FIGURE 11. *Coryphopterus glaucofraenum*, neotype, USNM 393907, Belize, 44 mm SL, DNA 6367: A, fresh; B, preserved.

Designation of Neotype for *Coryphopterus glaucofraenum*

FIGURE 11

Eschmeyer (2008) noted the need for designating a neotype for *Coryphopterus glaucofraenum* Gill, because the whereabouts of the holotype are unknown. He also noted that four MCZ specimens assumed to be syntypes do not constitute type material because Gill's (1863) description was clearly based on a single specimen. Because of the historical confusion regarding the validity of *C. tortugae* and *C. venezuelae* as distinct from *C. glaucofraenum*, and because the three species can be difficult to separate, we have elected to designate a neotype for *C. glaucofraenum* from which we have successfully obtained a COI sequence that places the specimen in the *C. glaucofraenum* clade. We hereby make the following type designation:

Neotype

Coryphopterus glaucofraenum Gill, USNM 393907, 44 mm SL, DNA 6367, Twin Cays, Belize, mangrove edge on interior channel, 0–6 ft. (GenBank accession no. GQ367355.)

SUMMARY AND FUTURE WORK

Cytochrome *c* oxidase I sequences (DNA barcoding) were useful in determining the number of distinct genetic lineages within Caribbean *Coryphopterus*. We used the neighbor-joining tree (see Figure 1) derived from those sequences to assemble voucher specimens (and color photographs of them taken before preservation) into clades and then compared the morphology of specimens among those clades. Assigning clades to species was relatively easy based

on review of original literature and examination of some type specimens (or photographs of them). Resolving the identities of many Caribbean *Coryphopterus* in the absence of the DNA data would have been extremely difficult.

We are continuing to expand our geographic coverage of *Coryphopterus* sampling and will continue sequencing COI, and ultimately other genes, from specimens from a diversity of locations. The precise geographic distributions of most western Atlantic *Coryphopterus* are not known, and our genetic analyses have revealed the presence of one or more additional cryptic species. Additionally, the existence of two morphological forms within the genetic clade identified as *C. venezuelae* warrants further investigation. Ultimately, our multi-locus data set will enable us to reanalyze phylogenetic relationships among *Coryphopterus* species, from which we can investigate patterns of speciation and morphological divergence. Finally, testing of the species identifications of *Coryphopterus* larvae proposed by Baldwin and Smith (2003) based on morphology is currently in progress based on COI sequences of larvae collected as part of this study.

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APPENDIX

TABLE A.1. *Coryphopterus* material. A number in the DNA column indicates that the specimen was analyzed for cytochrome *c* oxidase 1. An asterisk beside this number indicates the entry appears in the neighbor-joining tree in Figure 1; because of space constraints, not all specimens for which DNA was successfully sequenced are included in Figure 1. Extracting DNA was not attempted on formalin-fixed specimens. If the specimen was not sampled for DNA, "no DNA" is recorded in this column; BZE, Belize; FLA, Florida; CUR, Curacao; BAH, Bahamas; PAN, Panama; VEN, Venezuela.

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
C. lipernes	BZE 4067*	—	No voucher	No
	BZE 4082*	23	No voucher	No
	BZE 4083*	21	No voucher	No
	BZE 7729*	18	USNM 394796	Yes
	CUR 8051*	21	USNM 394895	Yes
	CUR 8326*	20	USNM 394896	Yes
	CUR 8327*	17	USNM 394894	Yes
C. hyalinus	BZE 4511*	15	No voucher	No
	BZE 4512*	15	No voucher	No
	BZE 5066*	13	No voucher	Yes
	BZE 6221*	13.5	USNM 394795	Yes
	BZE 6222*	14.5	USNM 394794	Yes
	BZE 7760*	7	No voucher	Yes
	CUR 8044*	20	USNM 394890	Yes
	CUR 8046*	19.5	USNM 394891	Yes
	CUR 8264*	19	USNM 394893	Yes
	CUR 8265*	17	USNM 394889	Yes
	CUR 8266*	16.5	USNM 394892	Yes
C. personatus	BZE 4014*	—	No voucher	No
	BZE 4079*	19	No voucher	Yes
	BZE 4307*	24	USNM 394756	Yes
	BZE 4308*	21	USNM 394757	Yes
	BZE 4309*	18	USNM 394758	Yes
	BZE 5067*	19	USNM 394913	Yes
	BZE 7163*	15	USNM 394742	Yes
	CUR 8045*	19.5	USNM 394897	Yes

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
	BAH 8263	23	USNM 394904	Yes
	BAH 8264*	22	USNM 394905	Yes
	PAN 7712-1*	22	AMNH 247346	No
	PAN 7712-5*	22	AMNH 247346	No
C. tortugae	BZE 4016*	28	No voucher	Yes
	BZE 4530*	40	USNM 394730	Yes
	BZE 5237*	34	USNM 394743	Yes
	BZE 5238*	30	USNM 394731	Yes
	BZE 7106*	20	USNM 394732	Yes
	BZE 7107*	36	USNM 394733	Yes
	BZE 7333*	25	USNM 394744	Yes
	BZE 7677*	31	USNM 394801	Yes
	BZE 7690	37	USNM 394878	Yes
	BZE 7691*	29	USNM 394802	Yes
	BZE 7692*	36	USNM 394879	Yes
	BZE 7693*	20	USNM 394800	Yes
	BZE 7708*	33	USNM 394877	Yes
	BZE 7709*	29	USNM 394798	Yes
	BZE 7734*	26	USNM 394799	Yes
	BZE (no DNA)	40	USNM 329834	No
	BZE (no DNA)	33	USNM 334838	No
	CUR CG25*	—	No voucher	No
	CUR CG26*	—	No voucher	No
	PAN 7725-6*	36	AMNH 247347	No
	VEN (no DNA)	45	USNM 194103	No
	VEN 7736-1*	33	AMNH 247340	No
	VEN 7736-4*	37	AMNH 247340	No
	VEN 7736-6*	46	AMNH 247340	No
	Bermuda (no DNA)	9 (15–31)	USNM 330023	No
	FLA (no DNA, photo of holotype)	—	SU 08363	No
C. glaucofraenum	BZE 6037*	35	USNM 394347	Yes
	BZE 6367*	44	USNM 393907	Yes
	BZE 7343*	6	No voucher	Yes
	BZE 7351*	35	USNM 394353	Yes
	BZE 7352*	25	USNM 394354	Yes
	BZE 7353*	17.5	USNM 394355	Yes
	BZE 7733*	25	USNM 394748	Yes
	BZE 7768*	22	USNM 394792	Yes
	BZE 7769*	17	USNM 394793	Yes
	BZE 7796*	8.5	No voucher	Yes
	BZE 7798*	8.5	No voucher	Yes
	FLA 7341	49	USNM 394348	Yes
	FLA 7342	42	USNM 394349	Yes
	FLA 7343*	35	USNM 394350	Yes
	FLA 7344	36	USNM 394351	Yes
	FLA 7345	30	USNM 394352	Yes
	FLA 7674	49	USNM 394356	Yes
	FLA 7675	44	USNM 394357	Yes
	FLA 7676	38	USNM 394358	Yes
	FLA 7677	32	USNM 394729	Yes
	PAN 7701-1*	39	AMNH 247334	No
	PAN 7701-2*	40.5	AMNH 247334	No
	PAN 7701-3*	32	AMNH 247334	No
	PAN 7701-4*	26.5	AMNH 247334	No
	PAN 7701-5*	33	AMNH 247334	No
	PAN 7712-2*	35	AMNH 247335	No

continued

TABLE A.1. *continued*

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
	VEN 7729-1*	31	AMNH 247336	No
	VEN 7729-2*	30	AMNH 247336	No
	VEN 7729-3*	31	AMNH 247336	No
	VEN 7736-2*	37.5	AMNH 247337	No
	VEN 7738-1*	38	AMNH 247338	No
	VEN 7738-2*	36	AMNH 247338	No
	VEN 7738-3*	39	AMNH 247338	No
	VEN 7744-2*	32	AMNH 247339	No
	VEN 7744-3*	27	AMNH 247339	No
	VEN 7744-4*	28.5	AMNH 247339	No
	Bahamas (no DNA)	31	USNM 386863	No
	Bahamas (no DNA)	2 (30–32)	USNM 386955	No
	Bermuda (no DNA)	4 (27–35)	USNM 178908	No
	Bermuda (no DNA)	2 (45–46)	USNM 178555	No
C. venezuelae	BZE 5099*	16	USNM 394735	Yes
	BZE 5319*	8.5	No voucher	Yes
	BZE 7248*	35	USNM 394736	Yes
	BZE 7362*	7.5	No voucher	Yes
	BZE 7704*	20	USNM 394880	Yes
	BZE 7728*	17	USNM 394881	Yes
	BZE 7797*	8.5	No voucher	Yes
	CUR 8052*	30.5	USNM 394737	Yes
	CUR 8053*	30	USNM 394764	Yes
	CUR 8054*	26.5	USNM 39475	Yes
	CUR 8055	28	USNM 394766	Yes
	CUR 8208*	31.5	USNM 394738	Yes
	CUR 8259*	29	USNM 394739	Yes
	CUR 8260*	29	USNM 394740	Yes
	CUR 8427*	35	USNM 394741	Yes
	BAH 8048*	43	USNM 394908	Yes
	BAH 8049*	42	USNM 394906	Yes
	BAH 8262*	39	USNM 394909	Yes
	PAN 7725-1*	42.5	AMNH 247341	No
	PAN 7725-2*	38	AMNH 247341	No
	PAN 7725-3*	33	AMNH 247341	No
	PAN 7725-4*	39	AMNH 247341	No
	PAN 7725-5*	42.5	AMNH 247341	No
	VEN 6670-3*	41	AMNH 247342	No
	VEN 6670-4*	45	AMNH 247342	No
	VEN 7733-1*	29	AMNH 247343	No
	VEN JV07*	20	AMNH 247344	No
	VEN JV08*	29.5	AMNH 247344	No
	VEN JV09*	36	AMNH 247345	No
	VEN JV10*	29	AMNH 247345	No
	VEN JV11*	29	AMNH 247345	No
	VEN JV12*	52	AMNH 247345	No
	VEN JV13*	50	AMNH 247345	No
	VEN JV14*	50	AMNH 247345	No
	VEN JV15*	50	AMNH 247345	No
	VEN JV16*	29	AMNH 247345	No
	VEN (no DNA; photo of paratype)	~42	MOBR-P-0867	No
	Puerto Rico; holotype of *C. bol** (DNA from Victor, 2008)	26.8	SIO 0869	No
	Saba (no DNA)	15	USNM 387726	No

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
	Brazil	4 (2–39)	USNM 357709	No
C. dicrus	BZE 4213*	22	USNM 394337	Yes
	BZE 5239*	27	USNM 394763	Yes
	BZE 6274*	25	USNM 394774	Yes
	BZE 6110*	13	USNM 394779	Yes
	BZE 7238	29	USNM 294338	Yes
	BZE 7266	24	USNM 294339	Yes
	BZE 7354*	22	USNM 394745	Yes
	BZE 7410	27	USNM 394746	Yes
	BZE 7700*	19	USNM 394778	Yes
	BZE 7701*	17	USNM 394776	Yes
	BZE 7707*	21	USNM 394777	Yes
	BZE 7745*	23	USNM 394780	Yes
	BZE 7818*	22	USNM 394775	Yes
	FLA 7346*	43	USNM 394343	Yes
	FLA 7347*	41	USNM 394344	Yes
	FLA 7348*	38	USNM 394345	Yes
	FLA 7680	39	USNM 394340	Yes
	FLA 7681	42	USNM 394341	Yes
	FLA 7682	44	USNM 394342	Yes
	CUR 8135*	30	USNM 394747	Yes
	BAH 8134*	43	USNM 394900	Yes
	BAH 8135*	38	USNM 394898	Yes
	BAH 8232	36	USNM 394899	Yes
	VEN 7736-3*	35	AMNH 247332	No
	VEN JV01*	33	AMNH 247333	No
	VEN JV02*	35	AMNH 247333	No
	VEN JV03*	36	AMNH 247333	No
	VEN JV04*	20.5	AMNH 247333	No
	VEN JV05*	21	AMNH 247333	No
	VEN JV06*	20	AMNH 247333	No
	Saba (no DNA)	4 (25–28)	USNM 388525	No
	Tobago (no DNA)	35	USNM 318808	No
	Tobago (no DNA)	3 (23–25)	USNM 318818	No
	Dominica (no DNA)	11 (13–27)	USNM 325165	No
C. thrix	BZE 6111*	15	USNM 394797	Yes
	BZE 7265*	10	USNM 394734	Yes
	BZE 7267*	30	USNM 394759	Yes
	BZE 7816*	23	USNM 394914	Yes
	BZE 7817*	22	USNM 394915	Yes
	BZE (no DNA)	3 (20–28.5)	USNM 328240	No
	CUR 8261*	16	USNM 394760	Yes
	CUR 8426*	23	USNM 394761	Yes
	Venezuela (no DNA)	26	AMNH 244983	No
	Navassa (no DNA)	31	USNM 359403	No
	Tobago (no DNA)	32	USNM 318811	No
	Tobago (no DNA)	2 (23–24)	USNM 317133	No
C. eidolon	BZE 4017*	31	USNM 394749	Yes
	BZE 4080*	20	USNM	Yes
	BZE 4081*	29	No voucher	No
	BZE 4089*	–	No voucher	No
	BZE 5070*	33	USNM 394750	Yes
	BZE 5099	16	No voucher	Yes
	BZE 6223*	18	USNM 394788	Yes
	BZE 6224*	24	USNM 394789	Yes
	BZE 6246*	25	USNM 394787	Yes
	BZE 6268*	23.5	USNM 394790	Yes
	BZE 6302*	33	USNM 394751	Yes

continued

TABLE A.1. *continued*

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
	BZE 7108	21	USNM 394785	Yes
	BZE 7109*	34	USNM 394752	Yes
	BZE 7152	19	USNM 394346	Yes
	BZE 7232*	31	USNM 394762	Yes
	BZE 7350*	36	USNM 394753	Yes
	BZE 7671*	28	USNM 394786	Yes
	BZE 7672	24	USNM 394784	Yes
	BZE 7673*	22	USNM 394781	Yes
	BZE 7702	31	USNM 394783	Yes
	BZE 7703*	26	USNM 394782	Yes
	BZE 7726	24	USNM 394912	Yes
	BZE 7727	17	USNM 394911	Yes
	BZE 7735	23	USNM 394791	Yes
	CUR 8047	37	USNM 394886	Yes
	CUR 8048*	39	USNM 394884	Yes
	CUR 8049	33	USNM 394883	Yes
	CUR 8050*	38	USNM 394885	Yes
	CUR 8262*	24	USNM 394887	Yes
	CUR 8263	33	USNM 394888	Yes
	BAH 8046*	41	USNM 394903	Yes
	BAH 8047*	37	USNM 394902	Yes
	Navassa (no DNA)	3 (32–33)	USNM 360458	No
C. alloides	BZE 7233*	24	USNM 394754	Yes
	BZE 7264*	19	USNM 394755	Yes
	BZE 7761*	12	USNM 394910	Yes
	BZE (no DNA)	21	USNM 267843	No
	CUR 8325*	18	USNM 394882	Yes
C. kuna	BZE 4586*	6	No voucher	No
	BZE 5134*	7.5	No voucher	Yes
	BZE 6049*	7	No voucher	Yes
	BZE 6387*	7.5	No voucher	Yes
	PAN; holotype* DNA from GenBank	17.1	SIO-07-5	No
C. punctipectophorus	FLA; paratype (no DNA)	28	USNM 179307	No
	South Carolina (no DNA)	28	USNM 315530	No

[a] USNM = U.S. National Museum (National Museum of Natural History), Smithsonian Institution; AMNH = American Museum of Natural History; MOBR = Museo Oceanológico Hermano Benigno Román, Venezuela; SIO = Scripps Institution of Oceanography.

Recent Insights into Cnidarian Phylogeny

Allen G. Collins

ABSTRACT. With representatives of more than 10,000 species from diverse clades scattered throughout the world's oceans, Cnidaria is a moderately diverse phylum of Metazoa. As such, various taxa within Cnidaria have been the subjects of recent phylogenetic analyses. Because of its diversity, it has not yet been possible to conduct any extensive phylum-level phylogenetic analyses. In addition, new information suggests that the large group of parasites known as Myxozoa is part of Cnidaria. The present contribution summarizes recent findings to create a picture of a current working hypothesis of cnidarian phylogeny. This summary, which treats the relationships among taxa down to the approximate level of order, likely provides a suboptimal estimation of cnidarian phylogeny as compared to a detailed phylogenetic analysis of data sampled densely from all the Cnidaria component clades. Nevertheless, it should provide points of comparison for upcoming efforts to more comprehensively assess cnidarian phylogeny. Even at the basic level of order, many taxa are thought to be polyphyletic. Understandably, current classifications are not fully reflective of recent phylogenetic advances.

