *Vimba vimba*). Overall parasite intensity was low (1–15 metacercariae per fish) and nine species of fish, for which fewer specimens were examined, were not infected (*Abramis brama*, *Barbus barbus*, *Gobio gobio*, *Leuciscus idus*, *Ponticola kessleri*, *Sander lucioperca*, *S. volgensis*, *Zingel streber* and *Zingel zingel*). Barcoding of representative isolates (*cox*1; n=18) provided molecular identification of three species: *D. spathaceum* in *L. aspius*, *B. bjoerkna*, *C. nasus* and *R. rutilus*; *D. pseudospathaceum* in *L. aspius*, *B. bjoerkna*, *G. schraetser*, *L. lota* and *V. vimba*; and "*D. mergi* Lineage 2" in *A. alburnus* and *B. sapa* (both new host records). This study is the first to establish a link between cercariae from *Radix auricularia* and metacercariae of the "*D. mergi* Lineage 2" in fish hosts thus partially elucidating the life-cycle of this putative species.

This study was funded by the project ECIP P505/12/G112.

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PARASITATION OF SEA TROUT (*SALMO TRUTTA TRUTTA* L.) FROM THE SPAWNING GROUND AND GERMAN COASTAL WATERS OFF MECKLENBURG-WESTERN POMERANIA, BALTIC SEA

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A total of 52 sea trout, *Salmo trutta trutta* were studied for parasites, originating from freshwater streams and the German coastal waters of the Baltic Sea. While 35 specimens were caught mainly close to the shoreline in the Baltic Sea, 17 specimens were sampled during their spawning migration in Warnow River and other neighbouring rivers of Mecklenburg-Western Pomerania. A total of twelve different parasite species were found in sea trout originating from the Baltic Sea, including five digeneans, two cestodes, three nematodes and two acanthocephalans. Below these taxa, marine and freshwater species were present. In the migratory trout, seven different parasite species were found, demonstrating lower parasite richness and load during the spawning migration compared with the fish from the Baltic Sea. The nematodes *Anisakis simplex* (sensu stricto), *Contraceacum rudolphii* and *Hysterothylacium aduncum* were identified by molecular analyses of the ITS-1, 5.8S, ITS-2 and flanking sequences of the rDNA. These sequences were deposited in GenBank (Accession numbers: KM406484, KP119838 and KP119839).Together with the digenean *Derogenes varicus*, *Hemiurus communis* and *H. luehei*, they represent six new host records for sea trout from the German part of the Baltic Sea.

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MACROPARASITES OF ALLIS SHAD (*ALOSA ALOSA*) AND TWAITE SHAD (*ALOSA FALLAX*) OF THE WESTERN IBERIAN PENINSULA RIVERS

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Samples of *Alosa alosa* (n= 163) and *Alosa fallax* (n= 223), caught in Western Iberian Peninsula Rivers from 2008 to 2013, were examined for macroparasites. *Alosa alosa* were infected with *Anisakis simplex* s.s., *Anisakis pegreffii, Hysterothylacium aduncum*, *Rhadinorhynchus pristis*, *Mazocraes alosae*, *Hemiurus appendiculatus*, *Ceratothoa italica* and an unidentified ergasilid copepod. The isopod *C. italica* represents a new host record for *A. alosa*. *Alosa fallax* were infected with *A. simplex* s.s., *A. pegreffii*, *H. aduncum*, *H. appendiculatus*, *Clavellisa emarginata* and an unidentified cymothoid isopod. This is the first report of the occurrence of *C. italica*, *C. emarginata* and *M. alosae* in the Iberian Peninsula. The phylogenetic positions of *M. alosae*, *H. appendiculatus* and *C. emarginata* were assessed using partial regions of the small and large subunit ribosomal RNA genes. Qualitative and quantitative differences in the parasite faunas of these two shad species are consistent with different feeding strategies (i.e. *A. alosa* zooplanktophagous and *A. fallax* ichthyophagous). The results provide information about host migration behaviour and transmission pathways through diet during the marine trophic phase of the shad's life cycle, their roles as paratenic or final hosts and transporters of parasites between seawater and freshwater environments. The presence of the zoonotic parasites *A. simplex* s.s. and *A. pegreffii* might entail a risk for consumers or riverine mammals that should be considered by authorities. Furthermore, the use of parasites as biological tags for shad stocks in Western Iberian Rivers could be a useful approach in multidisciplinary studies concerning fish stock delimitation and characterization.

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NEW PARASITE RECORDS FROM OCEAN SUNFISH *MOLA MOLA* (L.)

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During the study of 106 ocean sunfish *Mola mola* (L.) collected and analysed from 2005 to 2008 in La Azohía (Cartagena, Murcia, Spain) and Canet de Berenguer and Almazora (Valencian Community, Spain) a total of 21,703 metazoan parasites were collected and identified. All parasites were examined in saline solution under stereomicroscope (up to 80X) for initial classification and identification. Twenty seven species of metazoan parasites were identified: 10 trematodes, 1 monogenean, 4 cestodes, 1 acanthocephalan, 3 nematodes, 7 copepods and 1 isopod. With this study the richness of the parasite fauna of *M. mola* is increased, as 10 species previously not recorded in sunfish were identified: 1 trematode, 1 acanthocephala, 3 nematodes, 4 copepods and 1 isopod are new host records. To date, this is the most comprehensive study on the metazoan parasitefauna of this fish species worldwide.

Funded by AGL2010-20892, ISIC/2012/003 & PROMETEO 795/2015/018 projects.

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DIGENEAN PARASITES OF TWO CLOSELY RELATED SPARID FISHES, THE BOGUE *BOOPS BOOPS* AND THE BLOTCHED PICAREL *SPICARA MAENA* IN THE WESTERN MEDITERRANEAN OF ALGERIA

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The genus *Spicara* has recently been transferred from the family Centracanthidae to the family Sparidae. Although this genus is not monophyletic, in most of the published phylogenies *Spicara maena* and *S. smaris* appear closely related to species of the genera *Salpa*, *Spondyliosoma* and *Boops*. We carried out a comparative analysis of the digenean richness and abundance in two of these closely related sparid hosts, *Boops boops* and *Spicara maena*, in order to assess whether these differ in relation to trophically transmitted parasites. Totals of 237 *S. maena* and 238 *B. boops* were examined during 2013-2014 off the Algerian coasts. We identified 17 species (including metacercariae of 4 species) in *Boops boops* and 13 species (including metacercariae of 3 species) in *S. maena*. The two hosts shared 11 species representing 85% and 65% of the species found in *S. maena* and *B. boops,* respectively. Of these, four species (*Aphanurus stossichii*, *Hemiurus communis*, *Magnibursatus bartolii* and *Prosorhynchus crucibulum* met.) were more abundant in *B. boops* whereas three (*Ectenurus lepidus*, *Lepocreadium album* and juvenile lepocreadiids) were more abundant in *S. maena* (all $p<0.02$). Finally, two of the six species found in our study exclusively in *B. boops* and the two species found exclusively in *S. maena*, have been previously recorded in both hosts in the Mediterranean. The substantial overlap observed in the digenean fauna of the two hosts may be associated with both their phylogenetic proximity and the similar feeding habits (i.e. omnivorous diet based on suction feeding).

Partially funded by projects UV-INV-PRECOMP14-206687, Fundación Biodiversidad.

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DIGENEAN PARASITES OF THREE MARINE TELEOST FISH OF COMMERCIAL IMPORTANCE (*MERLUCCIUS MERLUCCIUS*, *PAGELLUS ERYTHRINUS* AND *TRACHURUS TRACHURUS*) FROM WESTERN ALGERIA

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The investigation into the digenean fauna of three teleost fishes (*Merluccius merluccius*, *Pagellus erythrinus*, *Trachurus trachurus*) was performed in the Algerian coasts of the western Mediterranean. These fishes of commercial importance represent an important biological resource in marine food webs and for the human population along Mediterranean coasts. However, the data on their digenean parasites in Algeria are incomplete. Therefore, the aim of this study was to provide new data on the diversity and species composition of digeneans from these hosts. A total of 720 fish (200 *M. merluccius*, 200 *P.erythrinus* and 320 *T. trachurus*) were collected off Oran, north western Algeria during 24 months (September 2010–October 2012). Ten species of digeneans were found in the three fish hosts: (i) *Aporocotyle* sp. (Aporocotylidae) and *Derogenes varicus* Müller, 1784 (Hemiuridae) in *Merluccius merluccius* (L); the former species is reported from Algeria for the first time; (ii) *Sphinteristomum* sp. (Apocreadiidae), *Lepocreadium pegorchis* Stossich, 1901 (Lepocreadiidae), *Ectenurus lepidus* Looss, 1907 (Hemiuridae) and *Opecoeloides* sp. (Opecoelidae) in *Pagellus erythrinus* (L); and (iii) *Ectenurus lepidus*, *Lecithochirium fusiforme* (both Hemiuridae), *Monascus filiformis* Rudolphi,1819 (Fellodistomidae) and *Pseudopecoeloides chloroscombri* Fischthal & Thomas, 1970 (Opecoelidae). *Aporocotyle* sp*. a*nd *Derogenes varicus* found in *Merluccius merluccius* are recorded from Algerian for the first time. Even though the fish species studied share the same habitat, they harbour different species of digeneans with the exception of the euryxenous *Ectenurus Lepidus.*

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BIODIVERSITY AND SPECIFICITY OF COPEPODS PARASITES OF CHONDRICHTHYAN FISH OFF THE TUNISIAN COASTS

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The parasitic crustaceans are common ectoparasites of fishes. Parasitic copepods on fish are well known in teleosts from the Mediterranean Sea and in Tunisia in particular, but those living on chondrichthyans remain poorly known. Thus, in order to update and complete the knowledge, we collect 300 chondrichthyans, belonging to five families and seven species, mainly from the gulf of Tunis and the gulf of Gabes. This study allowed us to collect seven species of parasitic copepods belonging to five families (Lernaeopodidae, Eudactylinidae, Kroyeriidae, Trebiidae and Sphyriidae). The Lernaeopodidae and Eudactylinudae were the more representative families. We report for the first time in Tunisia, the presence of *Tripaphylus musteli* on *Mustelus mustelus* (P=3.12% ; Im= 1 ; A= 0.031), *Nemesis lamna vermi* on *Dasyatis centroura* (P=12.76% ; Im= 6.33 ; A= 0.12). We also reveal the infection of *Dasyatis centroura* by *Eudactylina insolens* (P=2,12% ; Im = 1 ; A = 0.021) and by *Pseudocharopinus bicaudatus* (P=6.38% ; Im=2 ; A=0.063) for the first time in the Mediterranean area and off the Tunisian coasts. This work has contributed to the enrichment of the knowledge of the Mediterranean and Tunisian parasitic copepod diversity. We have found that parasitic copepods in chondrichthyans are generalist infecting from the largest to the smallest species.

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PARASITES OF FISH FROM TUNISIA

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In the past, little attention has been paid to parasites and their importance in the functioning of ecosystems. At present, parasites are considered as actors playing a crucial role in ecological studies because they provide valuable information on host populations, ecosystem structure, evolutionary hypotheses and biodiversity. Moreover, because of the complex life cycles of the majority of parasites, they can also provide important information (distribution, food habits and migration of the host) often unavailable by other means.

In fish, the most numerous and diverse vertebrate group, a high number of parasites (Protista, myxozoans, helminths, crustaceans and others) have been found, with most comprehensive data available from Europe and North America. In contrast, data on the parasites of fish in Africa are still scarce. In Tunisia, fish parasites have been studied since 1970, with focus on marine fish of commercial importance. Until now, about 6 species of protists, 30 species of myxozoans, 150 monogeneans, 110 digeneans, 90 cestodes, 10 nematodes, 10 acanthocephalans, 100 copepods and 8 isopods have been found. The parasites of freshwater fish are little known and only one species of nematodes and one species of tapeworms have been recorded from this country. Various approaches (morphological, ultrastructural and molecular) have been used to identify the various parasites groups of our coasts.

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THE PARASITE DIVERSITY OF *AMPHILIUS URANOSCOPUS* AND *CHILOGLANIS PRETORIAE* IN THE VHEMBE BIOSPHERE RESERVE, LIMPOPO PROVINCE, SOUTH AFRICA

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The parasite diversity of smaller fish species from tributaries of the Luvuvhu River in the Vhembe Biosphere Reserve (VBR) is unknown. Despite considerable progress in fish parasitology around the world in the last decades, some gaps still exist in understanding of the taxonomy, epizootiology, biology and control of some fish parasites, especially in South Africa. In the present study, an assessment of the parasite composition of the Stargazer mountain catfish, *Amphilius uranoscopus* (Pfeffer, 1889) and Shortspine rock catlet, *Chiloglanis pretoriae* Van der Horst, 1931 was done during April and August 2014 from four tributaries (Barotta, Dzindi, Lutanandwa and Mvudi) of the Luvuvhu River in VBR. Fishes were collected using electro-fishing gear, weighed, measured and examined for endo- and ectoparasites. All parasites were fixed and preserved according to standard methods for each parasite group. Parasites included digenean metacercariae, i.e. *Diplostomum* sp. (Diplostomatidae) and *Clinostomum* sp. (Clinostomidae) and nematode larvae *Contracaecum* sp. (Anisakidae) in the body cavity, recorded from both fish species. *Amphilius uranoscopus* was also infected by *Paracamallanus* sp. (Camallanidae) and an unidentified nematode of the Quimperiidae family in the intestine. No parasites were recorded from the latter fish species collected at Mvudi River (more polluted site). Moreover, *C. pretoriae* which occurred only in three rivers (Barotta, Dzindi and Lutanandwa) was infected with an unidentified monogenean from the Ancyrocephalidae family. The results of this study provide information on new distribution and host records of fish parasites from South Africa and can be implemented in the management and conservation of the VBR.

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PARASITE INFECTIONS OF *PSEUDOCRENILABRUS PHILANDER* AND *BARBUS PALUDINOSUS* FROM A RAMSAR WETLAND IN SOUTH AFRICA

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Barberspan Bird Sanctuary, a RAMSAR wetland of international importance, is situated in the North-West province of South Africa. Fish parasites have previously been recorded from many of the larger fish species from this locality but a paucity of information is available on parasites of the smaller fish species. *Pseudocrenilabrus philander* (Weber, 1897) and *Barbus paludinosus* Peters, 1852 were collected during October 2014 and March 2015 using small fyke nets, seine nets and electrofishing techniques. Collected fishes were comprehensively screened for metazoan parasites. *Pseudocrenilabrus philander* was found to be parasitised by Gryporhynchidae cestode larvae with prevalences of 77% and 67% in October 2014 and March 2015 respectively, with the intensity of infection (IF) ranging from 1 to 11 specimens per host. These cestodes were represented by 5 different species - *Paradilepis scolesina* (Rudolphi, 1819), *Paradilepis* sp., *Valipora campylancristrota* (Wedell, 1855), *Parvitaenia* sp. 1 and *Parvitaenia* sp. 2. Nematode larvae and cysts with metecercaria were also found in the mesenteria of a few specimens of *P. philander*. During March 2015, *Gyrodactylus* sp. (47% prevalence) and *Lernaea* sp. (6% prevalence) ectoparasites of *P. philander* were also recorded. Only ectoparasites were collected from *B. paludinosus* during both sampling periods. Monogeneans of *Dactylogyrus* spp. were present in prevalences of 100% (2014) and 53% (2015), respectively, with IF ranging from 2 to 18 specimens. Moreover, Myxozoan plasmodiums, *Dolops* sp. and *Ergasilus* sp. were also found on this small cyprinid. This is the first study reporting parasite biodiversity of small fishes from this RAMSAR wetland.

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ASPECTS OF ECOLOGY OF METAZOAN PARASITES FROM *LABEO* SPECIES IN THE MIDDLE LIMPOPO RIVER BASIN

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The present study reports metazoan fish parasites from four *Labeo* species in the middle Limpopo River Basin which forms international boundaries between Botswana, South Africa, Zimbabwe and Mozambique. Totally 149 specimens of *Labeo cylindricus, Labeo molybdinus, Labeo rosae* and *Labeo ruddi* were collected from Bubiana Dam and Bubi River in Zimbabwe whereas in South Africa they were collected from Nwanedi River and Nwanedi-Luphephe Dam. Gill nets and electro-fishing were used for sampling. Fishes were sacrificed and parasites were isolated from the gills, skin, muscle, body cavity, gall bladder, stomach and intestine. Standard methods for fixing and preserving the parasites were followed and they were identified based on morphology features. Seventeen parasite species were found comprising monogeneans (*Dactylogyrus* spp., *Dogielius* spp. and *Diplozoon* sp.), copepods (*Lamproglena* sp.), nematodes (*Philometroides khalili*), digeneans (*Nematobothrium* sp. and one unidentified metacercariae). This current study contributes to knowledge of the parasitic fauna by recording new host species and new geographic records. Future studies are essential in order to verify the diverse spectrum of metazoan parasites infesting many *Labeo* fish species in this area.

METAZOAN PARASITES OF THE MOZAMBIQUE TILAPIA (*OREOCHROMIS MOSSAMBICUS*) FROM TWO WATER BODIES IN MOZAMBIQUE P-135

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Since fish parasitology has not received much attention in Mozambique this study investigated metazoan parasites associated with Mozambique tilapia (*Oreochromis mossambicus*) from Lake Urema (*n* = 30), which is located in Sofala Province during October 2011, and from Massingir Dam (*n* = 15) within Gaza Province during February 2013. Fish specimens were collected using gill nets. Monogenean parasites were removed and mounted on slides using glycerine jelly while others were fixed and preserved according to standard methods prescribed for each group. Results revealed eight different species, with three occurring at both localities. In both systems, *Cichlidogyrus halli* (monogenean) was reported from the gills, *Contracaecum* larvae (nematode) from the body cavity and *Lernaea cyprinacea* (copepod) on the skin of fish sampled. While in Lake Urema the trematode metacercaria *Euclinostomum* sp. was embedded in the muscle tissue with *Ergasilus mirabilis* (copepod) attached to the gills. From Massingir Dam an unidentified digenean metacercaria was recorded in the body cavity with gryporhynchid cestode larvae and *Acanthogyrus* sp. (achanthocephalan) occurring in the intestine. At Lake Urema the parasite community was dominated by ectoparasites with all hosts infested with *E. mirabilis* and *C. halli* and 86.7% with *L. cyprinacea*. Similarly in Massingir Dam all fish were infected with *C. halli*, however, endoparasites were dominant. This study provides a valuable contribution towards the known fish parasite fauna of Mozambique resulting in four new parasite species and three new host records for this country, as well as a first host record for *E. mirabilis* from *O. mossambicus*, being documented.

CAPTIVE AND WILD HOSTS OF *GYRODACTYLUS CORLEONIS* PALADINI, CABLE, FIORAVANTI, FARIA & SHINN, 2010 (MONOGENEA: GYRODACTYLIDAE) IN THE WESTERN MEDITERRANEAN SEA P-136

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Gyrodactylus corleonis Paladini, Cable, Fioravanti, Faria & Shinn, 2010 is a gyrodactylid monogenean originally described from captive *Syngnathus typhle* L. held in aquarium in Emilia-Romagna (Italy), and afterwards no more recorded. The finding of this monogenean in *Gobius cobitis* Pallas caught off northwestern Sardinia (western Mediterranean Sea, 41°08'05''N 9°06'05''E), represents a new host record, but above all the first description of this parasite in the wild.

Nine specimens of *G. cobitis* were examined for monogenean parasites, and several specimens of *G. corleonis* were collected from the body and fins. These specimens are practically identical in the morphology to the original description. A detailed comparison of *G. corleonis* ex *S. typhle* with all known *Gyrodactylus* spp. from syngnathids was already presented by Paladini et al. (2010). Taking into account the present finding of *G. corleonis* on a gobiid in the wild, its comparison with other *Gyrodactylus* spp. of gobies is presented. Among all known gyrodactylids from gobiidaes, *Gyrodactylus bubyri* Osmanov, 1965 from *Knipowitschia caucasica* (Berg) is the most similar to *G. corleonis*. Moreover, in accordance with the phylogenetic relationships of marine gyrodactylids indicated by DNA analysis (Ziętara et al., 2012), *Gyrodactylus* spp. from sand gobies are very close relatives to *G. corleonis* and two other species from syngnathids.

The finding of *G. corleonis* on *G. cobitis* suggests that genetic and morphological data may need to be looked at again in terms of the relationships of gyrodactylids from syngnathids and gobiids.

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THE CYPRINIDAE OF MOROCCO AND ITS GILL MONOGENEA: SYSTEMATIC AND BIOGEOGRAPHY

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The Moroccan continental water is rare, and the Cyprinidae the most frequent freshwater fish group. Native and endemic cyprinids in Morocco included 7 *Luciobarbus* species, two *Carassobarbus* species and *Ptercapoeta maroccana*. Climate change is likely to endanger the rare and precious water resources. If we want to mitigate their effects through a careful management, we should first identify fish species with certainty and also understand their complex evolutionary history (different origins), which is a challenge because of their polyploidization and intra- and inter-generic hybridization phenomena. One of the solutions, in addition to conventional methods (morphology and genetics), is to study host specific parasites (Monogenea), which can be used as biological markers (systematic, phylogenetic and biogeography of hosts). Three sampling campaigns were carried out in Southern part of Morocco in 2014-15. More than 500 fishes were sampled from 16 localities. Their examination confirmed that the fishes belong to three genera *Luciobarbus, Carassobarbus* and *Ptercapoeta* The examination of the gill arches revealed the presence of 24 *Dactylogyrus* species, including twenty likely new species. One monogenean species collected on the fish gills possessed no anchors in its haptor. Our study also shows a specificity of the parasites at a species level, but also at a lower level with a clear difference between Northern and Southern Atlas monogenean populations of *Carassobarbus fritshii.*

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SEASONAL DISTRIBUTION OF MONOGENEAN PARASITES GILL OF THREE TELEOST FISH (*DIPLODUS ANNULARIS*, *PAGELLUS ERYTHRINUS* AND *LITHOGNATUS MORMYRUS*) FROM THE GULF OF ANNABA (NORTHEASTERN ALGERIA)

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A seasonal distribution study of parasite populations of gills from 720 specimens belonging to three species of teleost fish: *Pagellus erythrinus, Diplodus annularis* and *Lithognathus mormyrus* (Sparidae) was realized from the gulf of Annaba (northeastern Algeria). A preliminary description allows to distinguish ten parasite species belonging to two subclasses: Monopisthocotylea and Polyopisthocotylea.Parasitic indices were calculated for the host species and parasite species, and a statistical test of independence highlighted a significant variation between the seasons and the parasitic indices for each fish species, probably caused to the period of reproduction of each hosts. The study of population structure of identified parasites (S, H ', H'max and E), shows maximum values in autumn.

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MONOGENEA OF *LABEO* SPECIES FROM THE OLIFANTS AND LIMPOPO RIVER SYSTEMS IN SOUTHERN AFRICA

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The knowledge of the diversity of monogeneans is important, but more so in their prevention or treatment when their fish hosts are subjected to aquaculture. This study identifies monogeneans on the gills of *Labeo* species found in the Olifants and Limpopo River systems in southern Africa. Fish hosts (n=177) were sampled from both river systems and consisted of *Labeo molybdinus* (n=35), *L. rosae* (n=46), *L. rudii* (n=34) and *L. cylindricus* (n=62). The hosts were collected using electro-fishing and gill nets. Fish were sacrificed by cutting the spinal cord just behind the brain, examined for gill monogeneans, and the parasites were mounted on slides using either glycerine jelly or glycerine ammonium picrate (GAP). The monogeneans were identified by morphometric variations of the hard parts of the opisthaptor and copulatory organ using a BX50 microscope fitted with a camera and a drawing tube.

There were eight species of *Dactylogyrus* (*D. brevicirrus, D. cyclocirrus, D. falcilocus, D. longiphallus, D. longiphalloides, D. senegalensis, D. pienaari* and *Dactylogyrus* sp.), three species of *Dogielius* (*D. dublicornis, Dogielius* sp. 1 and *Dogielius* sp. 2) and one *Diplozoon* sp.

This study has shown that each of the four fish species investigated harbours at least three to eight different monogeneans on its gills. Again, some of these monogeneans are genus specific and not species specific. Most of these monogeneans are first records for southern Africa and some are first host records.

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MONOGENEAN INFECTIONS ON SOME FISHES FROM LESSER ZAB RIVER, NEAR KYSINJAQ CITY, KURDISTAN REGION, IRAQ

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A total of 362 fishes, belonged to six species namely: *Barbus kersin*, *Capoeta trutta*, *Carassobarbus luteus*, *Cyprinion macrostomum*, *Cyprinus carpio* (Family Cyprinidae) and *Liza abu* (Family Muglidae) were collected from Lesser Zab river, southeast of Koysinjaq city, east of Erbil province, Kurdistan region, Iraq, from April 2012 to the end of January 2013. The fishes were examined for monogenean parasites. The study revealed the existence of 14 species including: six species of *Dactylogyrus* (*D. barburli*, *D. baueri, D. elegantis*, *D. extensus*, *D. formosus* and *D. vistulae*), two species of *Dogilus* (*D. mokhayeri* and *D. persicus*) four species of *Gyrodactylus* (*G. elegans*, *G. khathrineri*, *G. schulmani* and *G. sprostonae*), two species of *Paradiplozoon* (*P. homoion* and *P. tadjikistanicum*), and one species of *Microcotyle* (*M. donavini*). Three of these species (*G. khathrineri*, *G. schulmani* and *P. tadjikistanicum*) were recorded for the first time in Iraq, and two species (*M. donavini* and *P. homoion*) in Kurdistan region. Also, three species of fishes were regarded as new host in Iraq for nine species of the studied monogeneans.

ON THE ECOLOGY OF MONOGENEAN PARASITES OF FRESHWATER FISHES OF IRAN

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Monogenean parasites of freshwater fishes of Iran have received most attention from fish parasitologists in two last decades. While around 280 parasites species have been known so far, at least 140 species are belonging to class monogenea. The main reason of such attention was the rapid developing of fish culture, intensification and applying new technologies during 30 years or so in Iran, which consequently favoured various parasitoses mostly monogenoses appearing and endangering the success of production. Over 85% of known monogeneans belong to two families, *Dactylogiridae* and *Gyrodactylidae,* which are highly pathogenic for their fish hosts. It seems that problems specially appeared when common carp and Chinese carp where introduced to make progress in polyculture systems in Iran through zoogeographical barriers. Among these species *Gyrodactylus derjavini*, *Dactylogyrus extensus* and *D. vastator* can be mentioned. In kutum fish, *Rutilus frisii* kutum, *D. frisii* proved to be the most common species in both freshwater and Caspian Sea environment and found to be most invasive species in fingerlings during their short period (2-3 months) in ponds. Endemic monogenean parasites in Iranian freshwater fishes are apparently narrow specific species such type of specificity may also be encountered amongst the endemic host species which inhabit in old region like Tigris and Oriental where situated in east, west and south of the country. In the present paper species composition, specificity and endemicity of monogenean parasites (particularly *Dactylogyridae*) are discussed and their geographical distribution is stressed.

SEASONAL VARIATION OF MONOGENEANS ON THE YELLOWTAIL SCAD *ATULE MATE* (CUVIER, 1833) AND YELLOW-STRIPE SCAD *SELAROIDES LEPTOLEPIS* (CUVIER, 1833) FROM THE GULF OF THAILAND

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The seasonal occurrence of monogeneans was examined in two species of commercial carangid fishes, *Atule mate* and *Selaroides leptolepis*, collected monthly from the upper gulf of Thailand from December, 2013 to November, 2014. From a total of 203 *A. mate* samples two monogenean species were found, *Gastrocotyle* sp. and *Monaxinoides austronensis,* with prevalence and intensity of infection as 12.81%, 1.27 and 8.37%, 1.27, respectively. Both species of monogenean parasites from *A. mate* showed peaks of infection in winter and slightly decreasing in summer and rainy season. Examination of 265 *S. leptolepis* samples were investigated two monogenean species, *Pseudaxinoides vientnamensis* and *Pseudaxine bivaginalis* with the prevalence and intensity of infection as 17.36%, 1.50 and 30.19%, 2.88, respectively. The highest of prevalence and intensity of infection in both monogenean species from *S. leptolepis* occurred in summer, followed by winter and rainy season. The size of fish hosts did not have an influence on monogenean infection. These finding suggested that period of peak prevalence of discovered parasitic infestation were well corresponded to the spawning season of both *A. mate* and *S. leptolepis.*

MONOGENEANS OF THE ARMORED CATFISH *PTERYGOPLICHTHYS ANISITSI* EIGENMANN & KENNEDY, 1903 FROM THE AGUAPEÍ RIVER, AGUAPEÍ RIVER MOUTH, MUNICIPALITY OF CASTILHO, SÃO PAULO STATE, BRAZIL

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This study aims to characterize the composition and structure of the monogenean ectoparasites of the Neotropical armored catfish *Pterygoplichthys anisitsi*, one of the dominant species in the Aguapeí River, Aguapeí River Mouth, municipality of Castilho, São Paulo State, Brazil. A total of 80 fish specimens (standard length in centimeters 28.86±4.57 [20.5-45] and weight in grams 492.32±205.49 [191.5-1,520]) were collected in four sampling expeditions, from August/2013 to November/2014, two in the rainy season and two in the dry season. The skin, nasal cavity and gills were analyzed to find monogenean ectoparasites. The monogenean species found were: *Pavanelliella pavanellii* - Prevalence (P)=43.75%, Mean Intensity of Infection (MII)=4.11±0.68, Mean Abundance (MA)=1.8±0.37; *Heteropriapulus heterotylus* - P=66.25%, MII=12.32±2.45, MA=8.16±1.74; *Heteropriapulus simplex* - P=62.5%, MII=11.3±1.96, MA=7.06±1.37; *Heteropriapulus* n. sp. 1 - P=60%, MII=3.56±0.53; MA=2.13±0.37; *Heteropriapulus* n. sp. 2 - P=35%, MII=3.07±0.57, MA=1.07±0.25; *Trinigyrus* n. sp. - P=12.5%, MII=29.4±24, MA=3.67±3; *Unilatus brittani* - P=12.5%, MII=1.4±0.16, MA=0.17±0.05; *Unilatus unilatus* - P=10%, MII=3±1.03, MA=0.3±0.14; and Dactylogyridae gen. sp. - P=43.75%, MII=2.71±0.66 (1-20), MA=1.18±0.32. The monogenean species found are new geographical records, and the species *P. pavanellii, H. heterotylus* and *H. simplex* are new host records. This study represents an important contribution due to the new records and new species found, broadening the knowledge on fish monogeneans in the Neotropical region.

Acknowledgements: Fapesp Doctorate Fellowship 2012/22895-7; CNPq (307808/2014-9), and CESP (Electric Company of São Paulo State).

INFESTATION OF *CHAETODON STRIATUS* BY A MONOGENEAN GILL PARASITE (MONOGENEA: POLYOPISTHOCOTYLEA)

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Representatives of the Chaetodontidae (Actinopterygii: Perciformes) are widely appreciated in the ornamental fish market, frequently suffering from overexploitation. Aquaculture offers an alternative to extractive fishing activities, promoting a conservation strategy that is more reliable than catch restrictions. Nevertheless, in order to develop culture protocols for a fish, it is first necessary to understand aspects associated with its biology. In this study, we aimed to gather information on the parasitic fauna of the butterflyfish *Chaetodon striatus* Linnaeus, 1758. From May 2014 to February 2015, specimens of *C. striatus* were collected using fish traps in the southeast coast of Brazil and transferred to a recirculating aquaculture system (RAS) where they were kept for further research. Fifteen specimens were anesthetized, biometrically measured and euthanized for parasitological analysis of their gills. Gills were fixed in 10% formalin, scraped and analyzed under stereomicroscope for detection of parasites. Parasitological indexes were calculated according to Bush et al (1997) and parasites were mounted in Hoyer's medium on a slide for identification. Data is presented as mean ± standard deviation. Fish had total length of 12.7 ± 1.1 and weight of 60 ± 17.1. Prevalence of parasitism by Monogenea was 53%, mean intensity was 6 ± 1.8 and mean abundance was 2.7 ± 3.4. Monogenean parasites belonged to a single species, representative of the subclass Polyopisthocotylea and belonging to the Heteraxinidae family. This is the first report of this group of Monogenea in this fish species.

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MONOGENEAN ECTOPARASITES (GYRODATYLIDEA) FROM THE ANTARCTIC FISHES IN PRINCE GUSTAV CHANNEL, WEDDEL SEA

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Gyrodactylidea Bychowsky, 1937 (Monogenea) includes species that parasitize predominantly on the gills or the fins of fish. To date, only nine species of the genus *Gyrodactylus* Nordmann, 1832 (Gyrodactylidae Cobbold, 1864) and five species of the three genera of Tetraonchoididae (*Allotetraonchoides* Dillon & Hargis, 1968, *Neopavlovskioides* Dillon & Hargis, 1968 and *Pavlovskioides* Bychowsky, Gusev & Nagibina, 1965) have been described from fishes living in Antarctic waters. The fish were sampled in Prince Gustav Channel, Weddell Sea during the Antarctic summers of 2013 and 2014. Examined fish belong to genera *Notothenia* Richardson, 1844 and *Trematomus* Boulenger, 1902 (both Nototheniidae Günther, 1861) and *Parachaenichthys* Boulenger, 1902 (Bathydraconidae Regan, 1914). A prevalence of 38% (44/115) was recorded for monogeneans. Species identification based on morphometric analysis of hard parts of attachment organ shown the presence of seven species from genera *Gyrodactylus* and *Pavloskoides*. From *Notothenia coriceps* two *Gyrodactylus* species were identified, *Gyrodactylus coriicepsi and Gyrodactylus* sp. 1. From the fish of *Trematomus (T. newnesi*, *T. bernacchi*, *T. eulepidotus* and *T. hansoni)* four *Gyrodactylus* species and two *Pavlovskoides* species were recognized, namely *G. australis*, *Gyrodactylus* sp. 1, *Gyrodactylus* sp. 2, *Gyrodactylus* sp. 3 and *Pavlovskoides* sp. 1 and *Pavlovskoides* sp. 2. No monogeneans were observed from fish of the genus *Parachaenichthys*. The highest species richness, 4 species of *Gyrodactylus* and 2 species of *Pavlovskoides,* was observed on *T. bernacchi*. The highest prevalence of 54% was observed for *G. australis* on *T. newnesi*. This study significantly extends the knowledge about monogeneans in the Antarctic region.