INTRODUCTION

Early in the history of Metazoa, the nematocyst evolved. This capsular organelle encloses venom and a tightly coiled, hollow, dart-like thread that is discharged at incredibly rapid accelerations of up to 5 million *g* (Nüchter et al., 2006). This explosive discharge can be achieved because of extreme osmotic pressures (Holstein and Tardent, 1984; Weber, 1989) within the highly stable nematocyst wall, the molecular structure and function of which are becoming ever clearer (e.g., Meier et al., 2007). Cnida is the more general term for this organelle, the nematocyst being just one type. However, it is reasonably clear, based on the distribution across cnidarian taxa, that the ancestral form of the cnida was as a nematocyst (Marques and Collins, 2004). The lineage in which the nematocyst originated gave rise to the moderately diverse phylum Cnidaria, most likely during the Ediacaran period (Peterson and Butterfield, 2005; Cartwright and Collins, 2007). Since this time, cnidarians have evolved an enormous variety of forms and a great diversity of life history strategies. Representative cnidarians build reefs, fish the depths, and parasitize other species. Extant valid species number a bit more than 11,000 (Daly et al., 2007),

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or more than 13,000 when roughly 2,200 myxozoan species (Lom and Dyková, 2006) are included (see following), and can be found living in all marine environments. Many myxozoans infect freshwater taxa, but just a small number of other cnidarian species live in freshwater (Jankowski et al., 2008).

Daly et al. (2007), in honor of the 300th anniversary of the birth of Linnaeus, recently provided a summary of phylogenetic knowledge about currently recognized cnidarian taxa (exclusive of Myxozoa) typically ranked at ordinal and family levels in current classifications. I aim to provide a summary of recent insights into cnidarian phylogeny focusing on relationships among the different cnidarian orders. No phylum-level analyses of the evolutionary relationships among these taxa have been carried out, although attempts have been made to assess phylogenetic hypotheses for large subclades of Cnidaria; i.e., Anthozoa (Berntson et al., 1999; Won et al., 2001), Hexacorallia (Daly et al., 2003; Brugler and France, 2007), Octocorallia (Bertnson et al., 2001; McFadden et al., 2006); Medusozoa (Collins, 2002; Marques and Collins, 2004; Collins et al., 2006a; Van Iten et al., 2006), and Myxozoa (Kent et al., 2001; Fiala, 2006). In addition, several recent studies have assessed the phylogenetic affinities of taxa that have been problematic (Collins et al., 2006b; Van Iten et al., 2006; Dyková et al., 2007; Jiménez-Guri et al., 2007). The approach taken here is to cobble together results from these various analyses to provide a reasonable picture of our present understanding of cnidarian relationships (Figure 1). Representative cnidarians are illustrated in Figures 2 and 3.

As it concentrates on recent insights, the present paper does not provide a thorough review of the history of ideas about relationships among cnidarian orders, nor does it attempt to summarize what recent phylogenetic results tell us about cnidarian character evolution. For that type of information, one should consult the studies referenced herein. The working hypothesis of cnidarian relationships (see Figure 1), as well as the summary provided by Daly et al. (2007), should provide points of comparison for phylum-wide analyses of cnidarian phylogeny, which will soon be attempted by researchers engaged in the cnidarian tree of life project (http://CnidToL.com). Because it is a representation of a hypothetical history of Cnidaria, every node in Figure 1 is uncertain and is subject to change in light of new information. In a couple of instances, question marks are inserted on the working hypothesis to indicate relationships that are particularly tentative at present.

A WORKING HYPOTHESIS OF CNIDARIAN PHYLOGENY

Cnidaria is one of the earliest diverging clades within Metazoa, and surprisingly its precise position within the early diverging animal lineages—Porifera, Placozoa, Bilateria, Ctenophora, and Cnidaria—has remained elusive (Collins et al., 2005b; Dunn et al., 2008). That said, it has become ever clearer that Cnidaria is more closely related to Bilateria than is Ctenophora, a finding based on a synthetic consideration of morphology (Salvini-Plawen, 1978), later supported by 18S rDNA data (Wainright et al., 1993; Collins, 1998), and most recently confirmed by a large analysis of many sequences of data from expressed gene transcripts (derived from large-scale sequencing of messenger RNA; known as expressed sequence tags, or ESTs) (Dunn et al., 2008; although note that the analyses published therein suggest that Ctenophora is the earliest diverging extant metazoan lineage, which is either a radical new finding or an indication of bias in the results). Ribosomal data, both 18S (e.g., Collins, 1998) and combined 18S and 28S (Medina et al., 2001; Cartwright and Collins, 2007), strongly suggest that Cnidaria forms a clade with Bilateria and the little-known phylum Placozoa, and that Cnidaria may be the sister group of either taxon or both together. More recently, phylogenetic analyses using entire genomes (unfortunately without any representatives of Ctenophora) found Placozoa to be the sister group of a clade composed of Cnidaria plus Bilateria (Srivastava et al., 2008).

Myxozoa is an interesting group of parasites that very well may be part of Cnidaria. Although some early workers suggested that they are cnidarians, based on the similarity between nematocysts and myxozoan polar capsules (Weill, 1938), they were mainly considered as protists throughout the twentieth century. In 1995, an analysis of 18S and morphological data suggesting that myxozoans were derived from within Cnidaria was published (Siddall et al., 1995). However, this conclusion was doubted by many because the 18S gene of myxozoans appears to have evolved very quickly relative to that of most other metazoans, and different analyses involving different sets of taxa came to conflicting conclusions about the precise position of Myxozoa within Metazoa (Smothers et al., 1994; Siddall et al., 1995; Hanelt et al., 1996; Siddall and Whiting, 1999; Kim et al., 1999; Zrzavý and Hypsa, 2003). This uncertainty was claimed to have been resolved when it was discovered that an unusual worm-shaped animal known as *Buddenbrockia* was a myxozoan (Okamura

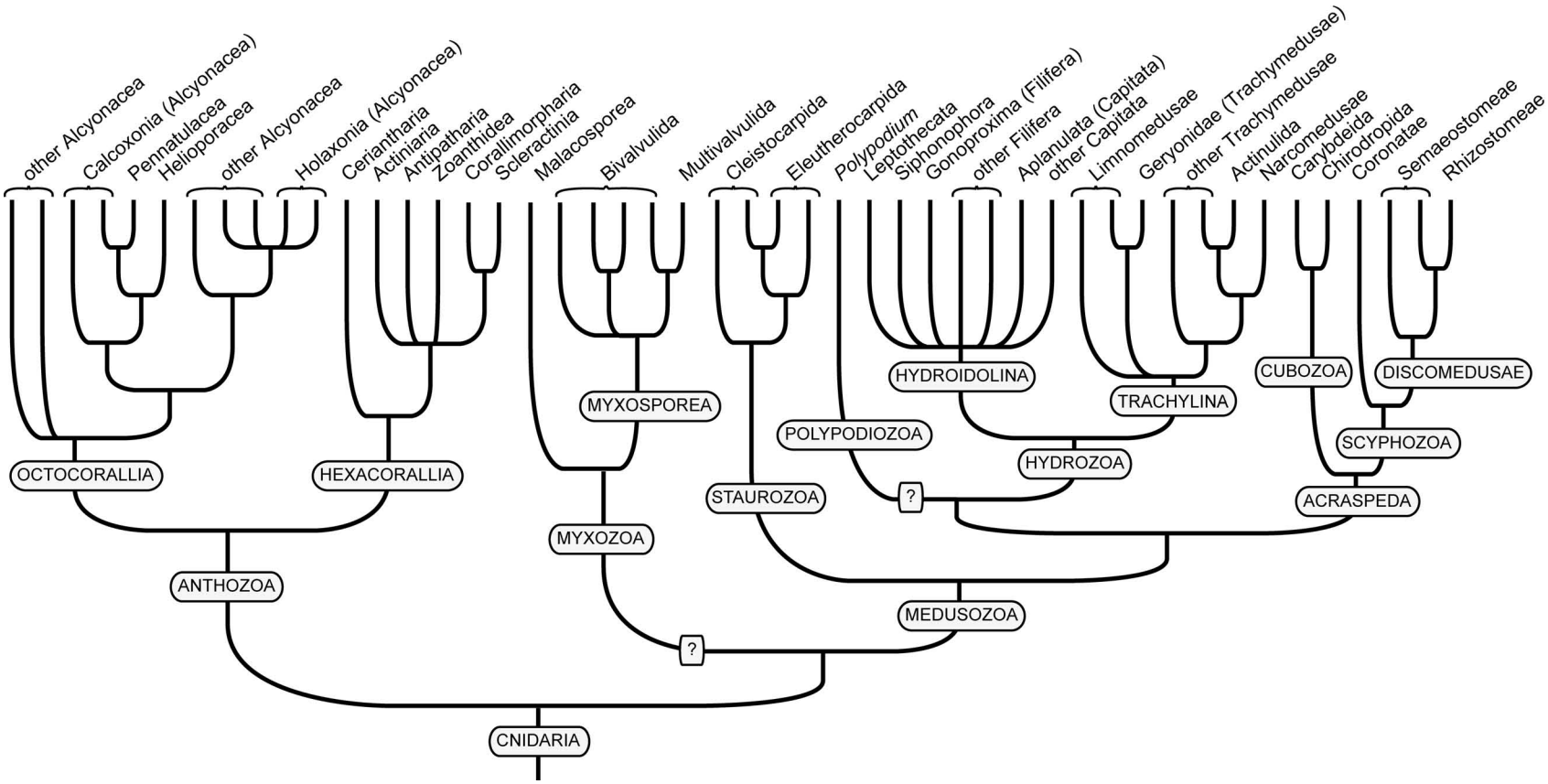

FIGURE 1. Working hypothesis of evolutionary relationships among extant cnidarian taxa usually classified at or near the rank of order.

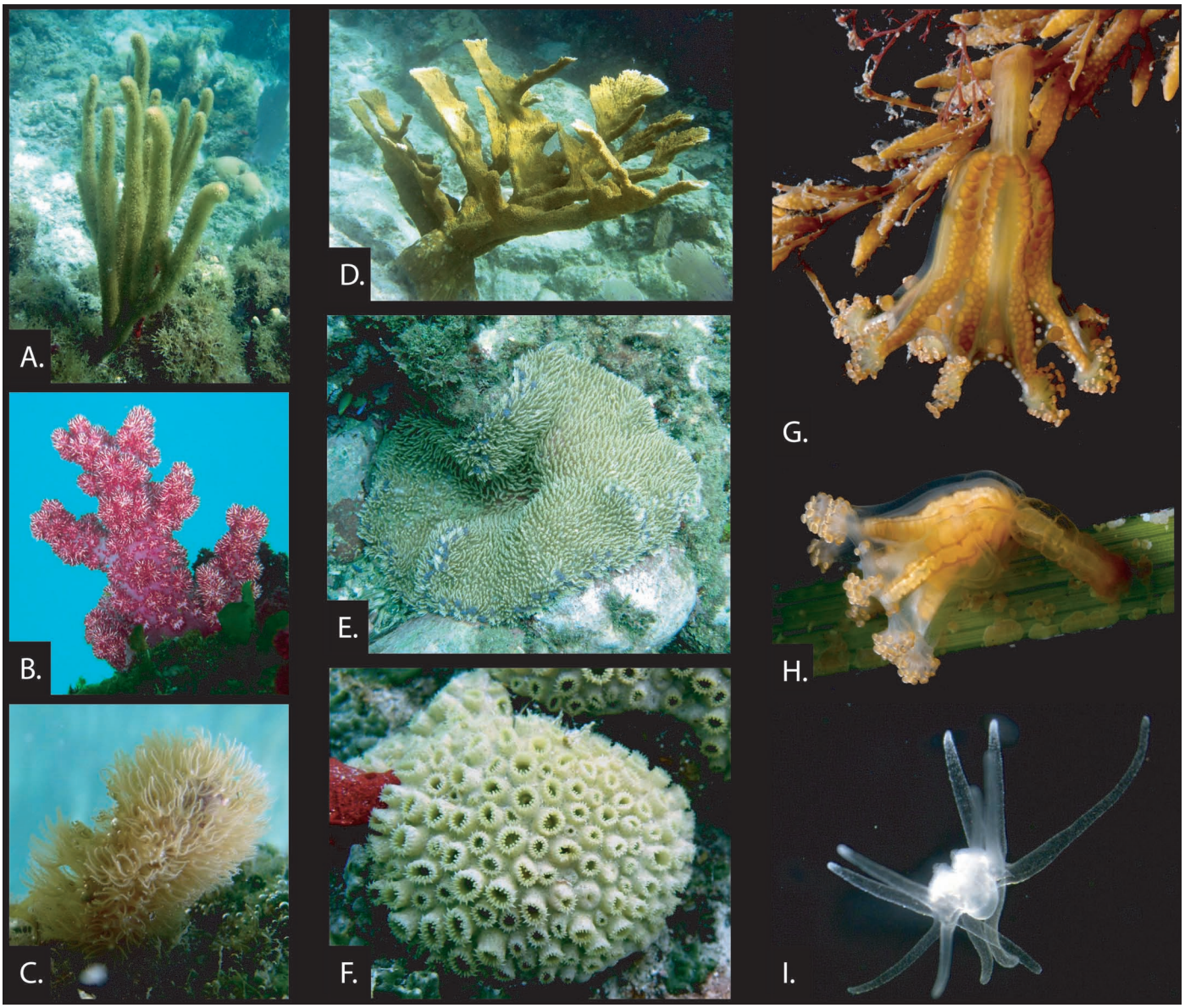

FIGURE 2. Representative cnidarians: Anthozoa, Staurozoa, and Polypodiozoa. A, Octocorallia, Holaxonia, *Plaxaura* from St. John, U.S. Virgin Islands; B, Octocorallia, Alcyonacea (part of the unnamed clade including Holaxonia), *Dendronephthya* from Shirahama, Japan; C, Octocorallia, Alcyonacea, *Briareum* from St. John, U.S. Virgin Islands; D, Hexacorallia, Scleractinia, *Acropora* from St. John, U.S. Virgin Islands; E, Hexacorallia, Actiniaria, *Thalassianthus* from Shirahama, Japan; F, Hexacorallia, Zoanthidea, *Palythoa* from St. John, U.S. Virgin Islands; G, Staurozoa, Eleutherocarpida, *Haliclystus* from Hokkaido, Japan; H, Staurozoa, Cleistocarpida, *Manania* from Hokkaido, Japan. I, Polypodiozoa, *Polypodium* (photographed by N. Evans).

et al., 2002; Okamura and Canning, 2003). Okamura and colleagues showed that the morphology and DNA of *Buddenbrockia* firmly placed it within Myxozoa. However, they also argued that the presence of four muscles located between the endoderm and ectoderm of *Buddenbrockia* indicated that it, and by extension Myxozoa as a whole, was a close relative of nematodes and firmly derived from within Bilateria. Most recently, this hypothesis was falsified by analyses of EST data taken from *Buddenbrockia* and other metazoans indicating that Myxozoa is part of Cnidaria (Jiménez-Guri et al., 2007), as suggested by earlier workers (Weill, 1938; Siddall et al., 1995).