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PROTEOCEPHALIDEAN TAPEWORMS (CESTODA) FROM FRESHWATER FISHES IN SOUTH AMERICA: WHAT DO WE KNOW?

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The order Proteocephalidea (= Onchoproteocephalidea *pro parte*) comprises 315 valid species with a worldwide distribution parasitizing freshwater teleosts, reptiles and amphibians; one species is known from a mammal. So far, freshwater catfishes (Siluriformes) inhabiting the major river basins of South America represent the dominant host group. Here we provide the information based on literature search of records of proteocephalidean tapeworms from freshwater fishes in South America. The compiled database provides records of 132 valid species representing 37 genera within 9 subfamilies and 1 family, associated with 64 species of fishes. Besides the order Siluriformes, which represents ≈89% of the hosts, members of the orders Atheriniformes, Characiformes, Gymnotiformes and Perciformes have also been found as hosts. The proteocephalidean with the highest number of host associations (5) is *Choanoscolex abscisus* reported from the Orinoco, Amazon and Paraná River basins, whereas most of the other taxa exhibit rather narrow host specificity, being recorded from a single host (oioxenous specificity; 44%) or a single host genus (mesostenoxenous specificity; 23%). The Paraná and Amazon River basins host the highest number of species; only three cestode species were described from other hydrological drainage basins. Since the Neotropical region hosts the highest diversity of freshwater fishes on the Earth, it can be assumed that the proteocephalidean biodiversity in South America in not yet well known. Some insufficiently studied hydrological drainages such as São Francisco and the Tocantins-Araguaia River basins may host unknown cestode taxa.

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COMPOSITION AND DIVERSITY OF HELMINTH FAUNA OF THE SHORTFIN MAKO (*ISURUS OXYRINCHUS*) IN THE NE ATLANTIC: AN EXAMPLE OF THE PELAGIC PREDATOR SYNDROME

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In the present study we describe the intestinal parasite community of 29 juvenile and adult specimens of shortfin mako (*Isurus oxyrinchus*). The specimens were captured by longline fishery in the northeast Atlantic Ocean open waters west from Portugal and Spain. Adult specimens of 4 species of cestodes were identified: *Molicola* sp. (Trypanorhyncha) with a prevalence of 3.4%, two species of *Rhodobothrium* (Tetraphyllidea) with prevalences of 82.8%, and 10.3%, respectively, and *Crossobothrium* sp. with a prevalence of 17.2%. Immature specimens of *Nybelinia* sp. (Trypanorhyncha, prevalence: 17.2%) and *Dinobothrium* sp. (Tetraphyllidea; 3.4%) were also found. Mean species richness (± S.D.) and mean total helminth abundance per host were 1.34 and 28.93, respectively. Species composition at infracommunity level did not depart from the null hypothesis of independent colonization of each species. Host size and sex were not significant predictors of the intensity of any cestode species or infracommunity species richness. The species-poor, randomly assembled helminth infracommunities found in shortfin makos contrast with those found in coastal, benthic-pelagic elasmobranchs, rather resembling those described in other large oceanic predators such as cetaceans, marine turtles and marine birds. We suggest that the vagile behavior and opportunistic diet of these predators, coupled with a "dilution" effect on infective stages in the pelagic realm, could generate poor and unpredictable helminth infracommunities, generating a "Pelagic Predator Syndrome".

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MORPHOLOGICAL STUDY OF *CUCULLANUS* SP. (NEMATODA: CUCULLANIDAE) PARASITIC IN *TILAPIA ZILLI* FROM LEKKI LAGOON, LAGOS, NIGERIA

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Based on the parasitologic examination of three hundred and twenty one specimens of *Tilapia zilli* (n=321), thirty three (n=33) individuals of *Cucullanus* sp. Muller 1777 were recovered. *Cucullanus* sp. had an overall prevalence (P) of 9.6%, with a P of 16.4% in female hosts and of 8.5% in male. The specimens of *Cucullanus* sp. were recovered from the fish host. SEM description of the recovered *Cucullanus* sp. shows some distinguishing features which place it apart from most of its congeners. The submedian papillae were located on the cuticle, single amphid in between the two upper submedian papillae, short spicule and 23 upper teeth and 34 lower teeth on the dorsoventrally elongate mouth. The tissue sections of the parasitized host revealed marked sloughing of cells into the lumen and intraluminal adult nematode mixed with necrotic debris.

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PRESENCE OF *ANISAKIS* SPP. LARVAE IN MESOPELAGIC AND BATHYPELAGIC FISH OF THE CENTRAL MEDITERRANEAN SEA

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Aim of the present paper was to report and identify by morphological and molecular methods the presence of anisakid larvae found in mesopelagic and bathypelagic fish belonging to the Myctophidae (*Electrona risso* and *Diaphus metopoclampus*) and Phosichthyidae (*Vinciguerria attenuata*) families. The study was performed in central Mediterranean Sea, precisely in the Strait of Sicily and the Strait of Messina where a lot of species of cetaceans known as host for Anisakids were reported (such as *Delphinus delphis*, *Stenella coeruleoalba*, *Tursiops truncatus*, *Physeter macrocephalus*, *Globicephalus melas* and *Ziphius cavirostris*). On the studied fish, a parasitological study was performed examining the coelomic cavity for nematode larvae, by visual and stereoscopic evaluation. Twenty two larvae isolated, fixed in ethanol and cleared in glycerol, were morphologically identified at genus level by light microscopy as L3 stages of *Anisakis* belonging to Type I and to Type II (sensu Berland 1961) and fixed in 70% ethanol for molecular characterization. In the molecular identification with PCR-RFLP analysis (D'Amelio et al 2000), the specific restriction profile obtained allowed to identify the anisakid parasites as *Anisakis pegreffii* and *Anisakis physeteris*. Particularly *A. pegreffii* larvae were isolated in 16/296 *D. metopoclampus*, *A. physeteris* larvae were found in 3/105 *E. risso* and in 3/36 *V. attenuata*. Anisakid larvae belonging to *Anisakis pegreffii* and *Anisakis physeteris* are here reported for the first time in these species of deep water fish. Some ecological considerations were consequently provided.

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DIVERSITY OF NEMATODES INFECTING THE GREATER WEEVER *TRACHINUS DRACO* L. FROM THE BAY OF BIZERTE

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Several studies have reported the diversity of parasitic nematodes from the marine fishes of the Mediterranean. In some cases, these parasites can cause massive infections with high fish mortality, leading to a noticeable decline in fish stocks especially species of Philometridae. On the other hand, some nematodes particularly Anisakid species are known for their zoonotic contribution to human anisakiasis. Between March 2013 and December 2014, 197 Greater weever *Trachinus draco* Linnaeus, 1785 (Trachinidae) were collected from the Bay of Bizerte (North-East of Tunisia). Fishes were dissected and all organs were inspected for the presence of parasitic nematodes. In the present study we report the presence of four nematodes species belonging to Anisakidae and Philometridae families. The nematodes species were found infecting the abdominal cavity, mesentery, liver, stomach, intestine and ovaries. The identification of nematodes species revealed the presence of *Anisakis simplex*, *Hysterothylacium* sp., *Hysterothylacium fabri* (3th and 4th larval stages) and *Philometra* sp. (gravid females). Morphological and morphometric characteristics of those species are presented in this work. *Anisakis simplex* appeared with a prevalence (P) of 79 %, mean intensity (MI) of 34.2 and mean abundance (MA) of 32.6; *Hysterothylacium* sp. with P = 78 %, MI= 46.1 and MA= 20.7; *Hysterothylacium fabri* (3th larval stage) with P= 52 %, MI= 68.1 and MA= 16.4; *Hysterothylacium fabri* (4th larval stage) with P= 64 %, MI= 72.3 and MA= 20.3 and *Philometra* sp. with P= 15.7 % , MI= 16.2 and MA= 12.8. Two species of the genus *Philometra* Costa, 1845 were previously reported from *Trachinus draco*: *Philometra filiformis* (Stossich, 1896) infecting the gonads of their host from the Adriatic Sea and Italy (Stossich, 1896; Orecchia and Paggi, 1978) and *Philometra globiceps* (Rudolphi, 1819) parasitizing the body cavity. We report for the first time the presence of *Philometra* sp. in *T. draco* from Tunisia coasts. The present material cannot be identified to species due to the absence of the males. In the Mediterranean Sea, anisakid nematodes have been found in several economically fish species and in many countries (Italy, Greece, Turkey, Spain, France, Tunisia…). *Anisakis* and *Hysterothylacium* larvae have been recorded in 43.8% of all fish marketed in two towns of the Adriatic coast of central Italy (Fioravanti et al., 2004). *Contracaecum fabri* (Rudolphi, 1819) was reported in *T. draco* from Turkey (Akmirza, 2004). In Tunisia, only the work of Farjallah et al. (2008) has reported the presence of larval forms of *Anisakis* species in several fish and *Anisakis pegreffii* in *T. draco*. However, species of the genus *Hysterothylacium* were not recorded. In the present work we report for the first time the presence of two *Hysterothylacium* spp. in *Trachinus draco* from Tunisia waters.Data on the prevalence of anisakid spp. found in *T. draco* in Mediterranean region are scarce. However, the prevalence of anisakid spp. is relatively higher in the present study.

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NEMATODE PARASITES OF CONGER EEL *CONGER CONGER* FROM SICILIAN WATERS (ITALY)

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The authors report preliminary data on the occurrence of nematode parasites in *Conger conger* sampled in Sicilian waters. Between December 2013 and January 2014, 25 specimens of conger eel *Conger conger*, caught from waters near Sciacca on the south coast of Sicily, were examined for parasitic infections. A total of 42 nematodes (larval forms and adults) was detected in visceral cavity in 13 samples by visual and stereoscopic evaluation: the parasites isolated, fixed in ethanol and cleared in glycerol, were morphologically identified at genus level by light microscopy. Five taxa of Nematoda, belonging to different families, were morphologically identified: L3 *Anisakis Type I* and *Anisakis* Type II (*sensu* Berland 1961) (Anisakidae), *Hysterothylacium* sp (L3, L4 and one adults) (Rhapidascarididae), *Cucullanus* sp. (Cucullanidae) and *Cristitectus congeri* (Cystidicolidae). Cucullanid nematodes (28.6%) were morphologically similar to *Cucullanus bioccai* which was previously reported by Orecchia & Paggi (1978). The Anisakidae larve recovered were identified and characterized at species level by molecular techniques. Molecular analysis with PCR-RFLP identified the Type I larvae as belonging to *A. pegreffii* (28.6%) and the Type II larvae belonging to *A. physeteris* (2.4 %). The nematodes identified as *Hysterothylacium* (38.1%), were characterized by sequencing of nuclear (internal transcribed spacer, ITS) markers as *Hysterothylacium fabri* compared with the most similar sequences available in GenBank. Most of this nematode parasites have been previously reported in conger eel from Mediterranean Sea and Atlantic Ocean. The authors also report the molecular characterization of Anisakidae.

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REPRODUCTION AND INFECTIOUS BIOLOGY OF CAPSALID MONOGENEA *NEOBENEDENIA GIRELLAE*

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Skin fluke infections have long been one of the most problematic parasitic diseases in aquaculture. Since the introduction into Japanese water in 1990's *Neobenedenia girellae* has become widespread due to its low host specificity and high reproduction rate. We have investigated various aspects of its biology by a series of *in vivo* and *in vitro* experiments. *N. girellae* inseminates by planting spermatophores on the body of itself or other individuals. The egg production occurs throughout the day regardless of light condition with the maximum daily egg output of over 700 per worm. The eggs are easily attached to the culture cage with a filament. In a culture cage, significantly higher proportions, 50-80%, of eggs were found near the surface. Hatching is strongly influenced by light, showing a monomodal daily peak in the morning. The oncomiracidia possess positive phototaxis and the majority of infection at farming sites occurs close to the water surface, but the larvae are capable of infecting fish in the darkness. In the experiment using circulating water channel, infections occurred under high water current of 50 cm/sec, suggesting the mode of infection is not only a simple attachment onto host's body surface.

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RELATIONS BETWEEN THE MORPHOLOGY OF MONOGENEA EGGS AND HABITAT OF FRESHWATER FISH

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The eggs of monogeneans have great diversity of shapes, sizes and appendices; they may have mainly oval, tetrahedral shape, appendages hook shape, appendage with adhesive material, without appendices, or some may have more than one filament. It is assumed that these shapes and appendices serve to facilitate the attachment of eggs to new hosts. Thus, the objective of this work was to compare the different shapes and the presence of appendages of Monogenea eggs, predicting that the kinds of host habits are the main factors responsible for the morphological changes observed in the eggs. For this, scientific publications were obtained in ISI Web of Knowledge, Scielo, BioOne, Scopus and Biomed. Some specimens of monogenean were obtained from fishes in the Ichthyoparasitology Laboratory of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia). The ten kinds of eggs found were categorized according with their morphology e with appendages. The appendices of monogeneans appear to have evolved independently, because correlated species have different shapes appendages. This shows that the similar appendages are the result of similar environmental selective pressures. Throughout their evolution, the eggs of monogeneans had to adapt to the host and the environment in which they live. During that time various appendages have developed eggs in order to facilitate the infestation of the host and become specialized for different types of environment host.

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THE ROLE OF THREE SEAGULL SPECIES IN THE TRANSMISSION OF DIGENEANS INFECTING COMMERCIAL FISH IN WESTERN MEDITERRANEAN

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Several digenean species infecting seagulls use fish as second intermediate hosts, often manipulating fish behaviour to enhance transmission to their definitive hosts, and sometimes causing also zoonotic diseases. In this study we report on potentially harmful digeneans infecting three species of seagull from the Mediterranean coast of Spain, an area where aquaculture and fishery are important economic activities. We examined the intestinal helminth fauna of 56 yellow-legged gulls (*Larus michahellis*), 6 Adouin's gulls (*Larus audouinii*) and 6 black-headed gulls (*Chroicocephalus ridibundus*) collected in a costal lagoon and surrounding coastal area near Valencia (Spain), i.e. “La Albufera” and “El Saler”. *Larus michahellis* was heavily infected with *Cardiocephaloides longicollis* (prevalence [95% C.I.]: 60% [50%-70%]; mean intensity [95% C.I.]: 11.5 [7.8-17]), and had lower infection levels of Ascocotyle sp. (4% [1%-13%]; 1261.5 [2-1261.5]), Diplostomum pseudospathaceum (8% [3%-18%]; 4.3 [1-9.3]) and Galactosomum sp. (21% [12%-34%]; 1.6 [1.2-2.3]). Individuals of L. audouinii harboured C. longicollis (33% [6%-73%]; 24.5 [5-24.5]) and Galactosomum sp. (17% [1%-59%]), whereas only D. pseudospathaceum was found in C. ridibundus (50% [15%-85%]; 8 [1-12.7]). Infection levels of C. longicollis and D. pseudospathaceum were significantly higher in L. michahellis than in the other seagulls, suggesting that this host species might act as an important transmission vector of digeneans infecting fish of commercial interest in the area, including several sparid species.

Funded by ISIC/2012/003 and PROMETEO 795/2015/018 projects.

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APOROCOTYLIDS IN SCIAENID *CYNOSCION NEBULOSUS*: EVIDENCE FOR TWO SPECIES AND ONE LIFE CYCLE

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Aporocotylids are fish blood flukes whose larval forms develop in polychaetes or bivalves. Recently we identified two terebellid polychaetes, *Enoplobranchus sanguineus* and *Amphitrite ornata*, as intermediate hosts for blood flukes along the Atlantic coast of South Carolina (SC) U.S.A. Analysis of ITS-2 and lsrDNA sequences from sporocysts and cercariae dissected from the coelom of both polychaetes showed 100% identity. Whereas all sequences obtained revealed close identity with the *Cardicola* clade of the Aporocotylidae, these were not identical to any sequence available from GenBank or to sequences obtained from flukes collected from two other local fishes. Sequences however matched specimens collected from seatrout, *Cynoscion nebulosus*. *Cardicola laruei* is the only species described from *C. nebulosus* but no sequence data is available in GenBank for this organism. We extended our observations of *C. nebulosus* from four sites along the SC coast and found adult flukes in seatrout from all four sites. However, two genotypes that differed by ~10% in both ITS-2 and lsrDNA were identified and found in different frequencies at the sites sampled. One genotype was identical to sequences obtained from larvae in polychaetes. Our data strongly suggest there are two *Cardicola* species that infect *C. nebulosus* and that one of these species uses *A. ornata* and *E. sanguineus* as intermediate hosts. No polychaetes were yet found infected by the second species. Differences in ecosystems, and thus annelid habitats, between sites sampled could explain the heterogeneous distribution of seatrout fluke genotypes observed.