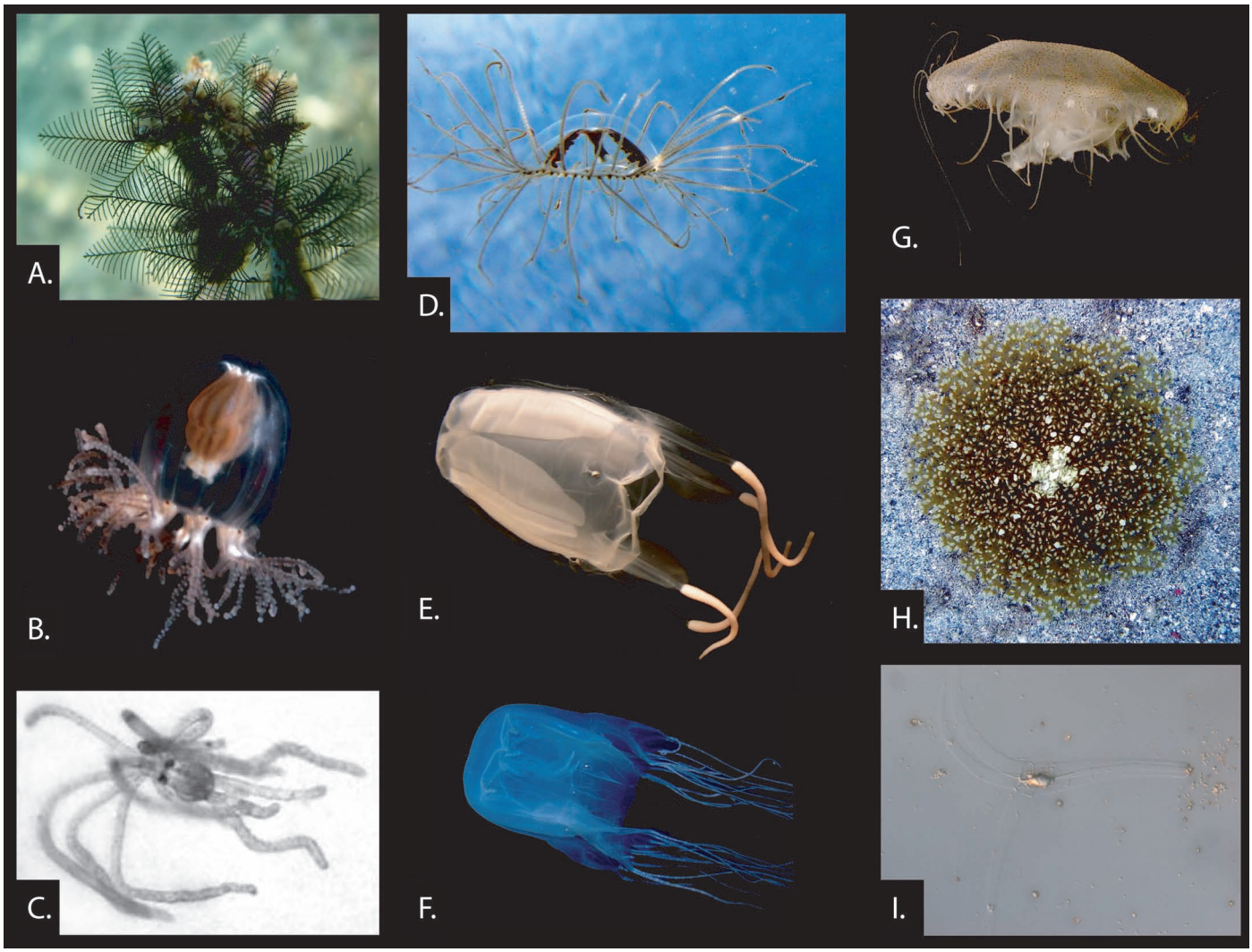

FIGURE 3. Representative cnidarians: Hydrozoa, Cubozoa, Scyphozoa, and Myxozoa. A, Hydroidolina, Leptothecata, *Lytocarpia* from Shirahama, Japan; B, Hydroidolina, other Capitata, *Cladonema* from Hokkaido, Japan; C, Trachylina, Actinulida, *Halammohydra* from Bocas del Toro, Panama (still taken from video by J. Norenburg); D, Trachylina, Limnomedusae, *Gonionemus* from Hokkaido, Japan; E, Cubozoa, Carybdeida, *Carybdea* from Bocas del Toro, Panama; F, Cubozoa, Chirodropida, *Chironex* from Southern Japan (photographed in Enoshima Aquarium); G, Scyphozoa, Semaeostomeae, *Chrysaora* from Bocas del Toro, Panama; H, Scyphozoa, Rhizostomeae, *Cassiopea* from St. John, U.S. Virgin Islands; I, Myxozoa, Myxosporea, Bivalvulida, actinospore stage of *Myxobolus* (photographed by A. Nawrocki and N. Evans).

Not surprisingly, given the type of data analyzed, taxon sampling for the EST analysis was rather limited, with just two anthozoans, two hydrozoans, and one scyphozoan included. The myxozoan was shown to fall as the earliest diverging lineage in a clade including the two hydrozoans and the scyphozoan (Jiménez-Guri et al., 2007). In contrast, even more recent analyses of rDNA data with excellent taxon sampling resulted in best trees in which Myxozoa was the sister group of Bilateria, a result thought to be biased by long-branch attraction (Evans et al., 2008). Figure 1 shows Myxozoa as the sister lineage of Medusozoa, as indicated by the EST results, but the branch also includes a question mark because of the small number of taxa included in the analysis by Jiménez-Guri et al. (2007). Knowing whether myxozoans possess linear or the typical circular mitochondrial genomes could help place Myxozoa within Cnidaria, as this is a major distinction between Anthozoa and Medusozoa (Bridge et al., 1992, 1995; see following).

The inclusion of Myxozoa's 2,200 species (Lom and Dyková, 2006) within Cnidaria increases the richness of

the phylum significantly. As parasites with complex life cycles involving multiple hosts, the diversity of Cnidaria is substantially enriched as well. In recent years, relationships within Myxozoa have mainly been addressed using 18S rDNA data. These data give a strong signal that species parasitic of freshwater bryozoans in the class Malacosporea (=order Malacovalvulida) form a small clade (just three species are known) that is sister to the remaining myxozoans classified in the class Myxosporea (Canning et al., 2000; Kent et al., 2001). Recent classifications of Myxosporea break the class into two orders, Bivalvulida and Multivalvulida. While neither taxon as traditionally recognized is monophyletic, including one aberrant bivalvulidid member in Multivalvulida renders Bivalvulida paraphyletic and Multivalvulida monophyletic (as shown in Figure 1; Kent et al., 2001; Whipps et al., 2004; Fiala, 2006; Lom and Dyková, 2006). The great majority of myxosporeans appear to fall into two large clades, one dominated by species inhabiting freshwater hosts, and the other including Multivalvulida and other species that primarily infect marine hosts (Kent et al., 2001; Holzer et al., 2004). Examples of reversals in freshwater and marine habits continue to accumulate, and a third smaller clade has been identified (Fiala, 2006). Considerable diversity of Myxozoa remains to be sampled, but existing studies indicate that many myxozoan taxa, even genera, are polyphyletic. Continued efforts to identify morphological features reflecting shared ancestry, which could be used to improve the existing classification, are necessary (Fiala, 2006).

In analyses of Cnidaria (exclusive of Myxozoa), it has generally become accepted that Anthozoa is the sister clade of Medusozoa, a hypothesis that is buttressed by morphology (Salvini-Plawen, 1978; Bridge et al., 1995), mitochondrial genome structure (Bridge et al., 1992), and rDNA sequences (e.g., Berntson et al., 1999; Kim et al., 1999; Medina et al., 2001; Won et al., 2001; Collins, 2002). One recent exception is a study by Kayal and Lavrov (2008) based on complete mitochondrial genome sequences, which found Medusozoa (just two representatives) derived from within Anthozoa as the sister group of three sampled representatives of Octocorallia. Although certainly worthy of consideration and future testing, limitations in taxon sampling in the Kayal and Lavrov (2008) analysis cast some doubt on the veracity of this finding. Similar arrangements were also presented in early rDNA analyses that similarly suffered from poor taxon sampling, as shown by pioneering work of Bridge et al. (1995). As indicated in Figure 1, Anthozoa is hypothesized to consist of two well-supported sister clades with diverse representatives, Octocorallia and Hexacorallia. Anthozoa is usually considered to be a class within the phylum (e.g., Daly et al., 2007), but making it a subphylum, with Hexacorallia and Octocorallia as its classes, would go some way toward balancing the classification of Anthozoa with that of Medusozoa.

The phylogeny of Octocorallia has posed some of the most troublesome questions in recent cnidarian systematics because of a relatively dramatic incongruence between traditional taxonomy and molecular-based hypotheses of relationships (Berntson et al., 2001). Nevertheless, consistent progress has been made; many of the alliances first suggested by the rDNA analyses of Berntson et al. (2001) have been confirmed, and some morphological synapomorphies of recently recognized clades have been identified (McFadden et al., 2006). It has been premature, given the great diversity of Octocorallia remaining to be sampled, to erect a new classification for the group, but some patterns are emerging. There appear to be two major clades and a minor clade or grade that branched early in the history of Octocorallia (McFadden et al., 2006). One of the three octocoral orders, Alcyonacea (soft corals and sea fans), which is by far the most diverse and least distinctive, is clearly paraphyletic. The other two orders, Pennatulacea (sea pens) and Helioporacea (blue corals), are monophyletic, and each appears to be independently descended from a paraphyletic Calcoxonia (one group of sea fans), a suborder of Alcyonacea (McFadden et al., 2006). Another group of sea fans known as Holaxonia all appear in one of the major clades, along with other alcyonaceans, but there is no strong evidence for holaxonian monophyly.

In contrast to Octocorallia, the overall picture of the phylogeny of Hexacorallia has been relatively clear. Of the six hexacorallian orders, several lines of evidence indicate that Ceriantharia (tube anemones) is the earliest diverging lineage (Berntson et al., 2001; Won et al., 2001; Daly et al., 2002, 2003; Brugler and France, 2007). Similarly, this same set of studies all concur in finding a close relationship between Scleractinia (stony corals) and Corallimorpharia. However, there has been some confusion about whether Corallimorpharia might be derived from within Scleractinia, that is, one version of the "naked coral hypothesis," which posits that one or more hexacorallian groups without skeletons are derived from stony coral ancestors. Abundant evidence refutes the idea that Actiniaria (true anemones) or Zonanthidea are derived from scleractinian ancestors (Berntson et al., 2001; Won et al., 2001; Daly et al., 2002, 2003; Brugler and France, 2007), but several analyses of mitochondrial genes, including whole mitochondrial genomes, have found corallimorpharians to be derived from within Scleractinia (France et al., 1996; Romano and Cairns, 2000; Medina et al., 2006). In contrast,

however, better taxon sampling of mitochondrial genomes (Brugler and France, 2007) and analyses of other genes with better taxon sampling (Fukami et al., 2008) favor the hypothesis that Corallimorpharia and Scleractinia are monophyletic sister groups. No clear picture of the relationships between this clade, Actiniaria, Antipatharia (black corals), and Zoanthidea has emerged from recent work, as different data sets or analytical approaches have yielded conflicting results (Berntson et al., 2001; Won et al., 2001; Daly et al., 2002, 2003; Brugler and France, 2007).

In present classifications, Medusozoa is usually presented as including four classes: Cubozoa, Hydrozoa, Scyphozoa, and Staurozoa. As mentioned earlier, a recent analysis of EST data suggested that Myxozoa is derived from within Cnidaria, as the sister group of the three medusozoans included in the analysis (Jiménez-Guri et al., 2007). Another taxon, Polypodiozoa, which is sometimes considered a class because of the unusual nature of the parasitic species within its single genus, *Polypodium* (Raikova, 1988), has also recently been hypothesized to fall within Medusozoa, most likely as a close relative of Hydrozoa (Evans et al., 2008). Thus, Medusozoa may have as many as six classes representing rather distinct, evolutionarily independent lineages. Evidence for the monophyly of Medusozoa, albeit with the exclusion of Myxozoa and Polypodoizoa, comes from rDNA data (Collins, 1998, 2002; Medina et al., 2001; Collins et al., 2006a) and observations of morphology (Werner, 1973; Salvini-Plawen, 1978; Schuchert, 1993; Bridge et al., 1995).

Attempts to incorporate data from Myxozoa and Polypodiozoa in analyses of cnidarian phylogeny have been complicated by the relatively rapid rate of molecular evolution in these two taxa. In many analyses of rDNA, representatives of these two groups appear to be artificially attracted to bilaterian examplars and end up forming sister group relationships with Bilateria (Siddall et al., 1995; Kim et al., 1999; Zrzavý and Hypsa, 2003). In a recent study of 18S and 28S data, dense taxon sampling of medusozoans appears to have overcome some of this long-branch attraction problem, at least so far as Polypodiozoa is concerned (Evans et al., 2008). Although the optimal trees of Evans et al. (2008) had Myxozoa branching as the sister group of Bilateria, perhaps because the 28S marker was only partially sampled for the myxozoans, Polypodiozoa consistently fell within Medusozoa, as one would expect based on its morphology (Raikova, 1980, 1994). Unfortunately, however, the exact position of Polypodiozoa within Medusozoa was shown to be dependent upon method of analysis and the inclusion or exclusion of myxozoan representatives (Evans et al., 2008), prompting the question mark shown in Figure 1.

Among the taxa more traditionally considered as part of Medusozoa, Staurozoa (or Stauromedusae) may possibly be the earliest diverging lineage, a result obtained through the analysis of both molecular and morphological data (Collins, 2002; Dawson, 2004; Collins and Daly, 2005; Collins et al., 2006a; Van Iten et al., 2006). As benthic, so-called stalked medusae, the finding that Staurozoa might branch early in the history of Medusozoa was of some interest because it very clearly implied that the pelagic medusa stage was a feature derived within this clade. However, Collins et al. (2006a) noted that some methodological choices in their phylogenetic analyses impacted the position of Staurozoa. Further, although not specifically addressed, the position of Staurozoa was not stable in the analyses of Evans et al. (2008). Thus far, no strong evidence has been published suggesting that Staurozoa is not an early diverging lineage of Medusozoa. Within Staurozoa, there are two main taxa, Cleistocarpida and Eleutherocarpida, neither of which appears to be monophyletic despite the fact that taxon sampling was relatively limited (Collins and Daly, 2005).

Another small class of Medusozoa is Cubozoa (box jellyfishes). Although 18S data provide no clear signal about the precise position of Cubozoa within Cnidaria (Collins, 2002; Evans et al., 2008), 28S data strongly suggest that Cubozoa is the sister group of Scyphozoa (true jellyfishes), together forming the clade Acraspeda (Collins et al., 2006a; Evans et al., 2008). Both 18S and 28S strongly support cubozoan monophyly, as well as that of its two main subtaxa, Carydeida and Chirodropida (Collins, 2002; Collins et al., 2006a). Similarly, there is relatively strong and stable evidence concerning the evolutionary relationships among the scyphozoan orders Coronatae, Rhizostomeae, and Semaeostomeae, although it should be noted that taxon sampling has been sparse. The earliest diverging lineage is Coronatae, and Rhizostomeae is a well-supported clade that is derived from within Semaeostomeae (Collins, 2002; Collins et al., 2006a).

The largest and most diverse class within Medusozoa is Hydrozoa. As indicated in Figure 1, an ancient divergence within Hydrozoa divides the group into two clades, Trachylina and Hydroidolina (Collins, 2002; Marques and Collins, 2004; Collins et al., 2006a). Each clade has been the subject of recent papers aimed at bringing increased taxon and genetic marker sampling to bear on the evolutionary relationships among their respective component groups (Cartwright et al., 2008; Collins et al., 2008). As Figure 1 shows, relationships among the major lineages of

Hydroidolina are uncertain (Collins et al., 2006a; Cartwright et al., 2008). In terms of taxonomy, the clade includes Leptothecata (thecate hydroids and leptomedusae) and Siphonophora (colonial siphonophores including the Portuguese man o' war), two groups with ample evidence for monophyly (Collins, 2002; Collins et al., 2006a; Cartwright et al., 2008). Hydroidolina also includes the large and diverse taxon Anthoathecata (athecate hydroids and anthomedusae), which is typically subdivided into Capitata and Filifera. There is no evidence supporting the monophyly of Capitata, Filfera, or Anthoathecata (Collins, 2002; Collins et al., 2006a; Cartwright et al., 2008). Despite the difficulty in working out the relationships among hydroidolinan clades, some advances have been made in identifying large clades that had not been previously recognized. For instance, Capitata appears to be composed of two well-supported clades, one dubbed Aplanulata (includes the well-known model organisms of *Hydra*) in reference to the group's lack of a ciliated planula stage (Collins et al., 2005a, 2006a) and the other consisting of all the other capitate groups (Cartwright et al., 2008). The name Capitata has recently been applied to this more restrictive clade (Cartwright et al., 2008). Similarly, within Filifera, a previously unrecognized alliance of species that bear gonophores, but not on their hydranth bodies, has been given the name Gonoproxima. There is no support for the monophyly of the remaining filiferans.

Trachylina is composed of four orders: Actinulida, Limnomedusae, Narcomedusae, and Trachymedusae. The monophyly of Narcomedusae seems to be relatively certain (Collins, 2002; Collins et al., 2006a, 2006b, 2008), whereas the monophyly of Actinulida has yet to be tested because just a single representative has been included in any phylogenetic analysis (Collins et al., 2008). Trachymedusae, a group of pelagic species that lack polyp stages, appears to be polyphyletic. One family (Geryonidae) has a close relationship with a subgroup of Limnomedusae (Collins et al., 2006a, 2008), whereas another (Rhopalonematidae) may have given rise to the interstitial Actinulida (Collins et al., 2008). Limnomedusae appears to represent a grade at the base of Trachylina (Collins, 2002; Collins et al., 2006a, 2006b, 2008). As with many cnidarian groups, the classification of Trachylina requires refinement to better reflect our phylogenetic knowledge.

CONCLUSION AND CLASSIFICATION

The working hypothesis of cnidarian phylogeny presented here (see Figure 1), as do all others, requires continued testing and refinement. Many of the studies behind it have limitations, especially in taxon sampling, and the original papers should be consulted for more detailed assessments of strengths and weaknesses of the analyses that they report. As the working hypothesis results from no single analysis and was instead put together from numerous sources, some of my biases, in the form of judgments, have had an impact on the final form of the working hypothesis. This effect is certainly a weakness in such an exercise and demonstrates why it is less preferable than an analysis that relies on data sampled from diverse representatives across Cnidaria. When such an analysis is conducted, the working hypothesis presented here may provide a helpful reference point for comparison.

Figure 1 makes it clear that the current classification of Cnidaria, even at the basic level of order, has not kept up with phylogenetic advances. A new classification using taxa hypothesized to be monophyletic is not feasible until more thorough and robust phylogenetic analyses are conducted. Conflicting results from different phylogenetic studies create one hindrance to advances in classification, but this is not really new, as different taxonomists have always offered different classifications to reflect their changing perceptions of taxa. More detrimental to progress in classification is the lack of completeness in existing phylogenetic analyses. With molecular data, individuals are sampled, and assessments of the phyletic status (monophyletic, paraphyletic, or polyphyletic) of larger taxa are not very strong until large numbers of component species are included in an analysis. Moreover, the relevant morphological features that distinguish any particular clade (especially if not corresponding to a traditional taxon) are not easily discerned without thorough sampling and examination of its members.

Nevertheless, classifications are made to enhance communication. Therefore, it may be prudent to attempt classifications that better reflect ongoing advances in phylogenetics. Below I present one such attempt for Cnidaria. It is not meant to be adopted, as this author has little expertise in non-medusozoan cnidarians. Instead, it is presented to illustrate one possible system for classifying traditional taxa in light of ongoing phylogenetic advances. It relies on annotation indicating whether a given taxon is likely to be monophyletic, paraphyletic, or polyphyletic. Taxa for which reasonable evidence suggests monophyly are followed by a superscript M. Taxa thought to be paraphyletic are followed by superscript P and a list of taxa [in brackets] hypothesized to be derived from it. Taxa that are likely polyphyletic are placed in quotation marks. Finally,

taxa whose phyletic status is essentially unknown are left with no annotation.

Phylum Cnidaria[M]
- Subphylum Anthozoa[M]
  - Class Hexacorallia[M]
    - Order Actiniaria[M]
    - Order Antiptharia[M]
    - Order Ceriantharia[M]
    - Order Corallimorpharia[M]
    - Order Scleractinia[M]
    - Order Zoanthida[M]
  - Class Octocorallia[M]
    - Oder "Alcyonacea"[P] [Calcoxonia, Helioporacea, Holaxonia, Pennatulacea]
    - Order Calcoxonia[P] [Helioporacea, Penatulacea]
    - Order Helioporacea[M]
    - Order Holaxonia
    - Order Pennatulacea[M]
- Subphylum Medusozoa[M]
  - Class Cubozoa[M]
    - Order Carybdeida[M]
    - Order Chirodropida[M]
  - Class Hydrozoa[M]
    - Subclass Hydroidolina[M]
      - Order Aplanulata[M]
      - Order Capitata[M] (excluding Aplanulata)
      - Order Filifera (excluding Gonoproxima)
      - Order Gonoproxima[M]
      - Order Leptothecata[M]
      - Order Siphonophora[M]
    - Subclass Trachylina[M]
      - Order Actinulida
      - Order Limnomedusae (including Geryonidae)
      - Order Narcomedusae[M]
      - Order Trachymedusae[P] [Actinulida, Narcomedusae]
  - Class Polypodiozoa[M]
    - Genus *Polypodium*
  - Class Scyphozoa[M]
    - Order Coronatae[M]
    - Subclass Discomedusae[M]
      - Order Rhizostomeae[M]
      - Order Semaeostomeae[P] [Rhizostomeae]
  - Class Staurozoa[M]
    - Order "Cleistocarpida"
    - Order "Eleutherocarpida"
- Subphylum Myxozoa[M]
  - Class Malacosporea[M]
    - Order Malacovalvulida[M]
  - Class Myxosporea[M]
    - Order Bivalvulida[P], [Multivalvulida]
    - Order Multivalvulida[M]

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Biodiversity and Abundance of Sponges in Caribbean Mangrove: Indicators of Environmental Quality

Maria Cristina Diaz and Klaus Rützler

ABSTRACT. We have long been fascinated by the lush biological diversity seen on subtidal substrates in Caribbean saltwater mangroves. Several groups of plants and sessile invertebrates flourish on the submerged prop roots of red mangrove (*Rhizophora mangle*), competing for space and tolerating a stressful range of ecological variables (temperature, salinity, nutrients, sedimentation) that is quite different from the more stable climate on nearby coral reefs. To test the limits of tolerance, we monitored populations of these organisms, the abundant sponges in particular, at environmentally and geographically dissimilar locations in Panama and Belize. We used relative abundance estimates and frequency counts of major ecologically functional groups and common sponge species to establish baselines, and we repeated our surveys over long time spans (months to years) to find correlations between community and environmental changes. Both study locations demonstrated environmental quality decline during the time of observation, mainly through mangrove clear-cutting, followed by increase of suspended fine sediments from dredging reef sands and filling in intertidal land, and elevation of nutrient levels from terrestrial inputs. Although our methods are still in a stage of refinement, our data are leading the way to responsible monitoring of our most precious coastal resources in the tropics. We find that photosynthetic organisms (cyanobacteria, algae) and filter-feeding invertebrates (sponges, ascidians, bivalves, bryozoans) count among the "canaries in the coal mine" as effective indicators of environmental change.

INTRODUCTION

Red mangrove trees, *Rhizophora mangle*, grow along thousands of kilometers of Caribbean shorelines, protecting them from storm erosion and offering habitat to many organisms (Rützler and Feller, 1988, 1996; de Lacerda et al., 2002). Caribbean mangroves harbor from a handful to more than 100 sponge species at any one particular site (Table 1). Available data indicate that sponges may make up 10% to 70% of epiphytic species diversity on submerged mangrove roots. The best studied mangrove sponge faunas are described from islands off southern Belize, with species richness reported between 50 and 147 species (Rützler et al., 2000; Wulff, 2000; Diaz et al., 2004), followed by faunas from a few islands in the Bocas del Toro Archipelago, Panama, with 65 species (Diaz, 2005), from various mainland and island sites off Venezuela with 62 species (Sutherland, 1980; Diaz et al. 1985; Orihuela et al., 1991; Pauls,

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TABLE 1. Number of species of Porifera and other epifaunal taxa reported from Caribbean mangroves (n.a. = no data available).

Country	Locality	Porifera	Other taxa	Author
Antilles	Guadalupe, Trinidad, Puerto Rico	4–10	32–70	Toffart (1983)
Bahamas	Bimini	13	n.a.	Rützler (1969)
Belize	Four cays	24	59	Farnsworth and Ellison (1996)
	Twin Cays	54	n.a.	Rützler et al. (2000)
	Pelican Cays	147	217	See Macintyre and Rützler (2000)
Cuba	n.a.	48	n.a.	Alcolado (unpublished data)
Panama	Bocas del Toro	60	n.a.	Diaz (2005)
USA	Indian River, Florida	3	25	Bingham and Young (1995)
Venezuela	Buche Bay	16	32	Sutherland (1980)
	Morrocoy National Park	23	n.a.	Diaz et al. (1985)
	Turiamo Bay	10	n.a.	Pauls (2003)
	Cienaga Bay	26	n.a.	Pauls (1998)
	La Restinga National Park	18	35	Orihuela et al. (1991)
	La Restinga National Park	40	n.a.	Diaz et al. (2003)

1998, 2003; Ramirez, 2002; Diaz et al., 2003; Pérez, 2007), and various mangrove sites in Cuba, with 41 species (Alcolado, unpublished data). Other reports are from Colombia, with 26 species (Zea, 1987; S. Zea, National University of Colombia, personal communication, 2006); Jamaica, with 18 species (Hechtel, 1965; Lehnert and van Soest, 1998); and Trinidad and Guadalupe, with 6 species (Toffart, 1983) (clearly representing only a portion of the mangrove sponge diversity there).

Most of the mangrove systems in the Caribbean remain unexplored, leaving a large void in biodiversity information. Most studies just cited show that the more closely these communities are investigated, the more new species are being discovered. An example is the research by the Caribbean Coral Reef Ecosystems Program in Belize during the past 25 years (Rützler et al., 2000, 2004). In particular, specialists on certain sponge taxa discovered and described numerous species in the families Suberitidae (order Hadromerida) (Rützler and Smith, 1993), Chalinidae (order Haplosclerida) (de Weerdt et al., 1991) and Mycalidae (order Poecilosclerida) (Hadju and Rützler, 1998). A recent revision of Caribbean *Lissodendoryx* allowed the reinterpretation of *L. isodyctyalis* (Carter, 1882) and seven other species, four of them new to science (Rützler et al., 2007). Similarly, two unique haplosclerids were found in Belizean and Panamanian mangroves: a thin, erect, and fragile undescribed species of *Haliclona* from Twin Cays, and *Xestospongia bocatorensis*, a thin crust occurring in Bocas del Toro mangroves and reefs. Both are in an endosymbiotic relationship with filamentous Cyanobacteria, a very unusual occurrence in this order of sponges (Diaz et al., 2007, Thacker at al., 2007).

Besides the importance of sponges species richness, they may be one of the most abundant animal groups in mangrove habitats. In Belize, for instance, on the leeward sides of islands, sponges cover 10% to 50% of the root surfaces, followed in importance by sea anemones, ascidians, and algae (Farnsworth and Ellison, 1996; Diaz et al., 2004). In the Caribbean, epibiont mangrove communities have been interpreted as highly heterogeneous (Rützler, 1969; Sutherland, 1980; Alcolado, 1985; Alvarez, 1989; Calder, 1991a; Bingham, 1992; Diaz et al., 2004) as a result of low recruitment rates (Zea, 1993; Maldonado and Young, 1996), low and fragmented available space (Jackson and Buss, 1975; Sutherland, 1980), and stochastic processes in the long term (Bingham and Young, 1995; Ellison et al., 1996). Abundance and distribution for sponges and algae in these communities have been related to environmental factors, such as light intensity, tides, wave impact, air exposure, and sedimentation (Rützler, 1995), and to biological factors, such as larval supply (Farnsworth and Ellison, 1996), root abundance, competition, and predation (Calder, 1991b; Littler et al., 1985; Taylor et al., 1986; Ellison and Farnsworth, 1992; Rützler, 1995; Rützler et al., 2000; Wulff, 2000). Algae abound on the shallow, well-lit parts of stilt roots, and their abundance and species composition are highly susceptible to the presence of grazers. Sponges are most abundant on the lower subtidal portions of the stilt roots and dominate peat bank walls and undercuts.

The major physical and biological processes are modulated by competitive abilities, such as growth rates and chemical defenses against predation (Wulff, 2000, 2004, 2005; Engel and Pawlik, 2005). Short-term epibiont abundances are likely to be determined by interspecific competitive interactions and predation, while long-term abundances are limited by seasonal environmental changes, such as freshwater inputs during periods of rain, strong tidal currents, waves, and stochastic processes that make these communities unstable (Bingham and Young, 1995; Ellison et al., 1996). Despite important generalizations about mangrove benthos ecology, we lack understanding of the temporal or spatial variation within most epibiont groups and knowledge about species occurrence, abundance, dominance, and interactions. For example, we do not know which species are generally abundant in these communities, how the hierarchy changes with the year's seasons, and if there are predictable succession patterns. Our current lack of knowledge prevents us from discerning between natural variations, for instance, seasonal or yearly dynamics, and artificial disturbances caused by humans.

The present work pursues the overall goal of a better understanding of diversity, biogeography, and ecological dynamics and their causes among the sponges in Caribbean mangroves. It encompasses two major aspects: evaluation of our current knowledge of epiphytic sponge taxa and the contribution of new data on causes for species richness, distribution, abundance, and dynamics, particularly from the examples of mangrove in Panama and Belize. The survey carried out in Bocas del Toro (Panama) intends to follow short-term changes (over one year) in the epiphytic fauna of mangrove roots, whereas the study in Belize will clarify shifts in distribution of taxa over a longer period (four years).

METHODS

Sponge Species Distribution in Caribbean Mangroves

The distribution of species in Caribbean mangroves was determined from currently published data or unpublished data provided to the authors. Faunas from different regions were compared by using a cluster analysis with the Bray–Curtis dissimilarity coefficient, which is part of the Multivariate Statistical Package (MVSP 3.1) (van Soest, 1993).

Sponge Identification

Specimens were identified in situ or, when necessary, briefly characterized and photographed, with a sample preserved in ethanol. In the laboratory, routine microscope preparations were made by cleaning spicules in household bleach and hand-cutting perpendicular and tangential sections, which were dehydrated and mounted in Permount and examined under the light microscope.

Physicochemical Variables

Temperature and salinity were measured at 0 and 50 cm depth at the Belize sites (January and August 2004), and in Bocas del Toro (February, June, and September 2004). Sedimentation rates were estimated from accumulations in buried sediment traps (plastic pipes, 10 cm diameter, 50 cm length) left in place for 210 days in Belize and 150 days in Panama. The trapped sediment was oven-dried (50°C), and its composition was determined as percentage of mud (including the very fine clay fraction) (<0.002–0.05 mm) and sand (0.05–2 mm). Approximate values of calcium carbonate content were determined from weight loss after exposure to changes of dilute hydrochloric acid, and sediment deposition rates were calculated (g/m^2/day). Seawater samples (500 mL each) were taken in Belize (September 2003) and at Bocas del Toro (September 2004) at low and high tide, filtered (0.2 μm, GF/F filter) and frozen for nutrient analysis (Astor, 1996). Nutrient values were determined by spectrophotometric technique using the procedure described earlier (Diaz and Ward, 1997). Qualitative observations about habitat types surrounding the mangrove fringe were recorded, as well as an estimate of the level of human disturbance.

Survey sites, Bocas del Toro, Panama

Four sites within a perimeter of 10 km were selected and surveyed during 2004 (Figures 1, 2): (1) STRI Point: location of the Smithsonian Tropical Research Institute's laboratory, several mangrove stands close to reef patches in the southwest of Colon Island, and near a well-developed area with a housing complex that is part of the station; (2) Solarte In: a protected lagoon in the east of Solarte Island, site of a modest housing development; (3) Solarte Out: a pristine mangrove island close to reef patches to the west side of Solarte Island; and (4) Big Bight: a pristine, mangrove-lined lagoon surrounded by a well-developed terrestrial forest on Colon Island, less than 5 km northwest of STRI Point. General physicochemical characterization and geographic location of the sites are presented in Table 2 and the nutrient regime in Table 3.

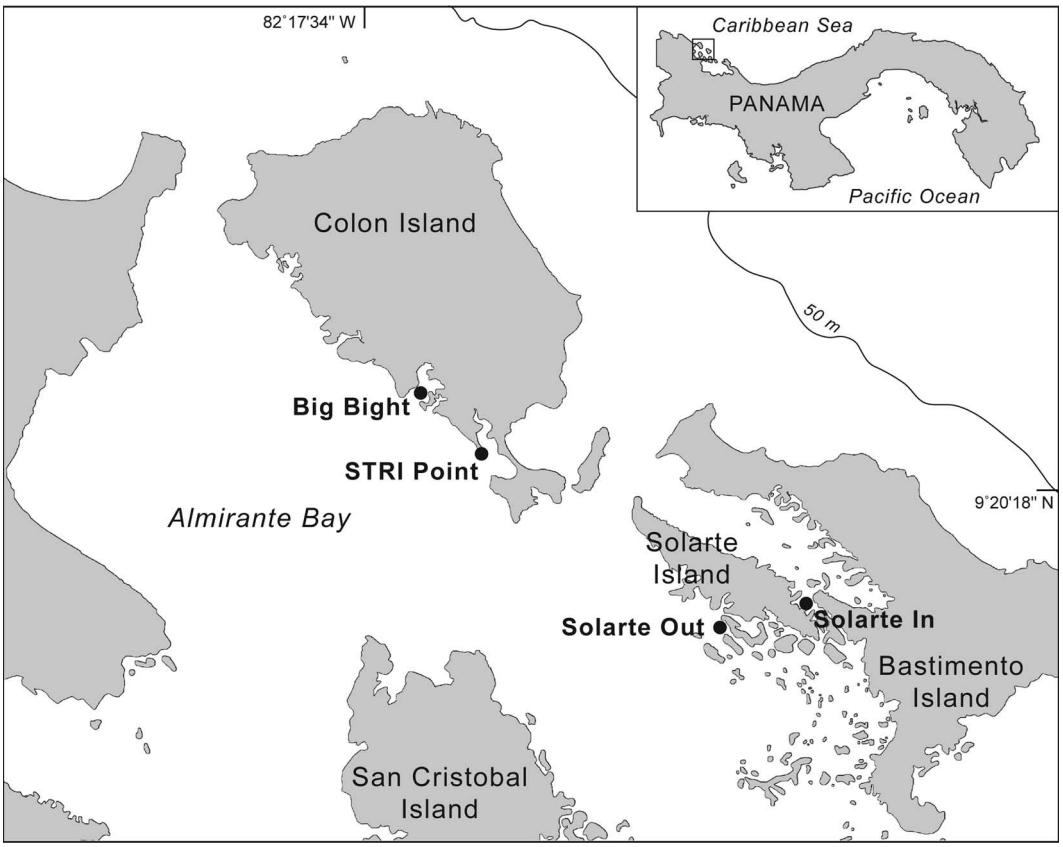

FIGURE 1. Map of research area at Bocas del Toro, Panama (STRI = Smithsonian Tropical Research Institute).