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DIVERSITY OF BLOOD FLUKE LARVAL STAGES IN TWO TEREBELLID POLYCHAETES IN U.S. ESTUARIES

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Annelids are known vectors to three aporocotylid blood fluke species that infect marine fishes. The scarcity of cycles resolved to date is in large part due to the impossibility of associating larval and adult stages using classical morphological characters. While such associations can nowadays be made via DNA sequencing, the challenge remains for the corresponding stages to be found in both hosts. In the context of a search for the annelid intermediate host of fish blood flukes from South Carolina U.S.A., we encountered multiple infections in the terebellid polychaetes *Amphitrite ornata* and *Enoplobranchus sanguineus.* Out of 322 annelids observed, 3% of *A. ornata* and 16.7% of *E. sanguineus* were infected by sporocysts or rediae in their hemocoel. For each individual, sporocysts and rediae were collected in filtered seawater and either observed fresh using a compound microscope, heat killed and fixed in neutral buffered formalin for morphological study, or fixed in 95% ethanol for molecular studies. We encountered five morphotypes, one of which was common to both *A. ornata* and *E. sanguineous*. ITS-2 and lsrDNA sequences of parasites from nine *A. ornata* and eight *E. sanguineus* annelids were obtained and the five morphotypes were each matched with a different set of sequences. Sequences were BLASTed on Genbank but no match closer than 96% identity was found. Sequences of sporocysts common to both *A. ornata* and *E. sanguineus* were matched to those from a blood fluke that infects the spotted seatrout but which remains to be identified.

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THE POTENTIAL INFECTION OF ELASMOBRANCHS WITH *NYBELINIA* SP. USING ITS INTERMEDIATE HOST, *OCTOPUS VULGARIS* CUVIER 1797 (CEPHALOPODA: OCTOPODIDAE)

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Trypanorhynch cestodes represent one of the most common metazoan parasites in marine fishes. Their life cycle is known to be highly complex, but the way in which it unfolds is still largely unknown. Elasmobranchs are the final hosts, whereas cephalopods or teleosts are the precedent intermediate or paratenic hosts.

The main aim of the present work was to determine the infection levels of *Nybelinia* sp. larvae for the octopus host, so that the risk of infection of the final hosts could somehow be evaluated. Samples of *Octopus vulgaris* Cuvier, 1797 (N=30/sample) were collected at two different localities off Portugal, i.e. Figueira da Foz and Portimão,

Prevalence and mean intensity ranged, respectively, from 12.5% (Portimão) to 50.0% (Figueira da Foz), and from 2.13 worms/host (Figueira da Foz) to 1.0 (Portimão) worms/host. Larvae were mainly found in the stomach and oesophagus, but they were also found free, within the body cavity. The low prevalence values recorded for Portimão, along with the low intensity values, suggest that the main route of infection of elasmobranchs is only occasionally related with the consumption of *O. vulgaris* (trophic transmission).

Acknowledgments: Financial support from Projects DIRDAMyx FCOMP-01-0124-FEDER-020726 (FCT–PTDC/MAR/116838/2010), AQUAIMPROV– Sustainable Aquaculture and Animal Welfare (NORTE-07-0124-FEDER-000038) and PEst-C/MAR/LA0015/2013.

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IS THE BLACKMOUTH CATSHARK *GALEUS MELASTOMUS* (CARCHARHINIFORMES; PENTACHIDAE) THE FINAL HOST OF *DITRACHYBOTHRIDIUM MACROCEPHALUM*?

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Several specimens of blackmouth catshark (*G. melastomus,* Rafinesque, 1810) were examined for the analysis of stomach contents and parasite fauna. Individuals were caught during the bottom trawl surveys carried out annually in the north of Spain waters (Cantabrian Sea) during 2012-2014. A total of 53 specimens (size range 17-54 cm) were examined on board. Sex ratio was balanced (♂ 49.1- ♀ 50.9). Only the stomach and spiral valve were scrutinized. Three parasitic taxa were identified: cestoda, nematoda and isopoda (only in one specimen). The main parasite taxa was cestoda (*Ditrachybothridium macrocephalum* Rees, 1959), both larvae and adult specimens of this species were observed in sharks of 15-32 cm. The abundance of larvae and adults was 0.35±1.03 and 5.58±10.89 and the prevalence 15% and 40.0% respectively. All cestoda and nematoda were observed free in the spiral valve/stomach not in the musculature. The stomach content analysis revealed crustacean, fish and cephalopoda as main prey. Among crustacean, euphasiids were the dominant prey (and the species *Meganyctiphanes norvegica* in particular). Among fishes the blue whiting *Micromesistius poutassou* was identified.

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THE ROLE OF MESOZOOPLANKTON AS INTERMEDIATE HOSTS OF SYMBIONTS IN NE ATLANTIC ECOSYSTEMS: PARASITES AND EPIBIONTS

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Worldwide, mesozooplankton is a key component in the dietary habits of the early stages of development of many cephalopods, fishes as well as some marine mammals, and occupies one of the lowest trophic levels, becoming an important link between parasite larvae and their definitive host through predator-prey interactions. Many fish species of commercial interest from Galician and European waters are affected by parasites that reduce flesh quality and cause diseases involving economic losses to the fishery industry. Some of these parasites constitute a potential risk for public health because they are responsible of gastroalergic disorders in fish-consuming population. Other parasites constitute a potential risk for host population increasing their predation risk, reducing fertility or castration. Nevertheless, both their arrival and impact into the highest levels of the food web, such as fishes, is unknown.
Our research revealed that the mesozooplaktonic euphausiid *Nyctiphanes couchii* plays an important role for acanthocephalans and nematode larvae transmission. Additionally, we identified *Muggiaea* sp. (Siphonophora) as the second intermediate host for trematoda. In addition to those, Calanoid copepods were encountered infected by protozoans, showing different specificity and intensity of infection. Finally, some Gnathiidae, Bopiridae, Epicaridean, Aegidae, Pennellidae and Caligus were detected among the mesozooplankton communities.

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EFFECT OF MONOGENEAN PARASITES ON THE GILLS TISSUES AND CHLORIDE CELLS IN THE CASPIAN SEA WHITE FISH (*RUTILUS FRISSI KUTUM*)

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The aim of the study was to identify Monogeneans and gill chloride cells changes in terms of number and diameter of their positions with respect to the density of parasites. Histopathological effects of the parasites in the gill tissue of also were studied. 33 *Rutilus frisii kutum* specimens were caught in December 2014 from the Southern coast of the Caspian Sea. The weight and length were 190 - 1200 gr and 20 -405cm. The gill filaments were collected and studied for parasites under the microscope, the collected parasites were fixed in Glycerin-Gelatin and the gills were fixed in 10% formalin. The 5µ tissue sections were made and samples stained by Hematoxylin-Eosin. Lesions caused by the presence of parasites, size and number of chloride cells was counting by optical microscope**.** According to the results three monogenean parasites were identified *Dactylogyrus frissi, D.nybelini* and *Diplozoon paradoxum*. Mean intensity and prevalence of *Dactylogyrus frissi, D.nybelini* were (118,100%) and *Diplozoon paradoxum* (4/53, 78/78) respectively. Lesions observed were: the destruction of the gill filaments and lamellae adhesion, epithelial cell hypertrophy and hyperplasia blades gill, detachment of the epithelial basement membrane blades, aneurysm and development of the clubbing. The results show; in the presence of parasites, the total number of chloride cells were reduced, but their size was unchanged. Correlation test results showed that the strong and significant inverse relationship between the number parasites and the number of total chloride cells. Reduce the chloride cells, may have a negative impact on migratory fish Osmoregulation.

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HISTOPATHOLOGICAL ANALYSIS OF SILVER CATFISH (*RHAMDIA QUELEN*) IMMUNIZED AND CHALLENGED WITH *ICHTHYOPHTHIRIUS MULTIFILIIS* THERONTS

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The development of vaccines as a prophylactic method in aquaculture has increased significantly. *Ichthyophthirius multifiliis* is the most important ciliate affecting the silver catfish (*Rhamdia quelen*) due to its low host specificity and wide geographic distribution. This study analysed the histopathology of fish immunized after challenge with live theronts of *I. multifiliis* in two assays: I - immunization via intraperitoneal injection (i.p.) and II- via immersion bath with the following groups: control (non-immunized no challenged); non-immunized and challenged with 12,000 theronts/fish; non-immunized and challenged with 22,000 theronts/fish; immunized and challenged with 12,000 theronts/fish; immunized and challenged with 22,000 theronts/fish. Six days after challenge, the samples for histopathological and parasitological analysis were collected. Either in the assay I or in the assay II, the prevalence of *I. multifillis* in the gills was higher in non-immunized fish (PBS) (33.33 % and 27.77 %, respectively). The melanomacrophages were present in 53 % and 50 % of the samples in non-immunized fish (PBS 12,000 and 22,000), respectively (assay I). Fish from the assay II, immunized and challenged showed more atrophied areas of the hepatocytes. Higher number of melanomacrophages in the kidney of non-immunized fish in the assay I was observed when compared to control. The results showed no difference in the gill lesions in both immunized and non-immunized fish compared to control. Histological alterations in the organs of silver catfish were considered light and of eventual occurrence except for the liver that presented significant atrophy and hypertrophy of hepatocytes after immunization via i.p.

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HISTOLOGICAL CHARACTERISTIC OF LEECH, *AUSTROBDELLA* SP. AND HOST REACTION OF THE FLOUNDER, *PARALICHTYS OLIVACEUS*

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This study described the morphological ultrastructure using the scanning electron microscope of the leech, which identified as *Austrobdella* sp. based on molecular analysis, and histopathological reaction of the host in Korea.

Seven flounder was found to be infected with thirteen leeches. Eight leeches on the caudal fin (61.5%), two leeches on the pelvic fin (15.4%) and three leeches on the body (23.1%) were found. The average total length of *P. olivaceus* and *Austrobdella* sp. were 40.2±3.8 and 12.13±0.21, respectively. Linear regression analysis showed that there was both no significant relationship between the length of host and the length of leeches ($r^2=0.271$, $F=4.085$, $p=0.068$), and length of host and number of leeches on parasitized host ($r^2=0.003$, $F=0.013$, $p=0.915$).

Infected region of the host is showing the tissue of the fin lifting into the posterior sucker, destruction of the epidermal layer, and inflammation and necrosis of connective tissue (Ct). H-E stain. Host-parasite interface is showing the T1 cell, T2 cell and epithelial cell on dorsal surface of the tegument. The fin attached parasite is showing the hypertrophy and hyperplasia mucous cell, and note that existed mucosubstance between posterior sucker and epidermal layer of the fin. The fin adjacent to posterior sucker note that pushed epidermal layer and showing the decreased mucous cell, karyolysis and pyknosis nucleus of epithelial cell, and necrotic region in the epidermal layer.

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NEW DATA ON THE MORPHOLOGY OF *ELECTROTAENIA MALOPTERURI* (FRITSCH, 1886) (CESTODA: PROTEOCEPHALIDAE) AND THE ATTENDANT HISTOPATHOLOGICAL CONSEQUENCES ON *MALAPTERURUS ELECTRICUS* (SILURIFORMES: MALAPTER)

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A parasitologic investigation was carried out on *Malapterurus electricus* from Lekki Lagoon (n = 340). The proteocephalidean cedstode, *Electrotaenia malopteruri* (Fritsch, 1886) (Cestoda: Proteocephalidae: Gangesiinae) which is the type and only species of *Electrotaenia* Nybelin, 1942 was the dominant parasites species recovered. The parasite is specific to the fish host. Scanning electron microscopy (SEM) revealed sharp differences in the scolex morphology of the new species with the existing species of *Electrotaenia malopteruri*.

The new sub-species have a wider spherical scolex, the four uniloculate suckers groove are bean shaped and not spherical in the existing species. The suckers of this new species are not widely closed to one another but distinctly large with a wider external surface and a wider groove. The disc shaped rostellum-like organ is also wider. There is no median line in-between the Uniloculate suckers. The overall incidence of infection was 30.2% with higher incidence of infection in the male (n = 210 (31.7%) and (n = 130 (27.6%) in the female specimens. A total of 254 helminthes were recovered from the three hundred and forty specimens examined of the fish hosts. The tissue sections of the fish host revealed diffuse oedema of epithelium with sloughing of epithelium into lumen, marked shortening and matting of villi with diffuse lymphocytic propria together with thickened wall of the intestine and the observation of the localized area of calcification.

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SURVEY OF INFECTIONS OF EYES CAUSED BY *DIPLOSTOMUM SPATHACEUM* (RUDOLPHI, 1819) METACERCARIA IN HASSANLOO RESERVOIR FISHES IN THE WEST AZERBAIJAN

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Infestation with *Diplostomum spathaceum* in 120 fishes (phytophage, common carp and caras) in West Azerbaijan Hasanloo reservoir was evaluated from spring to winter in 2008. The results indicate that 70% of fish (84 fish) showed infestation with this parasite which presents a high rate of infestation in the fishes in this water resource. Comparing sessions reveals the highest infestation rate of 96.7% in summer and the lowest rate of 46.7% in winter. The range of parasite number was between 1 and 259 with the mean abaundance was 20.04(±3.681) and the mean intensity was 28.631(±4.98)

Pathologically most lesions are characterized by penetration of metacercariae, subcapsular vacuolation and degeneration of lens fibers, liquefaction of fibers which gives them a basophilic appearance, and the acute parasitic cataract causes rupture of lens capsule and deposition of lens parts in vitreous body. In cornea pathologic lesions are characterized by keratitis by hydropic degeneration, infiltration of granulocytes containing orange granules with epithelial sloughing of epithelial cells. It is supposed that sloughing is due to host defense against parasite penetration. No characteristic lesion was observed in other parts of the eye. In a phytophage case, however, due to a heavy parasitic infection, lens in the left eye was completely destroyed such that only the compact nucleolus was remained and a constant miosis could be observed.

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PATHOLOGICAL ANALYSIS OF *EUCLINOSTOMUM HETEROSTOMUM* IN NATURALLY INFECTED *CHANNA STRIATUS* INHABITING CENTRAL INDIA

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Present study was conducted to reveal the pathogenicity of *Euclinostomum heterostomum* infection in *Channa striatus*, different infected tissues were assessed as histo-pathological biomarkers for detail comprehension of infection. Collected parasites (*E. heterostomum*) were fixed in AFA solution and stained with aceto-carmine, clarified in xylene and mounted in DPX. For pathological study the infected tissues were fixed in alcoholic bouin's fluid and processed to prepare paraffin blocks. They were cut at 4-5 µm in thickness for the preparation of histological slides. The percentage of infection was calculated as per Margolis *et al*. (1982). Encysted metacercariae of *E. hetereostomum* were found in fish flesh (muscles), liver and kidney showing a maximum prevalence and intensity of 48.0 % and 12.0, respectively. Pathological signs included degeneration, splitting and necrosis of longitudinal and circular muscles, presence of fat droplets, necrosis, indistinguishable and shrunk hepatocytes with nuclei shifted to cell periphery in the liver, while reduced size of glomeruli, severe degeneration and necrosis of haematopoietic tissue and detached epithelial cells of renal tubules in the kidney of *C. striatus* were observed. Metacercariae trematodes are extensively distributed in freshwater bodies and fishes inhabiting them. They damage the organ on which they subsist. The physiological activities of the parasitized fish are hindered and their growth is retarded which cause economic loss to the fishery industry.

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HISTOMETRY OF LIVER AND CYST-NEMATODES IN *GYMNOTUS INAEQUILABIATUS* (GYMNOTIFORMES: GYMNOTIDAE) OF BRAZILIAN PANTANAL

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Nematodes are typically found in fish digestive tracts, however eventually they can migrate to other organs as muscle, liver and serous membrane. The aim of this study was to evaluate some histometric features of the cysts-nematodes and liver in wild-fish from Pantanal biome. *Gymnotus inaequilabiatus* (125.5 ±43.4g; 32.8 ±4.5 cm), were collected in Paraguay River, Porto Morrinho, Brazil (21º41'56"S, 57º52'57" W) in the flood (n=40) and in the dry season (n=49). After biometric evaluation and euthanasia, cyst-nematodes were counted macroscopically/liver. Liver fragments were prepared for histological analysis in H&E and Masson Trichrome staining. In bright-field microscopy, different measurements on the cysts and liver structures were evaluated by histomorphometric digital technique. Cyst-nematodes count were higher in the dry season compared to flood (71.8 ±9.6 x 26.4 ±10.6, $p<0.01$). Diameter (µm), Cyst-wall (µm) and collagen layer (µm) were also higher ($p<0.01$) in dry season (323.8 ±12.0; 20.0 ±0.8 and 16.2 ±1.5, respectively) in contrast to flood season (259.7 ±19.2; 13.5 ±1.3 and 12.0 ±2.4, respectively). Percentiles of sinusoidal space, melanomacrophage centers (MMC) aggregates, hepatocytes and cysts differed ($p<0.05$) between seasons. Area (μm^2) of MMC in liver and mast cells count pericystic were higher in the dry season. The different values found in the cysts measurements, density of liver structures and immune response, suggest that *Gymnotus inaequilabiatus* is a paratenic host species of Nematodes in Pantanal, Brazil. River-pulse inundation is a critical factor to comprehend the biology of parasitism and pathogeny-dynamics of Nematode in wildlife fish.