Mangrove prop roots (25 per site) were haphazardly selected within a 30 m length of the mangrove fringe. The front side (facing the channel) of each selected root was photographed along its entire length and set to scale by tying a transparent measuring tape to the high-tide water mark. The tape was prevented from floating by attaching a metal weight to its lower end. Three to seven photographs were taken, depending on the root length. Roots were rephotographed four times during the year (February 2004, June 2004, September 2004, and February 2005). From these images, abundance values of epifauna were estimated by measuring the projected area of each species using the CPCe program. The area (cm^2) covered by each taxon was divided by the root length (m), so that the relative abundance values are related to a measure of available substrate. Cover of each taxon is reported as the sum of its abundances (cm^2/m root) on all roots at a particular site. Eight categories of epiphytes were distinguished: cyanobacterial mats (monospecific stands of cyanobacteria), green algae, red algae (including both crustose calcareous and fleshy species), turf (mixture of densely packed red, green, and cyanobacterial filaments), sponges, hydroids, bivalves, and ascidians; the ninth category was "empty" (spaces not occupied by macrofauna or macroalgae). When small organisms were found overgrowing a large one (such as *Spongia tubulifera*, *Hyrtios proteus*) the projected area of both species was included. The number of roots finally analyzed per studied site was reduced to 14–22 because there was some accidental loss of photographic data.

Survey Sites, Twin Cays and Pelican Cays, Belize

Three sites at Twin Cays and one in the Pelican Cays were surveyed in August 2003 and four years later in August 2007 (Figures 3, 4; see Tables 2, 3). Two of the Twin Cays sites, the Lair Channel and Hidden Creek, are deep creeks that branch off the Main Channel; Sponge Haven

FIGURE 2. Views of research locations at Bocas del Toro, Panama. Top row from left: STRI Point looking south, where the transect was located in the right foreground; Solarte Island, with transect location Solarte In near the center. Bottom row from left: mangrove fringe at Solarte In; underwater view of mangrove prop roots showing a specimen of sponge, *Chalinula molitba*; mangrove roots covered by the encrusting sponge *Halisarca* sp. (undescribed; note scale in centimeters [cm] to the left), along with bivalves, algae, bryozoans, ascidians, and other fouling invertebrates.

is a bay in the southwest of the Main Channel. The Pelican Cays site was in the northern part of the lagoon of Manatee Cay. Transects (30 m long) were placed along the red mangrove fringe at each site, with number of roots ranging between 52 and 143. In all, the presence of the six most conspicuous epiphyte categories was recorded on each root within each transect: cyanobacterial mats, macroalgae, sponges, sea anemones (*Aiptasia pallida*), bivalves, and ascidians.

RESULTS

Caribbean Mangrove Sponge Species Richness and Distribution

The distribution of 177 sponge species currently reported from Caribbean mangroves is presented in Table 4. A cluster analysis (Figure 5) of the best studied sites (Belize, Cuba, Panama, Venezuela) shows the highest similarity between Venezuela (62 species) and Panama (65 species).

TABLE 2. Characterization of study sites in Panama and Belize.

Country and locality	Habitat [a] (depth, m)	Human impact [b]	Temperature range (°C)	Salinity range (ppm)	Sedimentation			Turbidity [b]	Coordinates
					Type	$CaCO_3$ (% dry wt)	Rate (g/m²/day)		
Panama									
STRI Point	PR (1.5–2)	+	26–29	29–34	Mud	14–23	34–41	±/+	09°21′29.1″N, 82°16′28.9″W
Solarte In	SG (2–2.5)	−/±	26–29	27–32	Sand	4–24	28–58	−	09°17′05.0″N, 82°10′03.3″W
Solarte Out	PR (1)	−	27–29	29–33	Sand	80–98	88–248	−	09°17′35.6″N, 82°12′08.3″W
Big Bight	SG (1.5–2)	−/±	27–29	27–34	Mud/ sand	25	40	−/±	09°22′31.1″N, 82°17′38.3″W
Belize									
Sponge Haven	SG (1–1.8)	±	26–32	33–35	Mud	48.75	25	±	16°49′40.5″N, 88°06′16.5″W
Hidden Creek	TC (2–2.5)	±/+	25.5–33	32–36	n.a.	n.a.	n.a.	±	16°49′40.5″N, 88°06′16.5″W
Lair Channel	TC (1.5–1.8)	−	25.3–33	32–36	Mud	25.9	44	−	16°49′33.7″N, 88°06′11.6″W
Manatee Lagoon	PR/SG (1–2)	− [c]/+ [d]	25.5–32	35–36	Mud	38.9	45	−	16°40′03.3″N, 88°11′32.4″W

[a] Habitat abbreviations: PR = mangrove prop roots; SG = seagrass (Thalassia); TC = tidal creek with peat walls and undercuts.
[b] Human impact and turbidity designations: +, high; ±, medium; −, low.
[c] Survey of 2003.
[d] Survey of 2007.

TABLE 3. Ranges of nutrient concentrations (low tide to high tide) at the study sites: Panama samples taken in September 2004 and Belize samples taken in September 2003.

Country and locality	Phosphate (μmol/L)	Ammonium (μmol/L)	Nitrate (μmol/L)
Panama			
STRI Point	0–0.048	1.32–0.988	0.26–0.253
Solarte In	0.02–0.85	0.845–0.88	0.264–0.23
Solarte Out	0.024–0.048	0.096–0.071	0.345–0.276
Big Bight	0.048–0.048	1.55–1.100	0.345–0.230
Belize			
Sponge Heaven	0.528–0.624	4.79–1.88	0.5–0.41
Hidden Creek	0.336–0.786	3.19–2.35	1.1–0.39
Lair Channel	0.384–0.672	2.72–1.59	1.06–1.24
Manatee Lagoon [a]	0.576	1.41	0.32

[a] Only one sample taken, at intermediate tide.

These faunas were paired with Twin Cays (54 species) and Cuba (48 species). The most dissimilar fauna in the analyses resulted from comparison with the Pelican Cays mangroves (147 species).

Mangrove Surveys at Bocas del Toro, Panama

Changes in Abundance of Major Epifaunal Taxa

The relative abundance of major taxa at each of the four localities studied between February 2004 and February 2005 is shown in Figure 6. In terms of the hierarchy of major taxa, sponges were first or second in abundance on mangrove roots at all sites, followed by algal turfs. An exception to this pattern was found in Solarte In (see Figure 1), where large mats of green algae, mostly *Caulerpa verticillata* and *Halimeda* spp., dominated over the sponges in February 2004 and 2005. Bivalves were the third most abundant group, followed closely by unoccupied (empty) spaces.

The abundance of the two most dominant groups, sponges and algae/cyanobacteria, showed a considerable decrease at STRI Point and Solarte In by the end of the

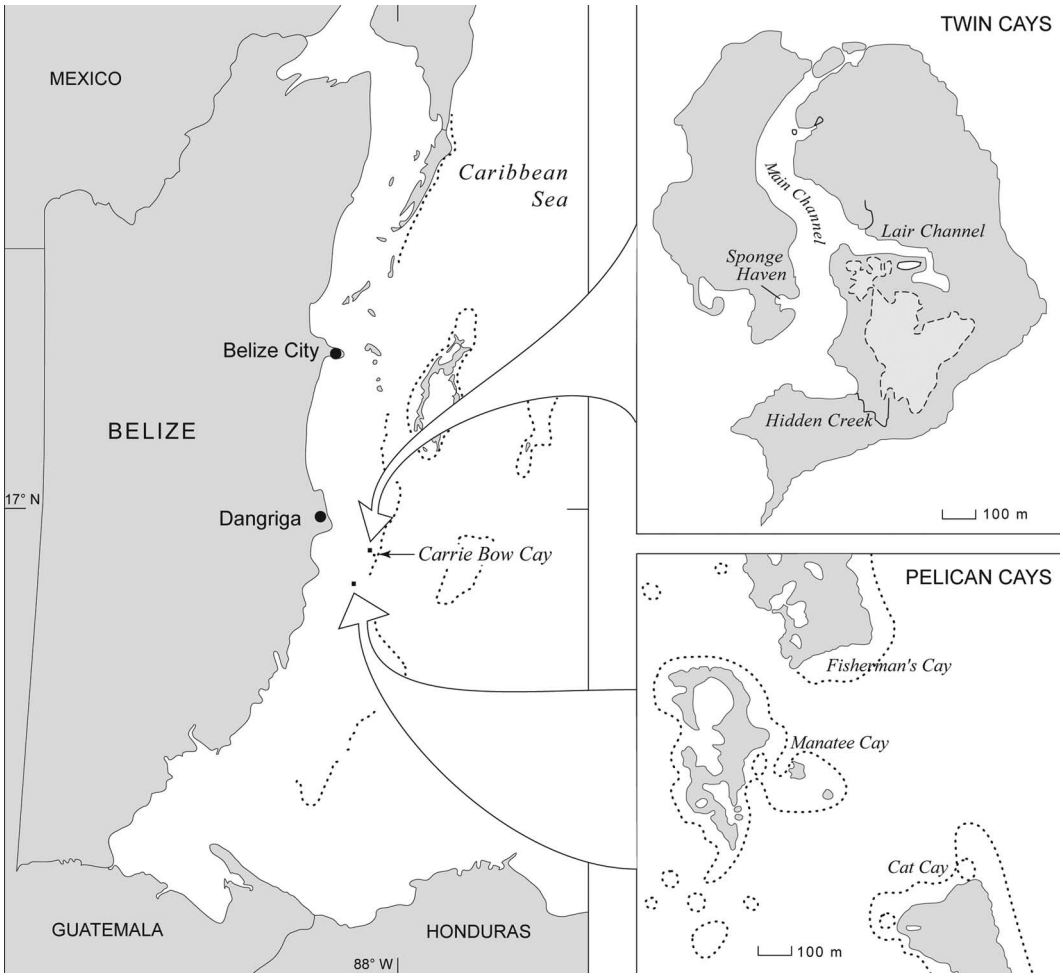

FIGURE 3. Map of research areas at Twin Cays and Pelican Cays, Belize.

study (February 2005), whereas abundance of both groups increased or stayed at similar levels at the other two sites.

Sponge Species Abundances per Site

Because of the high level of heterogeneity in sponge composition and dominance among sites, the relative abundance of the most conspicuous epiphytic sponge species is presented separately, by sites.

STRI Point

Sixteen of a total of 23 species found at this site comprise 99% of total sponge abundance. Most of them (13 species) belong to the order Haplosclerida, specifically the family Chalinidae, and to the order Poecilosclerida. *Tedania ignis* was the most abundant, followed by *Clathria schoenus*, *Spongia tubulifera*, *Mycale microsigmatosa*, *Chalinula molitba*, *Haliclona manglaris*, and *H. tubifera*. Figure 7a shows the relative abundance of the six most common species at this site, which added up to 87% of the total sponge abundance. It is interesting to note that the presence of both *T. ignis* and *Clathria schoenus* had decreased considerably by February 2005, whereas *S. tubulifera* remained with similar abundance throughout the year. *Chalinula molitba* shows a considerable increase (>200%) for June 2004 and a decrease to its initial values by February 2005.

Solarte In

Eight of 14 species found at this site constituted 99% of the total sponge abundance. Figure 7b demonstrates

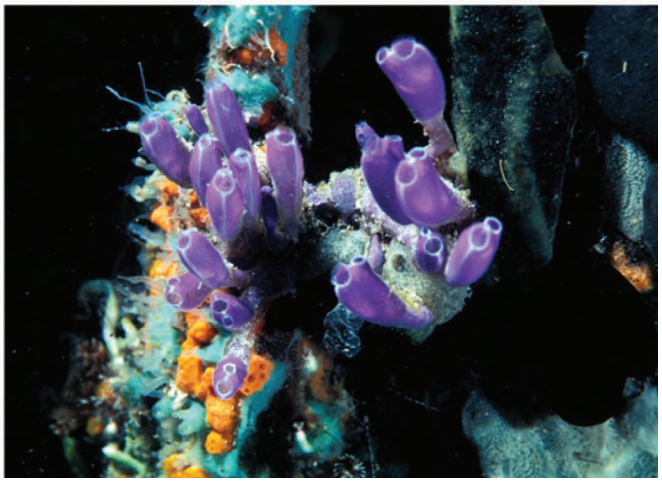

FIGURE 4. (*facing page*) Views of research locations at Twin Cays and Pelican Cays, Belize. Top left, aerial view of Twin Cays looking south, where transect locations were in the Lair channel (branching from the Main Channel center toward the left), in Sponge Haven (the small bay at the top right), and Hidden Creek (a narrow, deep tidal channel hidden by mangrove canopy, connecting the Main Channel in the far right background with Hidden Lake in the center background); top right, aerial view of Manatee Cay where a transect was placed in the large lagoon to the left (Cat Cay is in the background); middle left, mangrove fringe at Sponge Haven; middle right, red mangrove prop roots hanging free near the Pelican Cays site and covered mainly by the ropy sponge *Iotrochota birotulata*; bottom left, close-up of *Tedania ignis* and *Tedania* sp. (probably *T. klausi* Wulff, a species described after this survey was made), both red, attached to exposed roots in the main channel of Twin Cays; bottom right, close-up of purple ascidian (*Clavelina puertosecensis*) with sponges (turquoise *Haliclona curacaoensis*, primarily) on root at Manatee Cay lagoon.

TABLE 4. Distribution of sponge species reported from Caribbean mangrove localities by various researchers (X = presence). Localities are abbreviated as follows: BEL, Belize; TC, Twin Cays; PC, Pelican Cays; PAN, Panama; COL, Colombia; VEN, Venezuela; TRI, Trinidad; GUA, Guadalupe; JAM, Jamaica; and CUB, Cuba. Data sources are given in table footnotes.

	BEL [b]								
Species [a]	**TC**	**PC**	**PAN [c]**	**COL [d]**	**VEN [e]**	**TRI [f]**	**GUA [f]**	**JAM [g]**	**CUB [h]**
Plakina jamaicensis	—	X	—	—	—	—	—	—	—
Plakinastrella onkodes	—	X	—	—	—	—	—	—	—
Plakortis halichondriodes	X	X	—	—	—	—	—	—	—
Plakortis angulospiculatus	—	—	X	—	—	—	—	—	X
Oscarella sp. 1 (purple)	X	X	X	—	—	—	—	—	—
Oscarella sp. 2 (drab)	—	X	X	—	—	—	—	—	—
Cinachyrella apion	X	X	X	—	X	—	—	—	—
Ecionemia dominicana	—	X	—	—	—	—	—	—	—
Myriastra kallitetilla	X	—	—	—	—	—	—	X	X
Erylus formosus	—	X	—	—	—	—	—	—	—
Geodia gibberosa	—	X	—	—	X	—	X	X	X
Geodia papyracea	X	X	X	—	X	—	—	X	—
Dercitus sp.	—	X	—	—	—	—	—	—	—
Chondrilla caribensis	X	X	X	—	X	—	—	—	X
Chondrosia collectrix	—	X	X	—	—	—	—	—	X
Cervicornia cuspidifera	—	X	—	—	—	—	—	—	—
Cliona caribbaea	—	X	—	—	—	—	—	—	—
Cliona raphida	—	—	—	—	X	—	—	—	—
Cliona varians	X	X	—	—	—	—	—	—	X
Cliona sp.	—	X	—	—	—	—	—	—	—
Placospongia intermedia	—	X	X	—	X	—	—	—	—
Diplastrella megastellata	—	X	—	—	—	—	—	—	—
Spirastrella coccinea	—	X	—	—	—	—	—	—	—
Spirastrella hartmani	—	X	—	—	—	—	—	—	—
Spirastrella mollis	X	X	X	—	—	—	—	—	—
Aaptos duchassaingi	—	X	—	—	—	—	—	—	—
Aaptos lithophaga	—	—	—	—	—	—	—	—	X
Terpios fugax	—	X	—	—	—	—	—	—	X
Terpios manglaris	X	X	X	—	X	—	—	—	—
Prosuberites laughlini	—	—	X	—	X	—	—	X	—
Suberites aurantiaca	—	X	—	—	X	—	—	X	—
Tethya actinia	X	X	X	—	X	—	—	—	X
Tethya aff. *seychellensis*	—	—	X	—	X	—	—	X	—
Discodermia dissoluta	—	—	X	—	—	—	—	—	—
Paratimea ? sp.	X	—	—	—	—	—	—	—	—
Timea unistellata	—	X	—	—	—	—	—	—	—
Agela conifera	—	X	—	—	—	—	—	—	—

continued

TABLE 4. *continued*

Species [a]	BEL [b]		PAN [c]	COL [d]	VEN [e]	TRI [f]	GUA [f]	JAM [g]	CUB [h]
	TC	PC							
Phorbas amaranthus	—	X	—	—	—	—	—	—	—
Coelosphaera raphidifera	—	X	—	—	—	—	—	—	—
Lissodendoryx colombiensis	—	X	X	—	—	—	—	—	—
Lissodendoryx isodictyalis	X	X	X	—	X	—	X	—	X
Lissodendoryx sigmata	X	—	—	—	—	—	—	—	—
Monanchora arbuscula	—	X	—	—	X	—	—	—	—
Desmapsamma anchorata	—	X	X	—	X	—	—	—	—
Biemna caribea	X	—	X	—	X	—	—	—	X
Desmacella janiae	—	X	—	—	—	—	—	—	—
Desmacella meliorata	—	X	—	—	X	—	—	—	—
Neofibularia nolitangere	—	X	—	—	—	—	—	—	—
Hymedesmia sp.	—	X	—	—	—	—	—	—	—
Acarnus sp.	—	X	—	—	—	—	—	—	—
Artemisina melana	—	X	X	—	X	—	—	—	—
Clathria affinis	—	X	—	—	—	—	—	—	—
Clathria cf. *ferrea*	—	—	X	—	X	—	—	—	—
Clathria microchela	—	X	—	—	X	—	—	—	—
Clathria schoenus	X	—	X	—	X	—	—	—	X
Clathria aff. *schoenus*	X	—	—	—	—	—	—	—	—
Clathria spinosa	—	X	—	—	—	—	—	—	—
Clathria venosa	X	X	X	—	X	—	—	—	—
Clathria virgultosa	X	—	—	—	—	—	—	—	—
Mycale cf. *americana*	—	—	—	—	X	—	—	—	—
Mycale angulosa	—	—	—	—	X	—	—	—	—
Mycale arenaria	—	—	—	—	—	—	—	—	—
Mycale arndti	—	X	—	—	—	—	—	—	—
Mycale carmigropila	X	X	X	—	X	—	—	—	—
Mycale citrina	X	—	—	—	X	—	—	—	—
Mycale escarlatei	—	—	—	—	—	—	—		—
Mycale laevis	X	—	—	—	—	—	—	X	—
Mycale laxissima	X	X	—	X	X	—	—		—
Mycale magniraphidifera	X	X	X	—	X	—	—		X
Mycale aff. *magniraphidifera*	X	X	X	—	—	—	—		—
Mycale microsigmatosa	X	X	X	X	X	X	—	X	X
Mycale aff. *microsigmatosa*	—	X	—	—	—	—	—	—	—
Mycale paresperella	—	X	X	—	—	—	—	—	—
Iotrochota birotulata	—	X	X	—	X	—	—	—	—
Strongylacidon sp.	—	X	—	—	—	—	—	—	—
Ectyoplasia ferox	—	X	—	—	X	—	—	—	—
Eurypon laughlini	—	X	X	—	X	—	—	—	—
Tedania ignis	X	X	X	—	X	—	—	X	X
Tedania aff. *ignis*	—	X	—	—	—	—	—	—	—
Dragmacidon reticulata	—	X	—	—	—	—	—	—	—
Pseudaxinella ? sp.	—	X	—	—	—	—	—	—	—
Ptilocaulis walpersi	—	X	—	—	—	—	—	—	—
Dictyonella sp.	X	X	—	—	—	—	—	—	—
Scopalina hispida	—	X	—	—	X	—	—	X	X
Scopalina ruetzleri	X	X	X	X	X	—	—	—	X
Scopalina ? sp.	—	X	—	—	—	—	—	—	—
Ulosa funicularis	—	X	—	—	—	—	—	—	—
Amorphinopsis sp. 1	—	X	—	—	X	—	—	—	—
Amorphinopsis sp. 2	—	X	—	—	—	—	—	—	—
Ciocalypta ? sp.	—	X	—	—	—	—	—	—	—
Halichondria corrugata	—	—	—	—	—	—	—	—	X
Halichondria magniconulosa ?	X	X	X	—	X	X	X	—	X
Halichondria melanadocia	X	X	X	X	X	—	—	X	X
Halichondria poa ?	X	X	—	—	—	—	—	—	—

Species [a]	BEL [b] TC	BEL [b] PC	PAN [c]	COL [d]	VEN [e]	TRI [f]	GUA [f]	JAM [g]	CUB [h]
Hymeniacidon caerulea	—	X	—	—	—	—	—	—	—
Myrmekioderma rea	—	X	—	—	—	—	—	—	—
Topsentia ophiraphidites	—	X	—	—	—	—	—	—	—
Callyspongia arcesiosa	—	—	—	—	X	—	—	—	—
Callyspongia fallax	—	X	X	—	—	—	—	—	X
Callyspongia pallida	—	X	X	—	—	—	—	—	—
Callyspongia vaginalis	—	X	—	—	—	—	—	—	—
Haliclona caerulea	—	X	X	—	X	—	X	X	—
Haliclona curacaoensis	X	X	X	—	X	—	—	—	X
Haliclona aff. *curacaoensis*	—	X	—	—	—	—	—	—	—
Haliclona implexiformis	X	X	X	X	X	—	—	—	X
Haliclona aff. *implexiformis*	—	X	—	—	—	—	—	—	X
Haliclona magnifica	X	X	X	—	X	—	—	—	—
Haliclona manglaris	X	X	X	—	X	—	—	—	X
Haliclona mucifibrosa	X	X	X	—	—	—	—	—	—
Haliclona picadaerensis	X	X	X	—	X	—	—	—	—
Haliclona tubifera	X	X	X	X	X	—	—	—	X
Haliclona aff. *tubifera*	—	X	—	—	—	—	—	—	—
Haliclona twincayensis	X	X	X	—	X	—	—	—	—
Haliclona vermeuleni	X	X	X	—	—	—	—	—	—
Chalinula molitba	X	X	X	—	X	—	—	X	X
Chalinula zeae	—	—	X	—	—	—	—	—	—
Amphimedon compressa	—	X	—	—	X	—	—	X	—
Amphimedon erina	X	X	—	X	X	—	—	—	—
Amphimedon aff. *erina*	—	X	—	—	—	—	—	—	—
Amphimedon viridis	—	—	—	—	—	—	—	—	X
Niphates caicedoi	—	X	X	—	—	—	—	—	—
Niphates digitalis	—	X	—	—	—	—	—	—	—
Niphates erecta	—	X	X	—	X	—	—	—	—
Niphates sp.	—	X	—	—	—	—	—	—	—
Petrosia pellasarca	—	X	—	—	—	—	—	—	—
Petrosia weinbergi	—	X	—	—	—	—	—	—	—
Strongylophora davilai	—	X	—	—	—	—	—	—	—
Xestospongia carbonaria	—	X	—	—	—	—	—	—	—
Xestospongia muta	—	X	—	—	—	—	—	—	—
Xestospongia proxima	—	X	—	—	—	—	—	—	—
Xestospongia subtriangularis	—	X	—	—	X	—	—	—	—
Aka coralliphaga	—	X	—	—	—	—	—	—	—
Aka siphona	—	X	—	—	—	—	—	—	—
Aka sp.	—	X	—	—	—	—	—	—	—
Calyx podatypa	X	X	X	—	—	—	—	—	—
Oceanapia nodosa	—	—	X	—	X	—	—	—	—
Oceanapia oleracea	—	—	X	—	—	—	—	—	—
Cacospongia sp.	—	X	—	—	—	—	—	—	X
Fasciospongia? sp.	—	X	—	—	—	—	—	—	—
Hyrtios proteus	X	X	X	—	X	—	—	—	X
Hyrtios sp.	X	X	—	—	—	—	—	—	—
Smenospongia aurea	—	X	—	—	—	—	—	—	—
Ircinia campana	—	X	X	—	—	—	—	—	—
Ircinia felix	X	X	X	X	X	—	—	X	X
Ircinia strobilina	X	X	—	—	X	—	—	—	X
Spongia pertusa	X	X	X	—	X	—	X	—	X
Spongia tubulifera	X	X	X	X	X	—	—	—	X
Dysidea etheria	X	X	X	X	X	—	X	—	X
Dysidea fragilis	—	—	—	—	—	—	—	X	—
Dysidea janiae	—	X	—	—	—	—	—	—	X
Aplysilla glacialis	X	X	X	—	X	—	—	—	—
Chelonaplysilla aff. *erecta*	—	X	X	X	X	—	—	—	X
Darwinella rosacea	—	X	—	—	X	—	—	X	X

continued

TABLE 4. *continued*

Species [a]	BEL [b]		PAN [c]	COL [d]	VEN [e]	TRI [f]	GUA [f]	JAM [g]	CUB [h]
	TC	PC							
Halisarca caerulea	—	X	—	—	—	—	—	—	—
Halisarca sp. (white)	X	X	X	—	X	—	—	—	—
Aiolochroia crassa	—	X	—	—	—	—	—	—	—
Aplysina archeri	—	X	—	—	—	—	—	—	—
Aplysina fistularis	—	X	—	—	X	—	—	—	X
Aplysina insularis	—	X	—	—	—	—	—	—	—
Aplysina fulva	—	X	—	—	—	—	—	—	X
Aplysina lacunosa	—	X	—	—	—	—	—	—	—
Verongula rigida	—	X	—	—	—	—	—	—	—
Clathrina primordialis	X	X	—	—	—	—	—	—	X
Sycon sp.	X	—	X	—	X	—	—	—	—
Leucandra aspera	—	—	X	—	X	—	—	—	—

[a] Species are listed in taxonomic order according to class, order, and family.
[b] Rützler et al., 2000.
[c] Diaz, 2005; Lehnert and van Soest, 1998.
[d] Zea, 1987; unpublished data.
[e] Sutherland, 1980; Diaz et al., 1985; Orihuela et al., 1991; Pauls, 1998, 2003; Ramirez, 2002; Diaz et al., 2003; Perez, 2007.
[f] Toffart, 1983.
[g] Hechtel, 1965.
[h] Alcolado, unpublished data.

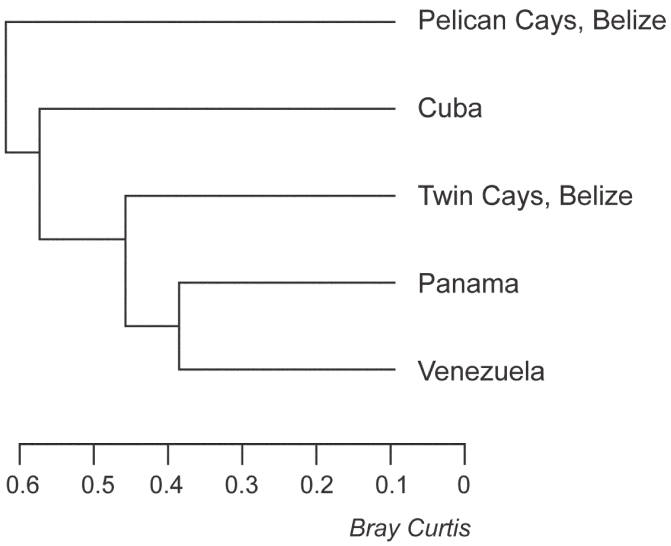

FIGURE 5. Similarities of mangrove sponge faunas from Belize, Panama, Venezuela, and Cuba. The dendrogram is built from a binary matrix (presence or absence) of species distribution using an unweighted pair-group method with arithmetic mean (UPGMA) clustal analysis program, with the Bray–Curtis distance index.

the relative abundance of the six most common species, which comprised 96% of all sponges. *Tedania ignis* and *Mycale microsigmatosa* were among the top species; *Halisarca* sp. (a species so far undescribed) and *Mycale carmigropila* appeared to be among the major components. Similar to STRI Point, most of the dominant species decreased in abundance or disappeared by the end of the study, while *Halisarca* remained steady in abundance throughout the study period. Three of the common species at this site (*Dysidea etheria*, *Haliclona curacaoensis*, and *Mycale carmigropila*) show an increase of sponge growth in the warmer periods (either June or September 2004), followed by a decrease in size during cooler periods (February 2005).

Solarte Out

Six of nine species found at this site made up 99% of total sponge abundance (Figure 7c). *Tedania ignis* continues to dominate, followed by three species not seen in the previously discussed sites: *Spirastrella mollis*, *Haliclona vermeuleni*, and *H. caerulea*. It is notable that the (projected) area coverage of the dominant species is much lower here than that at the other sites (most values are less than 500 cm^2/m).

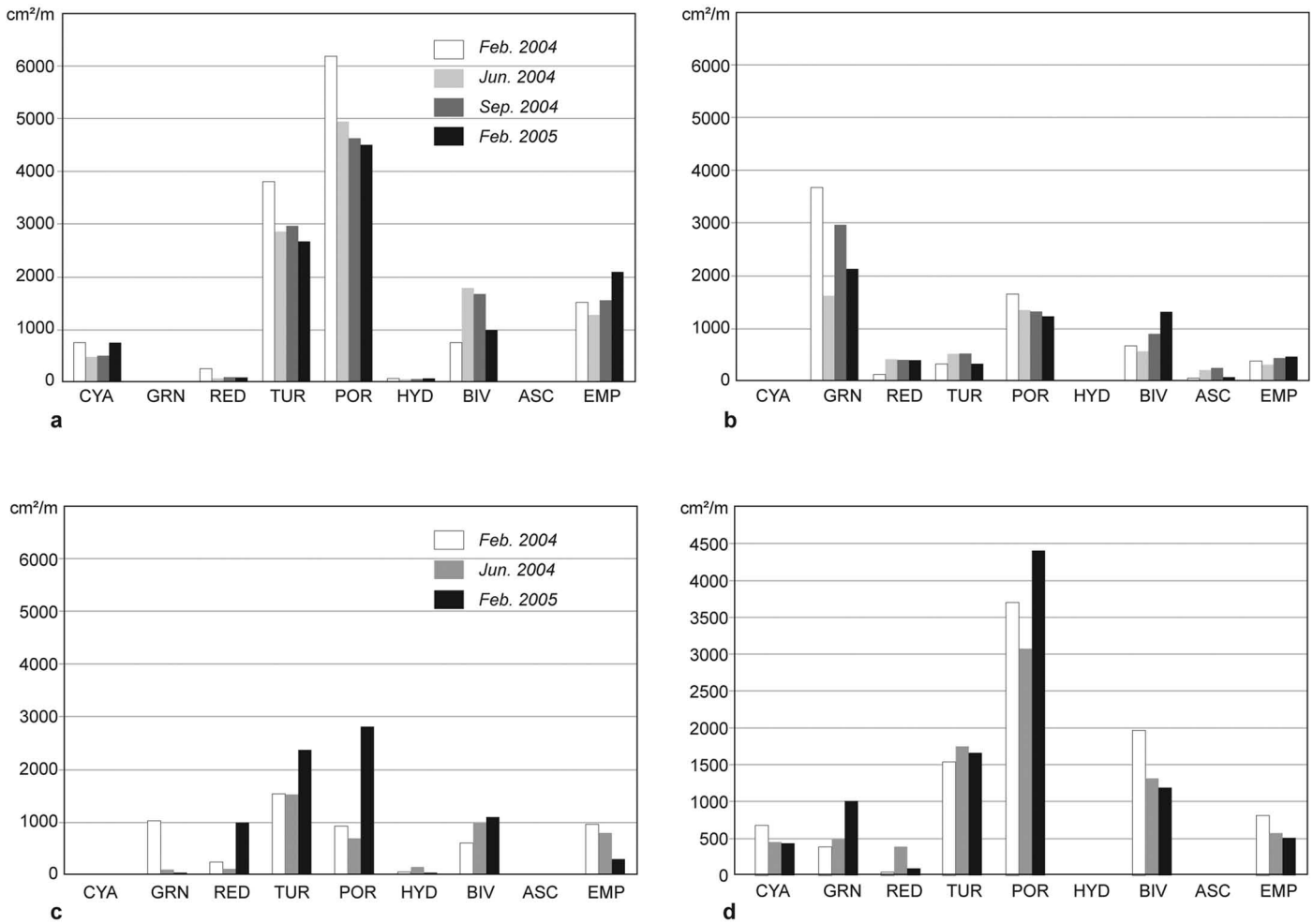

FIGURE 6. Relative abundance of major functional groups growing on mangrove roots (expressed as projected area [cm^2] per length [m] of root) at four Bocas del Toro sites, between February 2004 and 2005: a, STRI Point; b, Solarte In; c, Solarte Out; d, Big Bight. (ASC = ascidians; BIV = bivalves; CYA = Cyanobacteria; EMP = empty space; GRN = green algae; HYD = hydroids; POR = sponges [Porifera]; RED = red algae; TUR = algal–cyanobacterial turf.)

Big Bight

Twelve of 17 species found at Big Bight comprised 99% of the total sponge abundance; 6 of these amounted to 90% (Figure 7d). The most abundant species—*Tedania ignis*, *Mycale microsigmatosa*, and *Haliclona manglaris*—increased in size throughout the year, whereas *Lissodendoryx colombiensis* and *Dysidea etheria* gained in size up to September 2004 but disappeared altogether in February 2005. It is worth pointing out the large values for area coverage, as compared to the other locations. The September 2004 data from this site were accidentally lost.

Sponge Species Ranks

The most common sponges at each site amount to 21 species, from a total of 40 distinguished in the studied areas. Abundance ranks from each site are listed in Table 5. Only one species, *Tedania ignis*, maintained the same rank at all sites, as the most abundant species. The second and third most abundant species were *Clathria schoenus* and *Spongia tubulifera* at STRI Point, *Mycale microsigmatosa* and *Halisarca* sp. at Solarte In, *Spirastrella mollis* and *Haliclona manglaris* at Solarte Out, and *M. microsigmatosa* and *H. manglaris* in Big Bight. Seven of these 21 common sponges were only found at one site.