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POSITIVE IMPACT OF PARASITES ON HOSTS' PERFORMANCE: A NEGLECTED OCCURRENCE?

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The mechanisms underlying parasite-altered host behavior and fitness remain largely unanswered. We argue that performance capacity is an important target of parasitic manipulation and we aim to integrate the study of performance with that of parasitic manipulations of host behavior and fitness. We performed a meta-analysis of 101 measures of the effect of parasites on host performance capacity from the published literature. Although negligible-small effect sizes were detected in 40/101 measures, host performance capacity was overall affected by parasitic infection with a negative direction and medium-large magnitude in 58/101 measures and an increase in performance capacity in 3/101 measures. Diagnostic tests of these results suggested that the small number of cases of increased host performance capacity was due to bias in the published literature. Thus, it is expected that there are more cases of parasite-enhanced host performance capacity than reported and that such occurrences may be overlooked by scientists. We present results from a fish-myxozoan system in which increasing density of myxospores in the fish muscle results in increased fish performance capacity. We discuss parasite-enhanced host performance capacity in light of its possible adaptive value to some parasites and argue for the value to acknowledge and report such occurrences.

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METACERCARIAE OF *HYSTEROMORPHA TRILOBA* (DIGENEA: DIPLOSTOMIDAE) IN WILD AND FARMED FISH IN ITALY

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During 2013 two outbreaks of massive infestation by metacercariae in wild chub *Squalius cephalus* and farmed black bullhead *Ictalurus melas* were observed. The metacercariae were about 1 mm, whitish and scattered in muscle. The morphological study allowed identifying the parasites as *Hysteromorpha triloba*, a cosmopolitan Diplostomidae previously reported in Italy only at the adult stage in Ciconiiformes. Histological examination of skeletal muscle of infected chub revealed a massive presence of metacercariae within large elliptical cavities containing amorphous basophilic substance. At opposite ends of the cavities a mild inflammatory reaction with fibroblasts, lymphocytes, macrophages and rodlet cells was observed. Muscular fibres around the metacercariae presented hyaline degeneration and in some sections appeared replaced by granulation tissue. In black bullhead inflammatory reaction toward *H. triloba* appeared more severe than observed in chub; metacercariae were enclosed in roundish cysts characterized by a thick wall of granulation tissue, fibroblasts, epithelioid cells and macrophages. The differences observed regarding the tissue reactivity and pathogenic effects of *H. triloba* metacercariae in chub and black bullhead may be related to a different degree of adaptation of the parasite to autochthonous (the former) and allochthonous (the latter) host. The macroscopic evidence of metacercariae of *H. triloba* in the muscle, even in the absence of zoonotic implications, could adversely affect the marketability of parasitized fish species.

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BIOMARKER RESPONSES OF EELS AFTER INFECTION WITH THE NEMATODES OF THE GENUS *ANGUILLICOLA*

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Nematodes of the genus *Anguillicola* are swim bladder parasites of eels. During their life cycle they infect copepods as first intermediate hosts before they infect eels as definitive hosts. The oldest known definitive host of *Anguillicola crassus* is the Japanese eel (*Anguilla japonica*), while *Anguillicola novaezelandiae* appears to be an original parasite of the Short-finned eel (*Anguilla australis*). Both *Anguillicola* species have been recorded as nonindigenous parasites of the European eel (*Anguilla anguilla*) in Europe but differ with respect to their establishment success. Whilst *A. crassus* can be considered an established nonindigenous species, *A. novaezelandiae* was not able to build a stable population in Europe. A part of this establishment success is assumed to be associated with the physiological interaction between the host and its parasites. We evaluated a set of physiological responses (so called biomarkers) in European and Japanese eels infected by *A. crassus* and *A. novaezelandiae* under laboratory conditions using a common garden experiment including uninfected European and Japanese eels. Each group of infected eels was repeatedly investigated following initial experimental infection to elucidate the temporal development of biomarker levels. We focused on changes of the following biomarkers: catalase, lipid peroxidase, glutathione S-transferase and glutathione. Detailed results will be presented and discussed in the light of the establishment success differences of both *Anguillicola* species.

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ONCHOCLEIDUS DISPAR (MUELLER, 1936) (MONOGENEA: ANCYROCEPHALINAE) ON THE LARGEMOUTH BASS *MICROPTERUS SALMOIDES* IN SOUTH AFRICA: A TRANSCONTINENTAL TRAVELLER

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The largemouth bass *Micropterus salmoides* (Lacepède, 1802) is an alien invasive species in South Africa (SA) that was introduced into the country in 1928 from England. Interestingly, these fish were originally bred in Holland. Following introduction it was stocked for angling throughout SA. In North America, its native range, *M. salmoides* host several monogenean species. The aim of this study was thus to determine whether any of these monogeneans were introduced via its hosts into SA. *Micropterus salmoides* were collected during October 2014 from Boskop Dam, North-West Province, SA and screened for gill monogeneans. High prevalence (100%) as well as intensity of infections (up to 400 parasites per host) was found, without noticeable signs of pathological changes to the gills. Based on the morphometrics of the hard structures of the attachment organs, these monogeneans were identified as *Onchocleidus dispar* (Mueller, 1936). South African specimens were also compared with specimens of *O. dispar* previously found on *Lepomis gibbosus* L., 1758 from the Czech Republic, Slovakia, Bulgaria and Croatia. Molecular characterization of 18S and 28S regions of rRNA confirmed identification at the genus level as the only closest hit in the nucleotide database was *Onchocleidus similis* Mueller, 1936. This is the first time that this parasite has been confirmed from Africa and it’s evident that it has been introduced to South Africa with its host. More research should focus on the distribution of this parasite in other localities in order to manage its potential risk as invasive parasites of native fish.

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ECTOPARASITES OF *PLAGIOSCION SQUAMOSISSIMUS* (HECKEL, 1840) (PERCIFORMES, SCIAENIDAE) UNDER THE ENEMY RELEASE HYPOTHESIS PERSPECTIVE

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Biological invasions are considered one of the major leading causes of global biodiversity loss.When a non-native organism is inserted into a new area, many of its natural enemies can be left in the region of origin. The Enemy Release Hypothesis explains the success of the introduction of species by the loss of their natural predators and parasites. Thus, the aim of this study was to verify the parasitic fauna from gills of *Plagioscion squamosissimus* native from the Amazon and Negro rivers and introduced in Paraná and São Francisco rivers from the perspective of the Enemy Release Hypothesis. Twenty specimens of hosts from each river were analyzed and the total richness of parasites in native basin was significantly higher than that introduced in the basin, suggesting that during the introduction process the fish species lost over 90% of the richness of gill parasites. The only common species in the four basins was the monogenetic *Diplectanum piscinarius*. The successful establishment of *P. squamosissimus* is not only related to favorable environmental factors in the new region, but also to the loss of their gill ectoparasites.The introduction of piscivorous species such as *P. squamosissimus* have great potential to disrupt the native fauna, thus the better the knowledge on details of its introduction, the higher the chances of successful management and conservation plans.

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THE PARASITE COMMUNITY OF *CYPRINUS CARPIO* FROM THE RHINE RIVER

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The common carp, *Cyprinus carpio*, is distributed all over the world due to its commercial importance. Although the wild population in the Danube River, which is assumed to be the origin of the European lineages, is currently under threat populations in many other regions of the world are stable and even increasing. As a cosmopolitan fish species it might be expected that the common carb also harbors a variety of cosmopolitan parasites. However, after the inauguration of the Main-Danube-canal in the early 1990, a massive invasion of a large number of Ponto-Caspian species occurred. Various invertebrates (e.g. amphipods) as well as fish species, mainly gobies, from the Danube River system established and became dominant in the Rhine River system. Together with these free-living invaders various Ponto-Caspian parasites were also introduced into the Rhine River system.

Accordingly, the aim of the present study was to analyze whether the parasite community of carp is dominated by cosmopolitan parasites, by parasites from the Ponto-Caspian region or by parasites being native in the region of sampling. In order to address these aspects, we have sampled carp from the river Rhine in autumn und spring and investigated the composition of the parasite communities. Detailed data on the parasite composition of carp will be presented and discussed in the light of invasion biology.

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ARE EXOTIC AND NATIVE FISH SWITCHED THEIR DIGENEAN PARASITES? THE CASE OF HERBIVOROUS FISHES IN MEDITERRANEAN SEA

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Despite their potential ecological and environmental effects, parasites of exotic fish remain an underestimated component of ecosystem functioning. Among these parasites, Digenea have the most complex life cycles that make them difficult to be introduced. In the Gulf of Gabes (Central Mediterranean Sea), four herbivorous fish are present: two native *Sarpa salpa* and *Sparisoma cretense* and two lessepsian *Siganus rivulatus* and *Siganus luridus*. These fishes are assumed to have feeding analogies that increase the possibility to be infested by the same digenean species. Comparative parasitological studies of these fishes in sympatric sites are crucial to understand parasite circulation.

Thirteen *S. rivulatus,* 33 *S. luridus,* 30 *S. cretense* and 20 *S. salpa* were studied for their digenean parasites. The two lessepsian *S. rivulatus* and *S. luridus* have the same and only parasite *Aponorus sigani. Sparisoma cretense* has only one species *Schikhobalotrema sparisomae* and *S. salpa* is parasitised with 6 species *Centroderma spinosissima, Mesometra orbicularis, Mesometra brachycoelia, Wardula capitellata, Lepocreadium pegorchis, Robphildollfusium fractum*.

No parasites switching between these herbivorous fish were observed. Absence of intermediate host and phylogenetic barrier may prevent transfer of parasite. The introduced species was considerably less parasitised than those from native locality (10 species were reported from *S. rivulatus* and *S. luridus* of Red Sea and only one species from Mediterranean Sea). *A. sigani* is a co-introduced parasite established with its host and its intermediate host in Mediterranean Sea.

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A NEW SPECIES OF *MYRACETYMA* (COPEPODA: ERGASILIDAE: ACUSICOLINAE) FROM GILLS OF *PROCHILODUS LINEATUS* (OSTEICHTHYES: PROCHILODONTIDAE) IN THE MIDDLE PARANÁ SYSTEM, PROVINCE OF SANTA FE, ARGENTINA

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A new species of *Myracetyma* Malta, 1993 (Copepoda: Ergasilidae: Acusicolinae) is described from the gills of *Prochilodus lineatus* (Prochilodontidae). Seventy-three specimens of *Prochilodus lineatus* (Prochilodontidae) were collected in lagoons of the Northern Salado River (31°41'S, 60°44'W) and San Javier River (31°13'32"S, 60°10'20"W), both located in the Middle Paraná River floodplain (Santa Fe, Argentina) in March 2004 and June 2008. Specimens of copepod parasites were fixed in 5% formaldehyde solution and mounted on slides with Hoyer's medium to study their structures. *Myracetyma n. sp.* exhibits the main characteristic of the genus, namely, subcylindrical, slender and elongate second segment of the endopod of the first leg. The new species differs from other species of the same genus by possessing a larger egg-sac with up to 80 eggs per sac. Another remarkable difference is the presence of an expansion in the lateral inner margin along three-quarters of the total length of the third antennal segment. The three species described for this genus, *Myracetyma etimaruya* Malta, 1993, *M. kawa* Malta, 1993 and *M. piraya* Malta, 1993 were described from fish of the Amazon basin. This is the first record of this genus in the middle Paraná River system, Argentina.

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NEW COPEPOD PARASITES OF *ISACIA CONCEPTIONIS* (PERCIFORMES: HAEMULIDAE) COLLECTED IN NORTHERN CHILE AND A REEXAMINATION OF *CLAVELLA SIMPLEX* (CASTRO ET BAEZA, 1985) USING SCANNING ELECTRON MICROSCOPY

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Copepods were collected from recently captured fish, *Isacia conceptionis*, from northern Chile, fixed in 70% alcohol and then appendages dissected. Some fixed specimens were dehydrated in a series of increasing concentrations of ethanol, dried to a critical point and studied by scanning electron microscope. The first type of specimens were first determined as *Clavella simplex* Castro et Baeza, 1985, and presents more simple characteristics than other *Clavella* sp., including the armature and segmentation of antennules, the endopod armature of antenna, and diminutive bulla. These characteristics have precluded inclusion of this species in *Clavella* Oken 1815. Although this simple characteristic leads us to believe this species belong to a new genus in the *Clavella* branch. Another type specimen was detected inhabiting the olfactory sacs and included in the *Clavella* branch. Yet there is no other genus or species in the *Clavella* branch that colonizes such a particular site. Several characteristics differentiate this last specimen from all other *Clavella* branch genera, including antennae with exopods shorter than endopods, a large lobular projection and smaller subcircular projection at the base of the cephalothorax, a subspherical and flat bulla, mandibles without secondary dentition, and a suboval male body type with two modified caudal rami. Males of both types of specimens differ from each other in several ways (e.g. the position of the buccal area, oriented ventrally in the first while distally in the second type and this last with caudal rami).

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ADDITIONAL MORPHOLOGICAL INFORMATION ON YOUNG FEMALE *CALIGUS KUWAITENSIS* (COPEPODA, SIPHONOSTOMATOIDA) FROM AN EGYPTIAN MARINE FISH (*PAGRUS PAGRUS*)

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In the present study, identified *Caligus kuwaitensis* were detected from the gills of the Egyptian marine water fish (*Pagrus pagrus).* Additional morphological features of the young female *Caligus kuwaitensis* (Copepoda, Siphonostomatoida) were described with light (LM) and scanning electron microscopy (SEM). Sub-orbicular cephalothoraxes shield, with ridged inner wall of lunules were observed by SEM. The lunules appeared as finely striated and the distance between them larger than their diameters. The most characteristic feature of *C. kuwaitensis* was the distinct structure (arc-like) described as the sucker "apparatus" or "rugose" area in-between the lunules and used as a sensory organ.

On the first pair of legs, the first exopod bears a number of fine setules on the basal segment and has a distal spine-like bristle. The second exopodal segment was bearing four terminal optical stout setae nearly equal in length. In addition, the exopod of the second leg bears two unequal setae with large plumose seta on the posterior edge. Distinct spermatophores were attached with the genital complex of the young female *C. kuwaitensis*. These additional morphological features were important and significant in accurate identifications of this *Caligus* species. Furthermore, *P. pagrus* is considered as a new host in the new locality (Egypt) for *C. kuwaitensis*.

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BOMOLOCHUS BELLONES AND *CALIGUS DIAPHANUS* WITH MORPHOLOGIC CHARACTERS FROM TURKEY

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Bomolochus bellones Burmeister, 1833 (Copepoda; Bomolochidae) and *Caligus diaphanus* Nordmann, 1832 (Copepoda; Caligidae) were reported for the first time from Turkey. Morphological characters of these parasitic copepods are given in photographs and drawings. Hitherto, only ten species of the family Caligidae have been recorded parasitising fishes in Turkish marine habitats. They are *Caligus apodus*, *C. bonito, C. brevicaudatus*, *C. lagocephali*, *C. minimus*, *C. pageti*, *C. pelamydis*, *C. solea*, *C. temnodontis*, and *Lepeophtheirus europaensis* (Bakır et al. 2014, Demirkale et al. 2014, Öktener and Trilles, 2009). *Bomolochus soleae and B. bellones* were the most commonly-reported bomolochid species in the Mediterranean. 18 of tub gurnard, *Chelidonichthys lucerna* (Linnaeus, 1758) *and* 55 of garfish, *Belone belone* (Linnaeus, 1761) were collected by local gears from the Aegean Sea, Turkey, in 2014. The parasites collected were fixed in 70% ethanol. The drawings of appendages were carried out with the aid of a camera lucida. Photos were taken with the aid of a camera attached to a microscope. The antennae, maxillule, mandible, maxilliped, maxilla, caudal rami, setal and spinal formulae of the legs permitted identification of this copepod as *Bomolochus bellones*. It was recorded on gill filaments and inside the operculum of *Belone belone* with 43.6% prevalence. Morphologic features of all dissected parasites identified these copepods as *Caligus diaphanus*. It was recorded for the first time on gill chambers and inside the operculum of *Chelidonichthys lucerna* with 16.6% prevalence. Consequently, the report is very useful for updating the geographical distribution of these parasites in the Mediterranean.