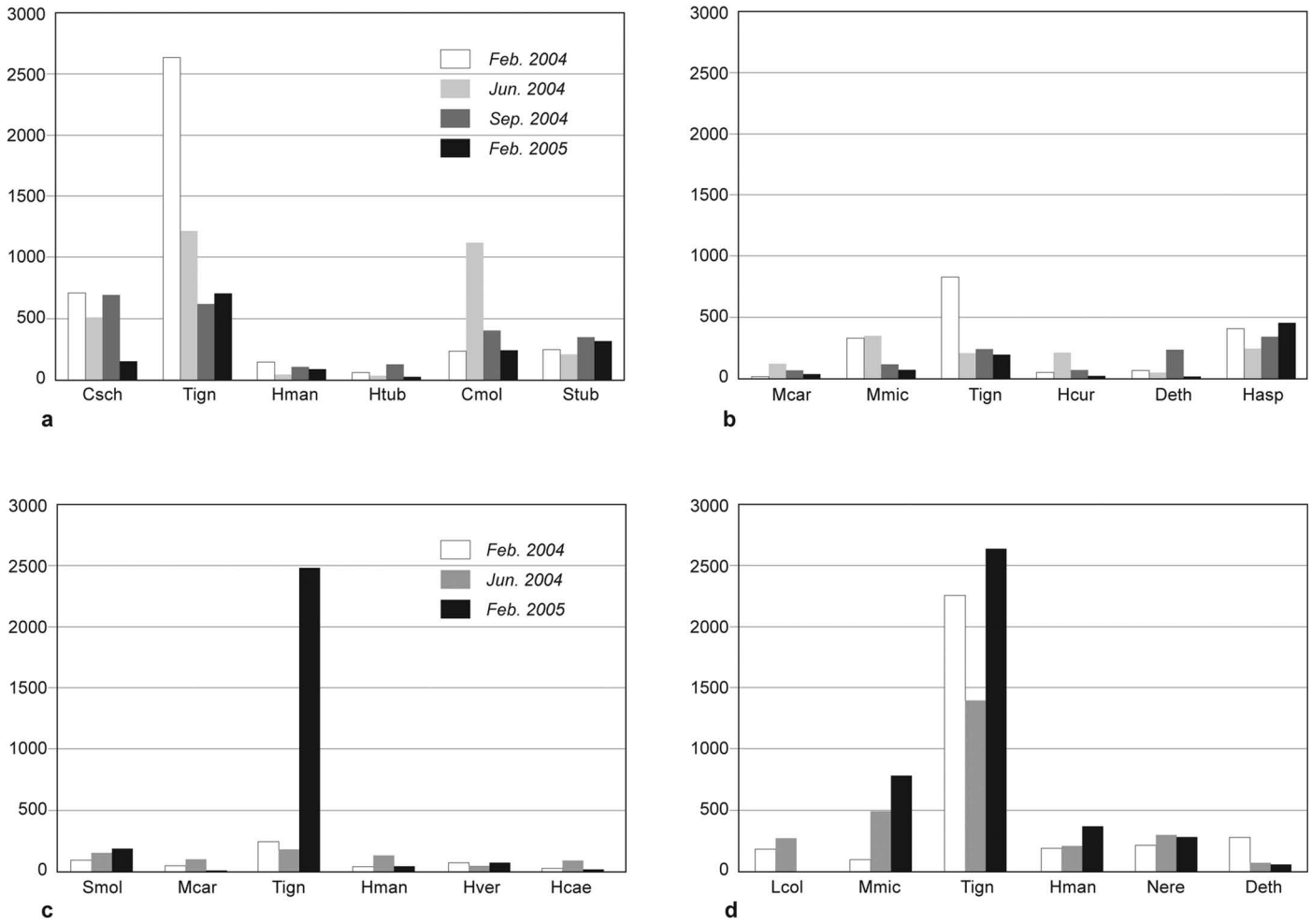

FIGURE 7. Relative abundance of most common sponge species growing on mangrove roots (expressed as projected area [cm^2] per length [m] of root) at four Bocas del Toro sites between February 2004 and 2005: a, STRI Point; b, Solarte In; c, Solarte Out; d, Big Bight. (Cmol = *Chalinula molitba*; Csch = *Clathria schoenus*; Deth = *Dysidea etheria*; Hasp = *Halicarca* sp.; Hcae = *Haliclona caerulea*; Hcur = *H. curacaoensis*; Hman = *H. manglaris*; Hpro = *Hyrtios proteus*; Htub = *Haliclona tubifera*; Hver = *H. vermeulei*; Lcol = *Lissodendoryx colombiensis*; Mcar = *Mycale carmigropila*; Mmic = *M. microsigmatosa*; Nere = *Niphates erecta*; Smol = *Spirastrella mollis*; Stub = *Spongia tubulifera*; Tign = *Tedania ignis*).

Mangrove Surveys in Belize

Changes in Frequency of Occurrence of Major Functional Groups

We determined the frequency of occurrence of important functional groups growing on mangrove roots to be able to assess changes over time (Figure 8). The six compound groups recorded in our surveys were cyanobacteria, algae, sponges, sea anemones, bivalves, and ascidians. In terms of the hierarchy, sponges were first or second at all sites, followed by colonial ascidians, macroalgae, and cyanobacteria. Only at Manatee Cay had sponge occurrence on roots decreased since 2003, whereas at the other three sites it either increased or stayed nearly the same. Ascidian occurrence decreased considerably (10%–26%) at all sites between 2003 and 2007. These changes in sponge and ascidian populations were accompanied by cyanobacterial blooms at three sites (Lair Channel, Hidden Creek, and Manatee Cay), where increases of 10% to 57% of these organisms were recorded. One of the less abundant

TABLE 5. Ranking of the most common sponge species according to their abundance at each studied site in the Bocas del Toro region, 2004–2005.

Species	Rank in abundance			
	STRI Point	Solarte In	Solarte Out	Big Bight
Spirastrella mollis	0	0	2	0
Lissodendoryx colombiensis	0	0	0	5
Lissodendoryx isodicyialis	11	0	0	11
Clathria schoenus	2	0	0	12
Mycale carmigrophila	16	4	6	16
Mycale microsigmatosa	5	2	0	2
Iotrochota birotulata	7	0	0	0
Tedania ignis	1	1	1	1
Haliclona caerulea	0	0	5	0
Haliclona curacaoensis	13	5	0	0
Haliclona implexiformis	10	0	0	0
Haliclona manglaris	6	7	3	3
Haliclona tubifera	8	0	0	0
Haliclona vermeuleni	0	0	4	0
Chalinula molitba	4	0	0	10
Amphimedon sp.	0	0	0	8
Niphates erecta	0	0	0	4
Hyrtios proteus	15	0	0	6
Spongia tubulifera	3	8	0	13
Dysidea etheria	0	6	0	7
Halisarca sp.	0	3	0	18

groups, the sea anemone *Aiptasia pallida* (Cnidaria), is worth mentioning for its striking change in occurrence at the Twin Cays sites. Although the population remained steady at Hidden Creek (8%), it doubled in Lair Channel (10%–24% of roots occupied), but it apparently disappeared from Sponge Haven where it had been present on 20% of the roots in 2003. The number of roots available for settlement per site increased considerably at Sponge Haven and Manatee Cay lagoon, although it decreased in Hidden Creek and Lair Channel.

Sponge Species Frequencies per Site

The distinctive species composition and richness at each site warrant separate presentations.

Sponge Haven

Most mangrove-specific species, such as *Halichondria magniconulosa*, *Haliclona curacaoensis*, *H. manglaris*, *H. implexiformis*, *Hyrtios proteus*, *Lissodendoryx isodyctialis,* and *Spongia tubulifera*, remained the most common among sponges, and some even increased in frequency between 2003 and 2007 (Figure 9a).

Lair Channel

In this mangrove channel most species remained in place between survey periods; some increased in root occurrence (*Tedania ignis*, *Haliclona manglaris*, *H. tubifera*, *Dysidea etheria*) and a few decreased (*Haliclona curacaoensis*, *H. implexiformis*, *Hyrtios proteus*) (Figure 9b). Overall, this change was accompanied by a slight decrease in root numbers (from 105 to 91) and an increase in all non-sponge groups except ascidians.

Hidden Creek

This tidal channel site is opposed to Sponge Haven in its changes between 2003 and 2007 (Figure 9c). Ten of the 12 sponge species found on the transect decreased considerably in occurrence on roots; only the opportunistic

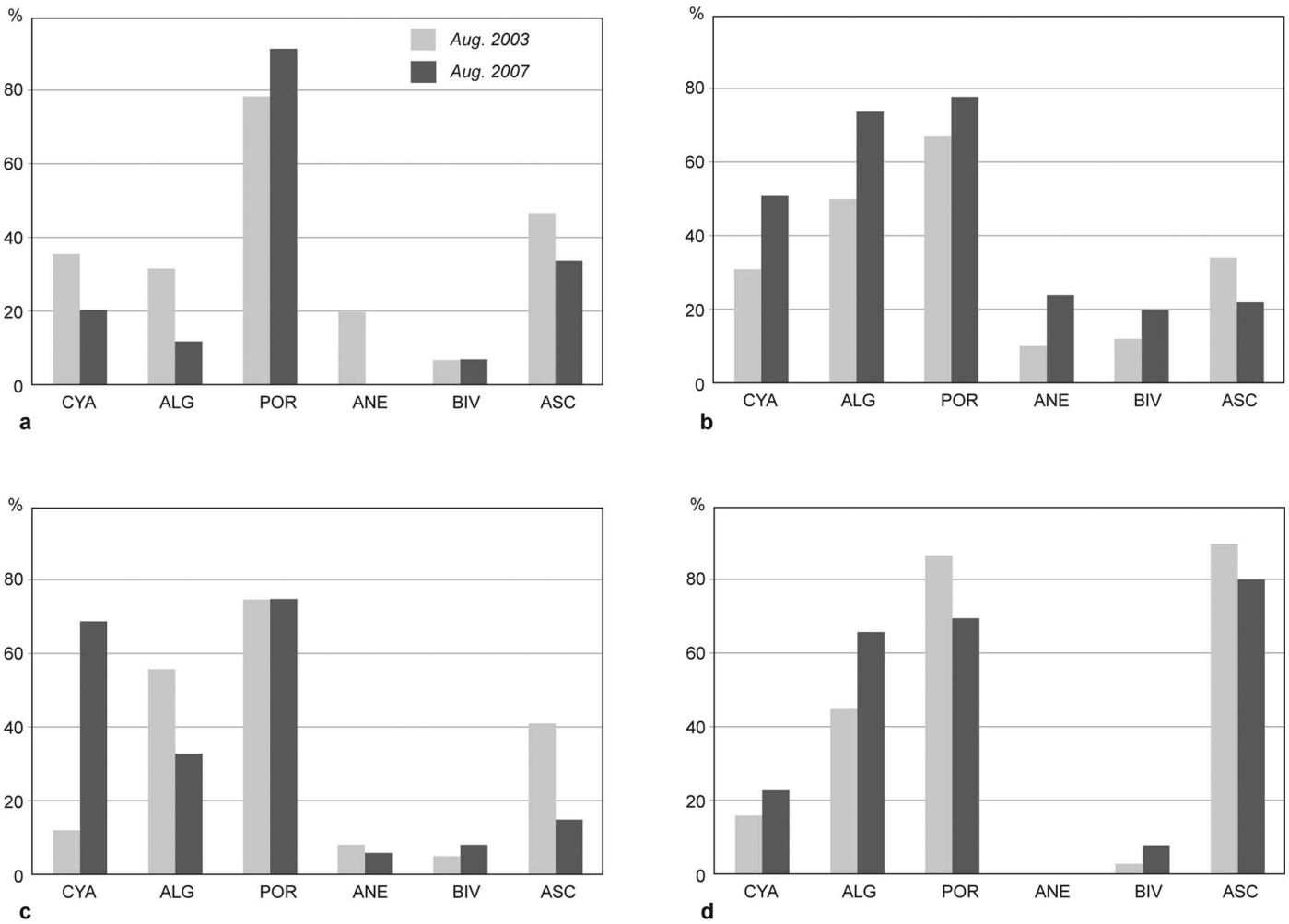

FIGURE 8. Frequency of occurrence (% of roots occupied) of major functional groups growing on mangrove roots at four sites in Belize: a, Sponge Heaven, Twin Cays; b, Lair Channel, Twin Cays; c, Hidden Creek, Twin Cays; d, Manatee Lagoon, Pelican Cays. (ALG = algae; ANE = sea anemones [*Aiptasia pallida*]; ASC = ascidians; BIV = bivalves; CYA = cyanobacteria; POR = sponges [Porifera]).

generalist *Tedania ignis* and *Lissodendoryx isodictyalis* increased slightly.

Manatee Cay

At this lagoon site, abundance of most typical mangrove sponge species decreased considerably during the survey period while two common opportunistic species (*Tedania ignis*, *Clathria schoenus*) experienced a considerable boost in their populations (Figure 9d). This trend coincided with a major increase in root numbers (from 89 to 123), similar to that which took place at Sponge Haven during the same time span.

DISCUSSION

Biogeography of Caribbean Mangrove Sponges

Available reports describing sponge species distribution in Caribbean mangroves suggest the importance of geographic vicinity, with high similarities between the faunas of Panama and Venezuela. On the other hand, this geographic concept is upset by the incongruence of faunas encountered at two nearby sites in Belize (Twin Cays and the Pelican Cays). This dissimilarity is caused mostly by the presence of several unique or usually coral reef-associated species in the mangroves of Manatee Lagoon, an environment of particular geomorphological structure and prevailing ecologi-

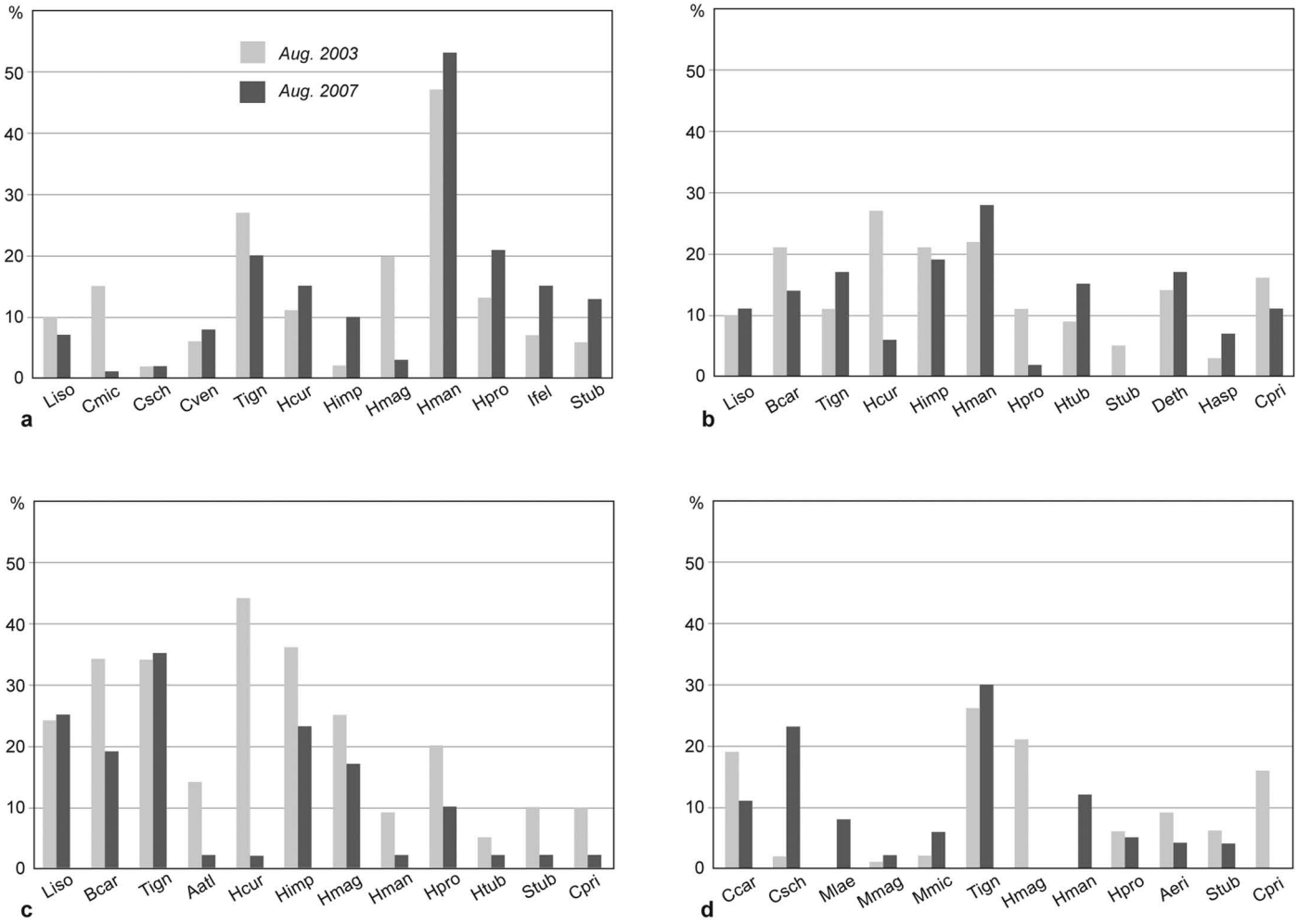

FIGURE 9. Frequency of occurrence (% of roots occupied) of sponge species growing on mangrove roots at four sites in Belize: a, Sponge Heaven, Twin Cays; b, Lair Channel, Twin Cays; c, Hidden Creek, Twin Cays, d, Manatee Lagoon, Pelican Cays. (Aatl = *Amorphinopsis atlantica*; Aeri = *Amphimedon erina*; Bcar = *Biemna caribbea*; Ccar = *Chondrilla caribensis*; Cmic = *Clathria microchela*; Cpri = *Clathrina primigenia*; Csch = *Clathria schoenus*; Cven = *Clathria venosa*; Deth = *Dysidea etheria*; Hasp = *Halisarca* sp.; Hcur = *Haliclona curacaoensis*; Himp = *H. implexiformis*; Hmag = *Halichondria magniconulosa*; Hman = *Haliclona manglaris*; Hpro = *Hyrtios proteus*; Htub = *Haliclona tubifera*; Ifel = *Ircinia felix*; Liso = *Lissodendoryx isodictyalis*; Mlae = *Mycale laevis*; Mmag = *M. magniraphidifera*; Mmic = *M. microsigmatosa*; Stub = *Spongia tubulifera*; Tign = *Tedania ignis*.)

cal conditions in the Pelican Archipelago (Macintyre and Rützler, 2000; Rützler et al., 2000; Wulff, 2000).