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OCCURRENCE OF *NAOBRANCHIA CYGNIFORMIS* AND *LERNAEOCERA BRANCHIALIS* FROM TURKEY

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Naobranchia cygniformis Hesse, 1863 (Copepoda, Lernaeopodidae) and *Lernaeocera branchialis* (Linnaeus, 1767) (Copepoda: Pennellidae) were reported for the first time from Turkey. The aim of this study is to contribute to the parasite fauna of fish from Turkey.

Eight species of the family Lernaeopodidae are reported from Turkey: *Clavellotis fallax*, *Clavellisa scombri*, *Clavellotis strumosa*, *Lernaeopoda galei*, *Parabrachiella impudica*, *Parabrachiella bispinosa*, *Parabrachiella exigua*, and *Clavellotis briani*. Five species of the family Pennellidae are reported from marine habitats of Turkey, *Pennella instructa*, *Pennella filosa*, *Lernaeenicus neglectus*, *Lernaeolophus sultanus*, and *Peniculus fistula*.150 of Poor cod, *Trisopterus minutus* (Linnaeus, 1758) (Pisces; Gadidae) and 63 of picarel, *Spicara maena* (Linnaeus, 1758) (Pisces; Centracanthidae) were collected by local gears from Aegean Sea, Turkey, in 2014. The parasites collected were fixed in 70% ethanol. Specimens were later cleared in lactic acid before dissection of the copepod appendages. The drawings of appendages were carried out with the aid of camera lucida. In addition photos were taken with the aid of a camera attached to a microscope.

Characteristic features of *Naobranchia cygniformis* were determined by the cephalothorax two times as long as the trunk and egg sacs posterolateral to trunk. The cephalothorax with a holdfast composed of one dorsal and two lateral branches/antlers, egg sacs, and the large size permitted identification of the other copepod as *Lernaeocera branchialis*.

The prevalence values of *Naobranchia cygniformis* and *Lernaeocera branchialis* were 12.6% and 6.15% respectively. *Naobranchia cygniformis* was found on gill filaments of *Spicara maena*, while *Lernaeocera branchialis* was on the mouth of *Trisopterus minutus.*

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ANALYSIS OF THE BIODIVERSITY OF THE HATSCHEKIIDAE (COPEPODA, SIPHONOSTOMATOIDA), ON PERCIFORMES FISHES OFF ALGERIA

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The Copepoda parasitic of fishes are still poorly known in Algeria. A survey was conducted by Rose & Vaissière in 1952 reporting 30 species belonging to 15 genera, which represent a small proportion of the copepods reported from Mediterranean sea (Raibaut et al., 1998). So in order to update and complete this research, we performed a new survey. This allowed us to collect 37 species belonging to 19 genus and 17 families. Among these species those belonging to the family Hatschekiidae Kabata, 1979, appeared particularly interesting. This group is currently represented in the Mediterranean by eight species of *Hatschekia* Poche, 1902 and a single species of *Congericola* van Beneden, 1854; Our study allowed us to collect, from the gills of the slender rockfish *Scorpaena elongata* (Cadenat) a new species called *Prohatschekia mediterranea.* This is the first record of this genus *Prohatschekia* Nunes-Ruivo, 1954 from the Mediterranean (Hamza, 2007). Four members of the genus *Hatschekia* Poche, 1902 were also collected: *Hatschekia cadenati* Nunes-Ruivo, 1954 from *Epinephelus guaza*, *Hatschekia ischnon* from *Pagellus erythrinus*, *Hatschekia mulli* van Beneden, 1851 from *Mullus barabatus* and *Mullus surmuletus*; *Hatschekia pagellibogneravei* Hesse, 1879 from *Diplodus annularis*, *Diplodus sargus*, *Diplodus vulgaris*, *Pagellus bogaraveo* and the new Mediterranean host *Diplodus cervinus*; *Hatschekia subpinguis* Brian, 1913 from *Symphodus tinca* and two new Mediterranean hosts, *Symphodus mediterraneus* and *Symphodus roissali* . A description of the female and unknown male is also given.

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SEA LICE (*LEPEOPHTHEIRUS SALMONIS*) AND ANCHOR WORMS (*LERNAEA CYPRINACEA*) FOUND ON SEA TROUT (*SALMO TRUTTA*) IN THE RIVER MINHO CATCHMENT, AN IMPORTANT AREA FOR CONSERVATION IN NW SPAIN

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The International Stretch of the River Minho (ISRM), in NW Spain, is an important area for marine and freshwater conservation. It constitutes the southern limit of distribution of migratory sea trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*), where their populations are considered as vulnerable and endangered respectively. A sample of sea trout from the River Minho catchment (NW Spain) was examined for ectoparasites. Sea lice (*Lepeoptheirus salmonis*) were found on 10/113 fish (9%). Infection levels ranged from 0–8 lice/fish. Anchor worms (*Lernaea cyprinacea*) were also found, on three fish (3%). Lice identifications were confirmed by taxonomic and molecular analysis. This is the first time the presence of *L. salmonis* has been confirmed in NW Iberia. The confirmed presence of these parasites, will inform conservation agencies, wild fisheries and sustainable aquaculture initiatives in this important area.

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INFESTATION BY *SPHYRION LUMPI* AND ABNORMALITIES IN *SEBASTES MENTELLA* IN ICELANDIC WATERS DURING 1995-2013

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Sphyrion lumpi is a marine ectoparasitic copepod that has a significant negative impact on fisheries of redfish, *Sebastes* spp. in the North Atlantic as it reduces the commercial value of the fillets. Long-term data of infestation by *Sphyrion lumpi* and abnormalities in *S. mentella*, in Icelandic waters were analysed. Five categories of external abnormalities were applied in this study; black spots, red spots, mixed spots, remnants or lesions caused by *S. lumpi* and the parasite itself. Infestation intensity of the copepod was not found to be related to redfish condition (K). During the period, the prevalence of *S. lumpi* infections have declined from 25% in 1995 to 9% in 2013. Significant differences in infestations were found between female and male fish and also between the pelagic and deep-sea stock. These results give a good overall view of trends in *S. lumpi* infestation, which could be a contributing factor towards defining *S. mentella* population stock structure, which remains a debate within the scientific community.

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USING INTEGRATIVE TAXONOMY TO DEFINE THE IDENTITY OF SPECIMENS IN THE GENUS *PENICULUS* AND *TRIFUR* (COPEPODA: PENNELLIDAE), PARASITES OF MARINE FISHES FROM ANTOFAGASTA BAY, CHILE

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Peniculus is a genus of copepod parasites of marine fishes of the family Pennellidae, widely distributed. *Peniculus* currently includes 8 species. These species are usually morphologically differentiated by the cephalothorax, neck, trunk and abdomen shape; however, these characters show high polymorphism and therefore the delimitation at level species of this genus is difficult using only this type of data. The genetic distances of 25 *Peniculus* specimens associated with nine marine fish species in Antofagasta Bay, Chile, was calculated from sequences of DNA barcoding marker (COI mt) (678 base pairs). The genetic distance among the specimens from the different host species was 0.10%. We analysed the barcoding gene fragment using Bayesian Inference (BI) for phylogenetic reconstruction (using three outgroups). Based on the phylogenetic analysis, an ultrametric tree was built and a general mixed Yule-coalescent (bGMYC) model for species delimitation was conducted. Results show that based on morphology, the individuals collected in this study can be assigned to *Peniculus* cf. *fistula*. However, the high morphologic polymorphism observed into the lineage of *Peniculus* associated to several host species of marine fishes, can be interpreted as speciation incipient pattern. Similar results were obtained for *Trifur tortuosus* parasites on Chilean marine fishes. Therefore, the molecular analysis for *Peniculus* species, especially for European *Peniculus fistula* is needed.

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MOLECULAR MARKERS: A TOOL FOR DISTINGUISHING SPECIES OF THE FAMILY ERGASILIDAE (CRUSTACEA: COPEPODA)

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In recent decades, Neotropical biodiversity surveys consider fish parasites an important component of species lists. The wide range of hosts and the parasitic fauna still unknown have been the subject of research aimed at identifying and studying new species. Among the most recorded parasites, copepods are the second largest group found in marine fish and the third largest in freshwater fish. The family Ergasilidae has been the focus of an increasing number of publications involving the taxonomic description. The identification of the group is extremely difficult and based on a set of morphological characters with small interspecific variations. Molecular biology techniques have played a key role in the study of phylogenetic relationships among species of parasites. This study assessed the effectiveness of sequence analysis of cytochrome C oxidase gene (COI) of the species *Ergasilus jaraquensis* collected from the gills of *Prochilodus lineatus* (curimba) in distinguishing this parasite species from this family. Fish were collected in the Upper Paraná River floodplain - Brazil. DNA extraction and amplification of the COI gene enabled the sequencing of a region of approximately 659 bp of 5 specimens. The interspecific *p*-distance was 0.002 for *E. jaraquensis.* When compared with the sequence of *Sinergasilus polycolpus*, members of the family Ergasilidae, available at NCBI, the sequence indicates a *p*-distance of 0.2. Data indicate a divergence that can distinguish the genera by means of these sequences, but first it is necessary to check the effectiveness of these sequences in distinguishing species within the same genus.

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SEASONALITY IN REPRODUCTIVE EFFORT OF *LEPEOPHTHEIRUS PECTORALIS* (COPEPODA: CALIGIDAE)

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Lepeophtheirus pectoralis is an ectoparasite of flatfish, for which, high infection levels were reported in the literature. Ovigerous females typically infect the host's pectoral and pelvic fins, and are easily obtained. This study aimed at developing the body of knowledge on its population dynamics. A total of 120 ovigerous females (30 per season), isolated from *Platichthys flesus* (Linnaeus, 1758), were characterised with respect to total length, genital complex length, egg sac length and width, mean egg length, egg volume, fecundity and total reproductive effort. Pairwise sample comparisons were conducted using the Tukey's test. Significant differences were recorded for all variables except genital complex length. For most variables, differences were greater between summer and winter. Moreover, summer females were smaller, had shorter and thinner egg sacs and a smaller number of eggs per sac, while winter females were larger, and had larger and wider sacs containing more eggs. Accordingly, the total reproductive effort was smaller for summer. Egg volume decreased from winter to spring, but no other significant differences were recorded for this variable. Autumn females were similar to those of winter in size, but were intermediate between summer and winter females for most variables; spring females ranged from large females, similar to those of winter, to small summer-like females. These trends suggest a pattern of at least two alternating generations: an overwintering generation with larger, more prolific females that endure autumn, winter and spring, and a summer generation with smaller, less fertile females, that undergo maturation more rapidly.

Acknowledgments: BYT/CIIMAR.

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PREVALENCE AND INTENSITY OF *CALIGUS ELONGATUS* (COPEPODA: CALIGIDAE) INFECTING *MULLUS SURMULETUS* (ACTINOPTERYGII: MULLIDAE) OFF AZORES ARCHIPELAGO, PORTUGAL

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Surmullet, *Mullus surmuletus* Linnaeus, 1758, ectoparasite infections are yet to be studied in detail, with only a few records for localities around the world; moreover, sea lice infections occurring on fish off the Azores Islands (Portugal) are still poorly investigated. With the aim of determining the prevalence and intensity of sea lice in surmullet, an ectoparasite survey was performed. Eight fishes, caught randomly with a barrier net off São Miguel Island (Azores archipelago, Portugal), were found infected with 60 specimens of *Caligus elongatus* Nordmann, 1832, which were 37 ♀♀ and 23 ♂♂. The prevalence recorded the maximum possible level, i.e. 100%; and the mean intensity + S.D. (range) were of 7.5 + 3.9 (3 – 14). These values are comparatively higher than those reported for North Sea; furthermore, it is noteworthy that the infection has not been recorded so far for Mediterranean Sea fish. *C. elongatus* is one of the most common species of sea lice infecting farmed salmon, and it is also widely distributed, with records found in the literature for several fish species. According to our results, wild surmullet may represent an important reservoir for those parasites.

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INVESTIGATION OF CRUSTACEAN PARASITES OF MARINE FISHES NEAR GÖKÇEADA, TURKEY İN THE NORTHEASTERN AEGEAN SEA

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In this study realized to determine the crustacean parasites of fishes caught near Gökçeada which is an important area in terms of fisheries, a total of 913 fish samples belong to 52 different fish species caught by using various fishing methods such as gillnet, longline, vertical longline, gig were investigated acording to standart methods. In the result of this study, a total of 12 species of crustacean parasites belonging to 5 species of Isopoda and 7 species of Copepoda were found on 21 fish species of 52 fish species. The found species, their host and prevalence are the following: *Ceratothoa oestroides* on *Diplodus annularis* (11.1%), *Diplodus vulgaris* (2.86%), *Trachurus mediterraneus* (3.57%), *Boops boops* (10.53%); *Ceratothoa paralella* on *D. annularis* (8.33%), *B. boops* (12.28%); *Anilocra physoides* on *Pagellus erythrinus* (7.69%), *D. annularis* (1.39%), *Oblada melanura* (2.86%), *B. boops* (3.51%); *Nerocila bivatata* on *Sciaena umbra* (1.89%), *Spicara flexuosa* (5.71%); Gnathidae gen. sp. praniza larvae on *D. annularis* (6.94%), *D. vulgaris* (8.57%), *Diplodus sargus* (16.67%), *Dentex macrophtalmus* (12.5%), *Sciaena umbra* (16.98%), *Conger conger* (11.54%), *Lithognathus mormyrus* (13.33%), *Symphodus sp*.(18.92%), *Phycis phycis* (43.75%), *S. flexuosa* (%2.86), *Coris julis* (33.33%); *Hatschekia mulli* on *Mullus surmuletus* (17.39%); *Hatschekia pagellibogneravei* on *D. annularis* (2.78%), *P. erythrinus* (7.69%), *C. conger* (46.15%); *Pennella instructa* on *Xiphias gladius* (16.67%); *Lernanthropus kroyeri* on *Dicentrarchus labrax* (20%); *Clavellopsis fallax* on *Diplodus sargus* (8.33%), *Spondyliosoma canthharus* (15.7%9), *Sarpa sarpa* (9.38%), *Pagellus erythrinus* (7.69%); *Sphyrion lump*i on *Squalus blanvillei* (14.29%); *Lernaea* sp. on *Boops boops* (1.75%), *O. melanura* (5.71%), *M. surmuletus* (8.69%). It was determined that Praniza larvae of Gnathidae gen. sp. was the dominant parasite species of this region; *D. anularis* was the fish species most parasitised, by 5 parasite species.

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CRUSTACEAN PARASITES OF *OTOLITHES RUBER* (PERCIFORMES: SCIAENIDAE) IN BANDAR ABBAS, IRAN

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Crustaceans are the most various and diversified in all the metazoan groups. Due to their feeding activities on the host they are economically important parasites and should not be neglected by researches. This study was conducted to determine the presence of crustacean parasites of *Otolithes ruber* (Bloch & Schneider) in coastal waters of Hormozgan province, Persian Gulf, Iran. A total of 118 specimens were caught seasonally by bottom trawl and by hook in Bandar Abbas from summer 2013 to spring 2014. All samples were immediately placed on ice and frozen samples were transferred to the laboratory of Shahid Beheshti University. After biometry of fish, parasitological examinations were conducted by stereo and light microscopes on the body surface, fins and gill cavities of the host. Three species of parasitic copepods *Lernanthropus* sp., *Caligus* sp. and *Parabrachiela* sp. and one species of parasitic isopod *Gnathia* sp. were removed from the inner surface of the operculum and gill filaments of the host. The prevalence, mean intensity and abundance of parasites are as follows: *Caligus* sp. (1.69%, 1± 0.31 and 0.01), *Parabrachiela* sp. (6.77%, 1.62 ± 0.12 and 0.11), *Lernanthropus* sp. (3.38%, 1± 0.52 and 0.03) and *Gnathia* sp. (1.69%, 1± 0.31 and 0.01). There were no significant differences among crustaceans abundance in different seasons. No significant differences were observed among the crustaceans analysed with respect to the sex of the host. And also no significant correlation was found between parasites abundance and total length of fish.

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ESTABLISHING INTRA-SPECIFIC VARIABILITY OF A FISH PARASITIC CYMOTHOID ISOPOD, *CYMOTHOA EREMITA* (BRÜNNICH, 1783), FROM SOUTHERN AFRICA USING MORPHOLOGICAL AND MOLECULAR TECHNIQUES

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Parasitic cymothoids from the order Isopoda are large obligate parasites on various fish species worldwide. They are found attached to the host's gills, external surfaces, in the mouth or inside the body cavity, and this attachment position can be genus or species specific. Cymothoids are notoriously known to exhibit a high level of variability and over the years intra-specific variation has often become confused with interspecific differences. One of the most poorly understood and least studied cymothoid genera is the mouth-inhabiting genus *Cymothoa* Fabricius, 1793. Currently there are six known species found in southern Africa including *Cymothoa borbonica* Schioedte & Meinert, 1884; *C. epimerica* Avdeev, 1979; *C. eremita* Brünnich, 1783; *C. hermani* Hadfield, Bruce & Smit, 2011; *C. sodwana* Hadfield, Bruce & Smit, 2013; and *C. selari* Avdeev, 1978. During a sampling trip to Santa Maria, Mozambique, parasitic cymothoids closely resembling *Cymothoa eremita* (Brünnich, 1783) were collected from *Liza macrolepis* (Smith, 1846), a mullet species. Extensive morphological techniques were used to accurately identify the species; however, many of the characters showed variability in certain species characteristics. Although minor, these variations observed in the Mozambique specimens have in the past been used to describe new *Cymothoa* species. Thus molecular studies were employed to prove the existence of intraspecific morphological variation within a cymothoid species and prevent erroneous identifications or dubious new species from being named. This is the first comprehensive work using both morphological and molecular techniques to identify a cymothoid and conclusively establish the intra-specific variability of a single species.

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FIRST MOLECULAR CHARACTERIZATION OF FISH PARASITIC MARINE CYMOTHOID ISOPODS FROM SOUTHERN AFRICA

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Isopods from the family Cymothoidae are parasitic on numerous marine, brackish and freshwater fishes where they can be found on the host's external surfaces, inside the body cavity, or within the gill or buccal cavity. Although easy to observe with the naked eye, these isopods are not often studied and as a result there are many aspects of their life history and taxonomy that remain unknown. One such area with a paucity of information is their phylogeny and taxonomy based on molecular data. With only a few species of cymothoids having been sequenced, and many genera without any sequences on GenBank, the need for good quality cymothoid sequences is evident. This is especially important due to the difficulty arising in accurately identifying species in this morphologically variable group of parasitic isopods. A number of cymothoid species from *Ceratothoa* Dana, 1852, *Cymothoa* Fabricius, 1793 and *Mothocya* Costa, in Hope, 1851 were collected from the south and east coasts of South Africa as well as from southern Mozambique. DNA was extracted and fragments of two mitochondrial genes (16S rRNA and cytochrome oxidase I) from each species were amplified and sequenced. Resulting sequences were compared to each other as well as selected sequences from GenBank and aligned using the software package Genious. This preliminary work resulted in a number of new cymothoid sequences being added to GenBank which will aid future research and molecular phylogentic studies in cymothoid isopods, as well as clarifying several morphologically ambiguous species based on their molecular configuration.

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OCCURRENCE OF CYMOTHOID ISOPOD PARASITES IN SOME COMMERCIAL FISHES FROM THE GULF OF THAILAND

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Two hundred and eighty samples of four commercial fish species; Yellow Scad (*Atule mate*), Torpedo Scad (*Megalapis codyla*), Butterfly Whiptail (*Pentapodus setosus*) and Bigeye Scad (*Selar crumenopthalus*) were collected from the Gulf of Thailand. The fish samples were examined for the cymothoid isopod infestation during December 2013 to November 2014. The results showed three species of parasitic isopods with their prevalence and intensity as follows: *Livoneca* sp. found in buccal cavity of *A. mate* (5.00%, 1.33) and in *M. codyla* (5.00%, 1.00), *Norileca indica* found on gills of *A. mate* (7.05%, 1.33) and on *S. crumenopthalus* (71.25%, 1.50). For the isopod *Smenispa irregularis* found in buccal cavity of fish species *P. setosus* (5.00%, 1.00). In this research, the results showed that Butterfly Whiptail, *P. setosus*, is a new host for the isopod *S. irregularis.*

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REVIEW OF THE PARASITIC MARINE CYMOTHOID ISOPODS FROM THE GENUS *MOTHOCYA* COSTA, IN HOPE, 1851 FROM SOUTHERN AFRICA

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Cymothoid isopods from the genus *Mothocya* Costa, in Hope, 1851 are known to occur inside the gill chambers of their host fish. Many of these gill-inhabiting species are recognised by the laterally twisted body shape of the female, a resulting character from the shape of the gill cavity in which they reside. After collecting infected fish from the Maputo fish market, Mozambique, as well as Sodwana Bay, St Lucia and Mhlathuze estuaries in South Africa, isopods were removed and identified. Three species were identified, two known and one species new to science. *Mothocya plagulophora* (Haller, 1880) was obtained from Maputo, Mozambique, from the gills of *Hemiramphus far* (Forsskål, 1775) and can be identified by the characteristically large and extremely wide pleon and pleotelson. *Mothocya renardi* (Bleeker, 1857) was identified from various localities in South Africa and Mozambique, from the hosts *Strongylura leiura* (Bleeker, 1850) and *Tylosurus choram* (Rüppell, 1837), and can be distinguished by their large size (24–36 mm in length) as well as the narrow pleon and extended uropods. Lastly, a new species *Mothocya affinis* Hadfield, Bruce & Smit, 2015, was discovered from Sodwana Bay, South Africa, from the gills of *Hyporamphus affinis* (Günther, 1866), possessing distinctively large coxae which extend over the pleon. This paper thus presents the first comprehensive revision of all the *Mothocya* species from southern Africa as well as a key to the south-western Indian Ocean *Mothocya*. The latter will aid all future researchers in identifying representatives of this important group of fish parasites.

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IMPACT OF OSTRACODS ON WILD *SERIOLA DUMERILI* FROM FISHERIES

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Feeding habits of marine ostracods are diverse, from herbivorism to carnivorism, opportunistic or parasitic. Many ostracods are scavengers capable of micropredator activity and cause extensive injuries, mostly on fish already harmed by natural causes or human actions (fisheries). The aim of this work is to describe invasion patterns and effects of large ostracods affecting greater amberjack *Seriola dumerili* in the western Mediterranean. To assess this objective, 60 fish from two different localities, Majorca and Alicante, were analysed in two different samplings during winter (2005-2006). Ostracods were collected and fish injures were registered. As a result of this study, 188 cypridinid ostracods were obtained. In spite of their low prevalence (10 out of 60 fish), infection sites varied among fish individuals: gills, oesophagus, stomach, pericardial cavity and visceral cavity. Concerning to injuries, ostracods dug circular orifices, similar in size to their body section, which were detected between buccal and pericardial cavities, as well as between pericardial and visceral cavities. These findings suggest that ostracods found in greater amberjack entered by the mouth and went through one cavity to another. The healthy external appearance of the affected greater amberjacks together with the fact that the ostracods were still alive when collected point to a fast invasion after fish capture. The significance of scavengers for fisheries has been disregarded to date but it is obvious that the presence of these ostracods on fish would devaluate them as products.

Funded by AGL2010-20892, ISIC/2012/003 & PROMETEO 795/2015/018 projects.

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ACANTHOCEPHALAN PARASITES OF *OTOLITHES RUBER* (OSTEICHTHYES: SCIAENIDAE) IN CHABAHAR, IRAN

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Parasites play a very important role in the lives of their hosts, not only in fish health but also to understand ecological problems. Acanthocephalans are relatively poorly known helminth group in marine fishes. A survey on acanthocephalan parasites of *Otolithes ruber* (Bloch & Schneider) was carried out in Chabahar, Sistan and Baluchestan province. Totally, 90 specimens were caught by gill net and hook seasonality from summer 2013 to spring 2014. Samples were immediately frozen and transferred to laboratory of Shahid Beheshti University. After measuring fish weight and length, parasitological survey was implemented by stereo-and light microscopes on internal organs of specimens. Two species of parasites were removed from stomach, intestine and abdominal cavity of the host. The parasites were *Tegorhynchus* sp. and *Serrasentis* sp. The prevalence, mean intensity and abundance of parasites were as follows: *Tegorhynchus* sp. (6.6%, 2 ± 0.71 and 0.13) and *Serrasentis* sp. (1.11%, 1 ± 0.03 and 0.01), respectively. A significant difference was observed among the acanthocephalans analyzed with respect to the sex of the host ($P < 0.05$). There were no significant differences between parasites abundance in different seasons. And also no significant correlation was found between parasites abundance and total length of fish.

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DIVERSITY OF ACANTHOCEPHALAN PARASITES OF TELEOST FISH FROM TUNISIAN LAGOONS

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Acanthocephala is a well studied group of fish parasites in North America, Europe and India; however researches concerning the diversity of this group in Africa are few. Hitherto, no study has been conducted on the acanthocephalans of teleost fish from the Tunisian coasts hence the importance of this study.

Fish were collected from two lagoons in northern east of Tunisia, Ichkeul and Bizerte; 98 specimens from Bizerte lagoon belonging to 4 families: Atherinidae, Carangidae, Mugilidae and Sparidae, and 70 specimens from Ichkeul lagoon belonging to Mugilidae.

Seven species of acanthocephalans belonging to 5 families were found in the digestive tract of examined fish: Neoechinorynchidae (*Neoechinorhynchus (Hebesoma) agilis*; *N.(Neoechinorynchus) rutili*), Arhythmacanthidae *(Acanthocephaloides propinqus, A.incrassatus*), Pomphorynchidae (*Longicollum pagrosomi*), Rhadinorhynchidae (*Rhadinorhynchus cadenati*) and Illiosentidae (*Telosentis exiguus*).

A. propinquus, *L. pagrosomi* and *R. cadenati* were restricted to the rectum and posterior intestine, whereas the remaining species colonized two or three parts of the intestine. The most diverse acanthocephalan fauna was recorded in *Lithognatus mormyrus* with three species.

L. pagrosomi and *R. cadenati* attained the lowest prevalence of less than 6% and were considered as rare species. *A. incrassatus*, *A. propinquus*, *T. exiguus* were satellite species. *N.(H) agilis* showed the highest prevalence (65%) and was considered as a dominant species. The diversity of the acanthocephalan fauna in the fish from the Bizerte lagoon is compared with previous studies from other localities. The report of *A. incrassatus*, *A. propinquus* and *N. (N.) rutili* in *L. mormyrus* presents new host records in Mediterranean waters.

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ULTRASTRUCTURAL CHARACTERS OF THE SPERMATOZOON OF THE DIGENEAN *TERGESTIA ACANTHOCEPHALA* (FELLODISTOMIDAE), PARASITE OF *BELONE BELONE GRACILIS* IN TUNISIA

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The contribution of ultrastructural studies of the spermatozoon to understanding the taxonomy and phylogeny of the parasitic Platyhelminthes is now widely accepted. In the present study, we describe the ultrastructural organisation of the spermatozoon of the digenean *Tergestia acanthocephala* (Gymnophalloidea: Fellodistomidae) by means of transmission electron microscopy (TEM). Adult specimens of *T. acanthocephala* were removed from the intestine of several specimens of *Belone belone gracilis* caught off the Gulf of Gabès in Chebba (Tunisia). The living digeneans were routinely processed for TEM examination.

The mature male gamete of *T. acanthocephala* presents most of the structures previously described in digenean spermatozoa such as two axonemes of different lengths of the 9+‘1’ trepaxonematan pattern, two bundles of parallel cortical microtubules, mitochondrion, nucleus and granules of glycogen. However, several characteristics of the spermatozoon differ from those described in other previously studied digenean species: (i) the filiform morphology of the anterior extremity and the presence of a continuous layer of cortical microtubules surrounding the axonemes at their anterior end, (ii) the presence of spine-like bodies, (iii) the presence of 2 mitochondria, and (iv) the morphology of the posterior extremity presenting only the nucleus with a very few granules of glycogen.

Study partly supported by the Spanish Project (no. A/023585/09) from the “Agencia Española de Cooperación Internacional para el Desarrollo (AECID)”.

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SPERMATOZOON ULTRASTRUCTURE OF THE DIGENEAN *LECITHOSTAPHYLUS RETROFLEXUS* (MICROPHALLOIDEA: ZOOGONIDAE), AN INTESTINAL PARASITE OF *BELONE BELONE GRACILIS* IN TUNISIA

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During last years, the use of spermatological data for phylogenetic inference in the parasitic Platyhelminthes has been widely demonstrated. Concerning the family Zoogonidae, the ultrastructural studies of the spermatozoon are currently limited to a single species, *Diphterostomum brusinae*. In the present work, we present the first ultrastructural results on the sperm cell of a second genus of this family with the study of *Lecithostaphylus retroflexus* by means of transmission electron microscopy (TEM). Adult specimens of *L. retroflexus* were removed from the intestine of several specimens of *Belone belone gracilis* caught off the Gulf of Gabès in Chebba (Tunisia). The living digeneans were routinely processed for TEM examination.

The mature spermatozoon of *L. retroflexus* presents two axonemes of different lengths with the 9+‘1’ Trepaxonematan pattern, a nucleus, two mitochondria, two bundles of parallel cortical microtubules and granules of glycogen. Additionally, the sperm cell of *L. retroflexus* exhibits the type 2 of the external ornamentation of Quilichini et al., spine-like bodies and a continuous layer of parallel cortical microtubules surrounding the axonemes at their anterior end. Moreover, the morphology of the posterior spermatozoon extremity corresponds to the fasciolidean type of Quilichini et al.

Study partly supported by the Spanish Project (no. A/023585/09) from the ‘‘Agencia Espanõla de Cooperación Internacional para el Desarrollo (AECID)’’.

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ULTRASTRUCTURAL CHARACTERS OF MATURE SPERMATOZOA OF THE HEMIUROIDEA (DIGENEA)

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The ultrastructural features of the spermatozoon are very good tools for establishing the phylogeny of Platyhelminthes. The phylogenetic relationships of many digenean parasites of teleostean fish are unknown. This is the case of Hemiuroidea. Thus, we focused on this group of helminths and performed the ultrastructural study of the spermatozoa of three species of Hemiuridae. These are *Lecithocladium excisum* from *Scomber japonicus* (Scombridae) *and Decapterus rhonchus* (Carangidae), *Parahemiurus merus* from *Sardinella aurita* (Clupeidae), *S. maderensis* (Clupeidae) and the Sclerodistomidae: *Prosorchis palinurichthi* from *Schedophilus velanei* (Centrolophidae), all captured in the Atlantic Ocean coast near to Senegal. Specimens were processed according to conventional methods for transmission electron microscopy and the grids were examined in a Hitachi H-7650 electron microscope operated at 80 kV, at the University of Corsica (France) and a JEOL 1010 transmission electron 85 microscope operated at 80 kV, at the "Centres Científics i Tecnològics de la Universitat de Barcelona (CCiT-UB)" (Spain). We show the most outstanding features of the spermatozooa of Hemiuridae and Sclerodistomidae, namely the lack of spine-like body, the presence of the external ornamentation of the plasma membrane in the anterior extremity of the spermatozoon without cortical microtubules, the disposition of the cortical microtubules in only one side of the spermatozoon when they are present, and the presence of a reduced quantity of glycogen granules. These ultrastructural characters can be good tools for phylogenetic purposes of the Hemiuroidea.

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PARADIPLOZOON HOMOION (MONOGENEA): DIGESTIVE TRACT ON THE FINE STRUCTURE LEVEL

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Diplozoons (Monogenea) are blood-feeding ectoparasites of fish with specific cross body arrangement of two permanently fused adult individuals. Each has two large bucal suckers and a muscular pharynx in the anterior part and an opisthaptor with scleroprotein clamps and hooks in the posterior part of body. Whereas these conspicuous morphological structures have been frequently studied, details of internal morphology and ultrastructure are little known.

Specimens of *P. homoion* were obtained from the gills of common bleak *Alburnus alburnus*; the fish were caught in the littoral zone of Mušov lowland reservoir (South Moravia, Czech Republic). The fish gills were checked using stereo microscope for the presence of all diplozoid ontogenetic stages. Found parasites were fixed by 2% osmium tetroxide, dehydrated through ascending acetone series and immediately embedded in a Spurr resin. All sections were obtained by Leica EM UC6i ultramicrotome. Semi-thin sections were stained by toluidine blue and ultra-thin sections were contrasted by uranyl acetate lead citrate. Ultra-thin sections were examined by transmission electron microscope operating at 60 kV (JEOL JEM-1010) equipped with Megaview II software.

In this study, the ultrastructural details of the gastrointestinal tract of this worm which represent a unique documentation of specialised structures such as buccal suckers, pharynx with pharyngeal glandular cells, haematin cells and the ultrastructural view of the gut lumen with intact erythrocytes, were documented. Our findings support the hypothesis that the mechanism of *P. homoion* digestion is predominantly intracellular.

This study was supported by the Czech Science Foundation – Grant no. GAP506/12/1258.

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THE ORGANISATION OF THE BODY WALL AND RELATED STRUCTURES IN *PARADIPLOZOON BLICCAE*

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The species *Paradiplozoon bliccae* belongs to the family Diplozoidae (Monogenea), representing blood-feeding gill ectoparasites of freshwater fish. Diplozoid monogeneans exhibit unusual body organisation and a life cycle involving a permanent fusion of two larval worms that subsequently transform into one individual. The body wall of parasitic Platyhelminthes, including adult monogeneans, is covered by a tegument consisting of a syncytial layer and underlying cell bodies, connected to the syncytium via cytoplasmic projections. The subtegumental position of their nuclei is thought to protect them from host defence. Besides conventional transmission electron microscopy, the freeze-etching technique proved to be a strong tool to reveal the membranous structures of tegumentary cells and body wall muscles. Direct labelling of filamentous actin with fluorescent phalloidin was used to visualize the general organization of the major muscular structures and three muscle layers separated from the syncytium by a *lamina basalis*. The body wall musculature appears well-developed and highly organized, comprising circular, intermediate longitudinal and inner diagonal muscle fibres. In contrast to other so far investigated diplozoids, the muscle fibres of *P. bliccae* are wavy. Scanning electron microscopy helped to explore the topology of tegument surface with sensory receptors. Combined microscopic approaches enabled a multidimensional depiction of the body wall organisation. For first time, the freeze-etching was used to investigate the tegument organisation in monogeneans. Comparison of data obtained from *P. bliccae* and other diplozoids allow to evaluate the degree of their diversification and adaptation to the ectoparasitic life.
Financed by the Czech Science Foundation project No. GAP506/12/1258.

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ACCUMULATION OF SOME HEAVY METALS IN *HYSTEROTHYLACIUM ADUNCUM* (NEMATODA, ANISAKIDAE) INFECTING THE COMMON SOLE *SOLEA SOLEA* (SOLEIDAE) AND ITS ROLE AS A BIOLOGICAL INDICATOR OF POLLUTION FROM MEDITERRANEAN

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*N*ematode parasites were isolated from the intestine of the common sole *Solea solea* (Soleidae) collected from coasts along Alexandria City at the Mediterranean Sea in Egypt. Light and scanning electron microscopy revealed that this nematode belongs to the family Anisakidae and to the genus *Hysterothylacium*. The type species is named *H. aduncum*, based on the presence of three interlocked lips with the interlabium in between, the presence of cephalic papillae, and large numbers of caudal papillae in males. The morphological characteristics of this species was confirmed by molecular analysis of 18S rDNA for these parasites followed by comparison with sequence data from the Genbank showing that *H. aduncum* is deeply embedded in the genus *Hysterothylacium* with a sequence similarity between 95.5–94.3% with close relationships to other *H. aduncum* specimens and *Hysterothylacium* sp. Concentrations of heavy metals (Zn, Cu, Mn, Cd, Ni, and Pb) accumulated in the parasites were higher than those in the tissues of the host fish with the exception for Zn which was found in higher quantity in fish kidney than in the parasite tissues. The results of this study supported the hypothesis that fish parasites can be regarded as a useful bio-indicator when evaluating environmental pollution of aquatic ecosystems by heavy metals.

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RELATIONSHIP BETWEEN THE CONCENTRATION OF HEAVY METALS AND PARASITES, FOOD AND HABITAT IN SOME MEDITERRANEAN EDIBLE FISHES

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Heavy metals are recognized as important pollutants in marine ecosystems that bioaccumulate in fishes, producing potential risk to human health. The capacity of some helminth parasites to bioaccumulate heavy metals has been widely demonstrated and it seems that some parasites (Cestoda) would reduce heavy metal load. We have evaluated concentrations of arsenic, cadmium, lead and mercury in muscle of several fish species from the South Western Mediterranean and we have compared infected fishes (79) and uninfected ones (143). The parasites found were mainly nematodes and some monogeneans and digeneans. The relationship between presence of parasites and the amount of heavy metals in flesh, altogether, showed that infested fishes had higher amounts of Pb, As and Hg. Contrary to the aforementioned antagonistic effect, in the present study both parameters seem to follow the same trend, and they will reinforce each other in weakening the fish. It is known that deep sea species show higher levels of Hg, and that trophic position should also explain different concentrations. In fact we found that there are higher lead and mercury concentrations in carnivorous species than the planktivorous ones. Pollutant levels also increase from pelagic to demersal and to littoral benthic species, the latter with higher metal concentrations.

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INFLUENCE OF INTESTINAL TAPEWORMS IN THE MUSCULAR SE:HG MOLAR RATIO OF *MERLUCCIUS MERLUCCIUS* FROM THE MEDITERRANEAN COAST OF GIRONA

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Fishes have been used to monitor marine mercury pollution which is related to the effect that methylmercury exerts on selenium-dependent enzymes. Selenium has some protective effect against mercury toxicity sequestering methylmercury and reducing its bioavailability. It has also been argued that higher Se:Hg molar ratios indicate a more protective effect of selenium against mercury toxicity. Nowadays, it is also accepted that some helminth parasites can accumulate high amounts of toxic elements.

In this context, we have quantified mercury and selenium as well as the Se:Hg molar ratio in the muscle of *M. merluccius* from the NW Mediterranean coast (50% infested by *Clestobothrium crassiceps*), in order to know if the presence of tapeworms modifies the concentrations of both elements as well as the Se:Hg molar ratio.

Total mercury in the muscle of non-parasitized hakes was higher than in infested specimens. Contrarily, concentration of selenium was higher in infested individuals by *C. crassiceps* than in specimens free of cestodes. Consequently, the Se:Hg molar ratio in infested hakes was higher (2.6 ± 0.8) than in non-parasitized ones (1.9 ± 0.9). These results indicate that fish parasites may be implicated in the amount of Se and Hg available in different tissues of their hosts modifying the Se:Hg molar ratios. Present data show that intestinal cestodes reduces the mercury level in hakes which by themselves tend to have large amounts of this element as a consequence of their predatory behaviour.

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CAN PARASITES AND EROD ACTIVITY IN FISH INDICATE ENVIRONMENTAL IMPACT IN TROPICAL/SUBTROPICAL RESERVOIRS?

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Fish parasites and pollution biomarkers have been considered useful tools to indicate environmental impact in freshwater ecosystems. We therefore examined whether the parasite burden and EROD activity of the South American silver croaker *Plagioscion squamosissimus* were affected in a pollution gradient, considering host related-factors, and seasonality. We collected fish and parasites and determined EROD levels from three localities during three seasons from Tietê River, Southeast Brazil. The monogenean *Diplectanum piscinarius* had the higher abundance in Barra Bonita reservoir (the most polluted locality) on the early-rainy and rainy seasons, with a significant decrease of this species in the dry season in all localities. *Austrodiplostomum* sp., abundance was very variable with high abundance on the early-rainy season at Barra Bonita and Bariri. We observed an influence of the higher pollution levels on the abundance of *D. piscinarius* and on EROD activity levels in comparison to Promissão reservoir (the reference site). We also found that reproductive status in croaker females and *D. piscinarius* had a significant negative effect on the seasonal variability of EROD levels in the most polluted locality (near São Paulo). Our results suggest that the pollutants could be acting as immunosuppressors in croaker, enhancing parasitism. Our results also suggest that parasites and reproductive status can be significant confounding factors for determining EROD activity in *P. squamosissimus* in freshwater ecosystems. Moreover, our results suggest that the presence of phenanthrene during the dry season at Barra Bonita reservoir might explain the highest EROD activity responses found in this locality

Financial support: FAPESP 2012/00561-0; CAPES/PDSE 13836/2013-07.

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PARASITE DIVERSITY AND HISTOPATHOLOGICAL INDICATORS OF METAL POLLUTION IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FROM A SUBTROPICAL RIVER SYSTEM IN ZIMBABWE

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African catfish *Clarias gariepinus* from the Gwebi, Manyame and Mukuvisi rivers in Zimbabwe were analysed to assess the effect of metal pollution on the histopathology and component parasite communities, as well as to determine selected metal levels in gill, liver, kidney and muscle tissues. The histopathology of these tissues was assessed while metazoan parasite diversity and species composition in fish along different sites of the rivers were compared. Levels of Cd, Fe, Pb and Zn were lowest in the Gwebi, Cr and Cu in the Manyame, and Ni in the Mukuvisi River. There were significant differences ($p < 0.05$) in concentration of iron and nickel in the gills and liver of fish among the three rivers while significant differences in concentration of iron and lead were observed in muscle tissue. Gill chronic inflammation and ossification were significantly different ($p < 0.05$) in fish from among the three rivers. Chronic inflammation, hemosiderin deposits and bile accumulation in the liver were also significantly different ($p < 0.05$) among the three rivers and so was the extent of chronic inflammation in the kidney. Lamellar fusion was slightly more prevalent in gills of catfish from the Mukuvisi than the Gwebi River. The parasite community of *C. gariepinus* comprised three monogenean, two cestode and three nematode species. The least polluted Gwebi River had the highest parasite diversity while the most polluted Mukuvisi River had the least. Fish parasite community structure is thus a potential indicator of river pollution, while metal pollution is a threat to public health in the system.

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PARASITES OF *EPINEPHELUS COIOIDES* (EPINEPHELIDAE) AS ENVIRONMENTAL INDICATORS IN INDONESIAN COASTAL WATERS: MISSING LINK JAKARTA BAY

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Fish parasites have been applied in many ways to indicate anthropogenic degradation of ecosystems. The approach of this study is to assess the pollution situation of marine areas by using definite parameters that describe the fish parasite fauna. *Epinephelus coioides* (Epinephelidae), from the heavily polluted Jakarta Bay as well as off Jakarta were analysed for metazoan parasites. The findings enabled us to develop a pollution indicator system for Indonesian coastal waters by taking into account the results of previous studies of less affected localities. The system is based on a total of 300 examined fish. The documented parasite fauna consisted of 38 taxa. Seven of these parasites and five parasitological indices were used to visualise the results with a star graph. The obtained two-dimensional chart, combining twelve selected parasitological parameters, illustrates the altered environmental conditions at the sampling sites. A highly significant difference (ANOVA: F= 6.47, P< 0.01) demonstrates that the megacity Jakarta heavily influences the environment, close to the coast as well as more off the coast towards the Thousand Islands National Park. The present study provides a fish parasite based indicator system of environmental conditions in Indonesian coastal habitats. It is suggested to apply this system in other marine and freshwater areas in the future.

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ECOLOGICAL ASPECTS AND ANTHROPOGENIC INFLUENCE ON THE PARASITIC FAUNA IN *HYPOSTOMUS ANCISTROIDES* (IHERING 1911) (OSTEICHTHYES, LORICARIIDAE) IN A NEOTROPICAL RIVER

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Parasites can be used as biomarkers because they are highly sensitive to environmental changes. In this context, 121 specimens of *Hypostomus ancistroides* were collected in the Pirapó river, Paraná State, Brazil, between Summer and Winter of 2014, and analyzed for parasites. Eight parasite species were recorded, of the groups Nematoda, Monogenea, Digenea and Acantocephala. The nematode *Raphidascaris* sp. was the dominant with DI=0.80. There was no significant difference between the prevalence and abundance of parasitism in males and females (G = 2.0472, p = 0.1525; Z (U) = 0.8562, p = 0.3919, respectively). A significant difference between the seasons was verified for *Rhaphidascaris* sp. (Z (U) = 2.9677, p = 0.033) with the summer season showing the highest abundance. Significant differences in the abundance of parasitism were observed between sampling points (H = 6.2415, p = 0.004), being more abundant in the intermediate and downstream sections of the river. There was a significant positive correlation between the prevalence/abundance of parasitism and host total length (r = 0.8931, p = 0.0005; rs = 0.3607, p = <0.0001, respectively). The increase in the prevalence and abundance of parasites may reflect the cumulative effect. The parasitized hosts presented relative condition factor greater than non-parasitized (Z (U) = 3.47694, p = 0.0005). No differences were found between sampling points, which may suggest that, despite the intermediate and the downstream points are under anthropogenic influence, this is not reflected in the general condition of the hosts. The presence of *Raphidascaris* in the sampling sites suggests that this parasite serves as pollution indicator, since it supports polluted environments.

MACROECTOPARASITES (COPEPODS AND ISOPODS) OF MUGILIDAE AND SPARIDAE, IN THE NORTH AND SOUTH LAGOON OF TUNIS, TUNISIA

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Macroectoparasites can be excellent bioindicators that are able to reveal possible changes in environmental conditions. Indeed, these parasites are in direct contact with the aquatic environment by their external location on their hosts. The environmental conditions of the aquatic environment have a great impact on the survival life cycle and population dynamics of these parasites. The Northand South lagoons of Tunis went through some environmental restoration work in 1988 and 2000. These changes led to a marked improvement of water quality, an intensified exchange with the marine environment and reduction of the degree of confinement. Therefore, it seems to be a good example to estimate the impact of these changes on the ichthyoparasitofauna. Accordingly, a comparative study on the diversity and ecology of ectoparasites of Mugilidae fishes was conducted, in comparison with a previous work to estimate the impact of the two restoration works. Similarly, the study of ectoparasites of Sparidae fishes has allowed us to compare this ichtyoparasite fauna with other Tunisian lagoons already scrutinized. This has permitted us to examine 1056 fish including 313 fish from North 743 from South lagoon. The Mugilidae family is represented by 5 species and Sparidae family by 6 species in the North lagoon and 13 species of fish in the South lagoon. We collected nine species of ectoparasites on the Mugilidae fishes and we found 11 species of ectoparasites, on the13 species of Sparidae studied, including 10 species that have been cited for the first time in the lagoons of Tunis. Five species of the parasites have been reported for the first time in Tunisian lagoons.

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ERRATUM AND LAST MINUTE MODIFICATIONS

NEW DATA ON GEOGRAPHICAL DISTRIBUTION AND HOST SPECIFICITY OF SOME CESTODES IN THE ANTARCTIC

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In 2010-2014 thirteen species of bony and cartilaginous fishes (*Dissostichus mawsoni*, *D. eleginoides*, *Macrourus whitsoni*, *Pogonophryne sp.*, *Muraenolepis marmorata*, *Muraenolepis* sp., *Chionobathyschus dewitti*, *Paraliparis* sp., *Amblyraja georgiana*, *Bathyraja maccaini*, *Bathyraja cf. eatonii* & *Bathyraja meridionalis*) were examined for cestode infestation in the Ross Sea, the Amundsen Sea, the Scotia Sea and the Weddell Sea. All fishes were caught during longline fishing at a depth from 552 to 1938 m. DNA of adult cestodes from different hosts - *Otobothrium antarcticum* (Tetraphyllidea), *Echeneibothrium maculatum* (Rhinebothriidea), *Calyptrobothrium* sp., *Clistobothrium montaukensis* (Tetraphyllidea), *Echinobothrium* sp. (Diphyllidea) and four morphological types of plerocercoids from intestine of the examined bony species were sequenced using primers 1200R (5`-GCATAGTTCACCATCTTTCGG–3`) (Lockyer et al., 2003) and LSU5 5`-TAGGTCGACCCGCTGAAYTTAAGCA–3`) (Littlewood et al., 2000). As a result three found morphological types of plerocercoids were unambiguously correlated to the adult forms. Nucleotide sequences of adult *Otobothrium antarcticum* collected from rays *Bathyraja meridionalis и Amblyraja georgiana* in the Ross Sea and the Scotia Sea showed exact match with sequences of large larvae with trilocular bothridia collected from *D. mawsoni* in the Ross Sea, the Amundsen Sea and the Weddell Sea. Sequences of small larvae with bilocular bothridia collected from intestine of all the examined species except rays showed exact match with *Calyptrobothrium* sp., collected from *Amblyraja georgiana*, *Bathyraja maccaini* and *Bathyraja cf. eatonii* at the Ross Sea. Sequences of plerocercoids whose bilocular bothridia bear loculi of a different size showed reliable similarity to *Clistobothrium montaukensis* (GenBank). Supported by RFBR 14-04-31950..

OCCURENCE OF ASCARIDOID LARVAE IN MARINE FISH OFF NEW CALEDONIA, WITH DESCRIPTION AND GENETIC CHARACTERISATION OF NEW HYSTEROTHYLACIUM LARVAL TYPES XIII AND XIV

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Here we report occurrence of various morphotypes of ascaridoid type larvae from 58 species of fish and adult nematodes from one species of whale collected from New Caledonian waters. So far we found *Anisakis* type I, *Hysterothylacium* type VI and new larval types XIII and XIV, *Raphidascaris* larval type and *Terranova* larval type II in fish. Representatives of each morphotype were subjected to the amplification of the second internal transcribed spacers (ITS-2) of ribosomal DNA (rDNA), and those sequences were compared with ITS-2 sequences of other ascaridoid nematodes previously deposited in GenBank. ITS-2 sequences of *Anisakis* larval type I were identical to those of *A. typica*. ITS-2 sequences of *Hysterothylacium* larval type VI in the present study were identical to those previously found in Eastern Australian waters. No match was found for ITS-2 sequences of *Hysterothylacium* larval types XIII and XIV; therefore, the specific identity of these larvae remains unclear. ITS-2 sequences of *Raphidascaris* larval type were identical to those of *R. trichiuri*, previously reported in Taiwan waters. *Terranova* larval type II in the present study had identical ITS-2 sequence with those *Terranova* larval type reported from Australian waters, however, the specific identity is unknown. Morphological and molecular results are yet to be obtained for many of the specimens. This taxonomic work is essential if further research on these zoonotic parasites is to be effective, including investigations into aspects such as life cycles, impacts on human health and risk assessment for their transmission to humans.

9th ISFP
VALENCIA 2015

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