Short-Term Dynamics of Mangrove Epifauna in Panama

Major Functional Groups

As previously reported, mangrove-root epiphytic communities in Bocas del Toro are dominated either by sponges or by algae/cyanobacteria (Farnsworth and Ellison, 1996; Diaz et al., 2004; Peréz, 2007). Elsewhere in the Caribbean, other groups, such as bivalves, anemones, or ascidians, may rival these taxa in abundance (Sutherland, 1980; Toffart, 1983; Bingham, 1992). The dominance of macroalgae at the protected lagoon of "Solarte In" might be a consequence of the eastward orientation of this site (as opposed to the westward orientation of the other three sites), which would expose the mangrove fringe to sunlight

for longer periods, thus promoting the growth of typical shallow-water algal species. However, further studies are required to sustain this hypothesis. There were no observations of seasonal changes in the composition of epiphytic taxa from one sampling period to the other at any site. The decrease in abundance (20%–35%) found for the most dominant groups at two sites (STRI Point and Solarte In) coincides with housing developments that occurred since the study started in 2004. Increases in suspended sediments and incidences of sponges covered by silt, which were observed at STRI Point during September and February 2005, may have impacted the community. In contrast, at the more pristine sites (Solarte Out and Big Bight), these same organisms demonstrated considerable quantitative increases.

Sponge Species

The six most common species at each site constitute from 87% to 99% of the total root area covered by sponges. These dominant species differed between sites, bringing the number of the most abundant sponges to 21, of a total diversity of 40 species. Only *Tedania ignis* was the most common species at all sites. At Solarte In the second most common species was a thin crust of the genus *Halisarca*, whereas at STRI Point it was *Clathria schoenus*, a species with a highly variable growth form (thick crusts to branching), supporting the common observation that mangrove fauna can be highly heterogeneous within one biogeographic region. It is interesting to note that 5 species that ranked near the top at the four sites were encrusting sponges (*Mycale microsigmatosa*, *Dysidea etheria*, *Haliclona manglaris*, *Halisarca* sp., *Clathria schoenus*, and *Spirastrella mollis*). This result suggests that, at least in Bocas del Toro, encrusting species are highly successful competitors. The dominance of *Tedania ignis* was also reported from other Caribbean locations (Toffart, 1983; Sutherland, 1980; Wulff, 2004; Diaz et al., 2004) and is probably related to its high and nearly year-around production of larvae (Ruetzler, unpublished data) and rapid growth rate (Wulff, 2005).

Dominant species were not always consistent in abundance at all sites. For example, during the observation time *Tedania ignis* decreased considerably at STRI Point and Solarte In but increased at Solarte Out and Big Bight. Furthermore, increase or decrease in abundance was not necessarily restricted to certain species or localities. Certain locality trends, however, were observed. At STRI Point, where *T. ignis* and *Clathria schoenus* decreased or disappeared entirely from the roots, the few large specimens of *Spongia tubulifera* remained with only slight size changes throughout the year. At least at one location, Solarte In, deterioration of sponges appeared to be coinciding with the aforementioned housing development, which caused an increase in suspended and deposited sediment.

An interesting trend is the predominance of large sponges at Big Bight versus the much smaller sizes at Solarte Out. Solarte Out is a shallow habitat in an exposed position and subjected to strong wave action and scouring by predominantly sandy sediments (see Table 2). These parameters must impede the growth of large individuals, with the result that small and better adapted forms, such as *Haliclona vermeuleni*, *H. caerulea*, and *Spirastrella mollis*, become very abundant. Even an opportunistic species such as *Tedania ignis*, common and large-growing elsewhere, tends to be considerably restricted in size there. On the other hand, Big Bight sponges were found to have rapid growth rates that can be attributed to high nutrient concentrations measured at this site, possibly related to runoff from the dense forest that surrounds this lagoon (see Table 3). The high variability of sponge species composition between contiguous sites corroborates previous reports that the mangrove sponge fauna is rather heterogeneous in species distribution and dominance within relatively small geographic areas (Farnsworth and Ellison, 1996; Rützler et al., 2000; Diaz et al., 2004). This characteristic is probably the result of low recruitment rate in most species studied and, in some cases, selective physicochemical variables, such as those described for Solarte Out. A third aspect that became evident in this study is the intrinsic growth dynamics of species over time, high in species such as *Tedania ignis* and *Chalinula molitba*, and low or barely noticeable in *Hyrtios proteus* and *Spongia* spp. It must be recognized that species have different lifespans, growth rates, growth periods, and frequency of reproduction. Understanding these processes is essential to the interpretation of community dynamics.

Long-Term Dynamics of Mangrove Epifauna in Belize

Major Functional Groups

The distribution of the four primary components of mangrove-root ephiphytic communities in Belize—cyanobacteria, macroalgae, sponges, and ascidians—varied differently at each of the four studied sites between August 2003 and 2007. Sponges were the most frequent occupants at all four locations in 2003; by 2007, the population had either increased (Sponge Heaven, The Lair), decreased (Manatee Lagoon), or remained steady. The decrease at Manatee Cay seemed to be related to macroalgal blooms

that coincided with the recent clear-cutting of the mangrove adjacent to this lagoon and to dredging for land-fill that released large quantities of fine sediments. Ascidian occurrence followed a similar pattern, indicating that all filter feeders are impacted by environmental events such as increase of sedimentation and blockage of vents by cyanobacterial blooms. The effect of changing root numbers seems to be obscured by the environmental factors, because there was no obvious relationship between changes in root number and frequency of any of the major taxa in the community.

Sponge Species

Comparing species composition and frequency at the four study sites in Belize, we found that they varied considerably during the four years between observation periods. The most obvious parameters affecting sponge populations were space competitors (cyanobacteria, macroalgae), number of roots available for settlement, and anthropogenic destructive events. The considerable decrease in cyanobacteria and macroalgae and increase in root numbers (from 99 to 143) in Sponge Haven may be related to the strong increase of mangrove-specific sponges because important competitors were no longer present and new substrata became available. In contrast, at Hidden Creek, the increase of filamentous cyanobacteria (to 57% of substrate area) and decrease in root numbers (from 59 to 52) must have caused the dramatic reduction of most mangrove-specific sponge species. In Manatee Cay Lagoon, mangrove-specific species lost in frequency while opportunistic species (*Tedania ignis*, *Clathria schoenus*) gained. Overall, however, there was a reduction of sponge populations despite an addition in root numbers. This trend can be explained by increased algal competition and an artificial incursion, the clear-cutting of mangrove trees and dredging of fill material for a housing development sometime before the 2007 survey. The dredge operation in particular can be blamed in the short term as it causes suspension of fine sediment, affecting the delicate filtration system of the sponges. A shift of species toward more robust opportunists rather than typical mangrove forms is therefore not surprising.

Comments on Methods for Evaluating Mangrove Prop-Root Communities

Two criteria were used in the present study to evaluate epiphytic communities on mangrove roots. To determine short-term dynamics (within one year; Bocas del Toro), it was expected that specimen size rather than numbers would change. Therefore, a photographic record was made of a specific number of roots (25) along their entire lengths (the side facing the open water), and planimetry was used to measure projected area cover of the fouling organisms. From these values and the record of root length, an index of species abundance could be calculated. Area cover has been extensively used to compare the abundances of plant and sessile animal communities, and it has been proven a most practical and reliable method for reef surveys (Weinberg, 1981). Considering that in mangroves substrate availability is quite low, measuring area cover gives a good indication of how important an organism is in this community. The limitation of this method applies mostly to stoloniferous organisms for which cover may underestimate their importance. The photo-transect method proved to be most useful in areas where visibility was very good, but it was problematic in locations with high freshwater or sediment input. Such conditions caused whole sets of photographs to be impossible to interpret. This method is also time consuming, both the work underwater and that during photo analysis in the lab. For this reason there was a limit to the root numbers that could be included in each survey. Usually, to complete a survey of 25 roots in one site it was necessary to visit twice, and evaluation of all (3–8) photos for one root took from 30 to 60 min. In the end, after excluding useless images, the data set was reduced to only 14 to 22 recorded roots, depending on the site.

Alternatively, in Belize we used data on the presence or absence of taxa on each root and thus were able to survey a much larger number of roots (50–150), from which we determined the frequency of occurrence of major taxonomic groups and species of sponges. These data allowed monitoring the presence of each group or species and change in distributions over time. This type of survey follows the fate of the community rather than fluctuations in biomass. The method also aids detection of a species or community reaction to particular environmental disturbances. In terms of time investment, it takes only 2 to 5 h to obtain frequency data from a 30 m transect along the mangrove fringe. The data were in hard copy once the fieldwork was completed and were independent of visibility conditions and other variables that may ruin photographic data.

CONCLUSIONS

Many more Caribbean mangroves must be studied before we can expect a full understanding of the biodiversity and the biogeographic relationships of their unique and

fascinating prop-root fouling communities, particularly the sponges. The rather disjunct pattern of sponge species distribution found in the Panama and Belize study sites suggests that biodiversity is better evaluated by surveying extended stretches of mangrove fringe at numerous sites in any region rather than short lengths of transects. Interpretation of species composition and interactions can be based on smaller-scale levels of inquiry. The most abundant organisms in the studied sites were sponges, macroalgae, cyanobacteria, ascidians, and bivalves. The hierarchical ranking of these groups showed great variability on spatial and temporal scales, making generalization and prediction of structure and dynamics of communities very difficult.

The one-year study of four sites in the Bocas del Toro region, Panama, showed various important aspects of abundance changes in these fouling communities. First, a few sponge species contribute most of the abundance; second, the identity of major community components varies within a small geographic scale; third, species have adopted distinct life strategies (in growth potential, recruitment rates, and asexual reproduction capabilities) that allow for adaptations to resist stressful environmental variables; and fourth, the combination of the factors of large sediment grain size and energy from wave or current action limits species habitat access, survival, and growth, as demonstrated by the increase in turbidity from landfilling and development in some areas.

The four-year observations in Belize made it evident that the frequency of occurrence of sponge species and other taxa, such as cyanobacterial and macroalgal blooms, is a relatively simple and fast measure to detect major environmental changes. Even if sponge frequency on the roots is not much affected by algal blooms, the presence of mangrove-specific species certainly shows a decline; only a couple of generalist species seem to profit from such stressful events. The degree of disappearance of ascidians at all four sites in Belize suggests that these organisms may be even more sensitive to algal and cyanobacterial competition, as well as suspended fine sediments, than sponges. We find, both in Belize and in Panama, that two sponge growth forms are highly successful among sponge root occupiers: encrusting and irregularly massive. This observation is in contrast to open reef environments where tubular and ramose forms predominate.

Close monitoring of the abundance and frequencies of key mangrove benthos at specific sites and their correlation with short-term or long-lasting environmental impacts and stress will be a useful tool for assessing mangrove health throughout the Caribbean region in the future.

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Internal Transcribed Spacer 2 (ITS2) Variation in the Gorgonian Coral *Pseudopterogorgia bipinnata* in Belize and Panama

Daniel Dorado and Juan A. Sánchez

ABSTRACT. One of the most intriguing aspects of molecular evolution is the concerted evolution of ribosomal genes, yet the presence of intragenomic rDNA variants is still not well understood. We studied the intragenomic variation of the internal transcribed spacer 2 (ITS2, rDNA) in the gorgonian coral *Pseudopterogorgia bipinnata* (Gorgoniidae: Octocorallia) using a combined approach of denaturing gradient gel electrophoresis (DGGE), DNA sequencing, and RNA secondary structure prediction. We examined intragenomic variants of colonies from Carrie Bow Cay (Belize) and Bocas del Toro (Panama). Despite frequent intragenomic ITS2 variation in *P. bipinnata*, predicted RNA secondary structures exhibited no signs of including pseudogenes and comprised functional copies. Given the low divergence among the ITS2 sequences recovered from DGGE gels, intragenomic variation was restricted to a few mutations that did not compromise the functionality of the ITS2 secondary structure. The presence of common ITS2 intragenomic variants at two distant populations raises new questions such as whether sharing similar copies can be the product of gene flow. Regardless of the limited number of individuals analyzed in this study, the method used here, excising bands from DGGE gels for further amplification and sequencing, examined the reliability of the technique to separate intragenomic variants with up to one nucleotide difference. Studying the intragenomic variation of ITS2 has potential to provide us with information on recent population events such as introgressive hybridization.

INTRODUCTION

Ribosomal DNA (rDNA) intragenomic variation has puzzled molecular systematists and ecologists during the past few years. The rDNA is a multigene family arranged in tandem repeats, frequently achieving several hundreds of repetitions per chromosome. Each repetition is composed of three ribosomal subunits (18s, 5.8s, and 28s), separated by two internal transcribed spacers (ITS1 and ITS2, or ITSs), an external transcribed spacer (ETS), and the non-transcribed intergenic spacers, IGS. The ITS1 and ITS2 spacers form secondary structures that are crucial for ribosomal maturation as well as important for the maturation of the rRNA (Coté and Peculis, 2001). The ITSs are known to have conserved core structures throughout the metazoans (see reviews in Coleman, 2003; Schultz et al., 2005). Changes in the ITS secondary structure are known to produce inhibition of the maturation of rRNA as a consequence of

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coevolution between RNA secondary structures and the processing molecular machinery responsible for its removal (Van Nues et al., 1995). As multicopy genes, the rDNA is assumed to evolve via concerted evolution, resulting in the homogenization of the sequences throughout the genome (Harris and Crandall, 2000; Hillis and Davis, 1988), that is, homogenization of copies through unequal crossing-over and gene conversion processes (Liao, 2000). However, variations within individuals have been reported primarily as a result of slow concerted evolution (Harris and Crandall, 2000; Coté and Peculis, 2001), hybridization, or the presence of pseudogenes (Marquez et al., 2003; Harpke and Peterson, 2006). The latter can appear because of the presence of highly divergent rDNA types in different chromosomes (Arnheim et al., 1980), which retain ancestral rDNA polymorphisms for long periods of time (Marquez et al., 2003). Hybridization phenomena between species per se could increase the rDNA diversity in an individual, but as an additional consequence could result in silencing some rDNA loci by chromatin modifications in a nucleolar dominance process (Chen et al., 1998; Frieman et al., 1999; Muir et al., 2001), which can drive some rDNA loci by neutral selection toward pseudogenes (Muir et al., 2001). However, the presence of ITS2 intragenomic variants is a phenomenon that we do not clearly understand.

Pseudopterogorgia bipinnata Pallas is one of the most abundant shallow-water gorgonian corals in the Caribbean Sea (Bayer, 1961; Sánchez et al., 1997). This species has two particular characteristics: it exhibits large phenotypic plasticity along the depth–wave exposure gradient, and it presents clear intragenomic variation in the ITS2 sequence (Sánchez et al., 2007). Consequently, *P. bipinnata* constitutes an appropriate model species to study the nature and genetics of ribosomal intragenomic variation. In this study we had two main objectives: (1) to isolate sequences of intragenomic ITS2 variants in *P. bipinnata* from populations at Belize and Panama and (2) to examine if intragenomic ITS2 variants were functional copies using predicted RNA secondary structures.

MATERIALS AND METHODS

Samples from *Pseudopterogorgia bipinnata* colonies were obtained by scuba diving at Carrie Bow Cay ($n = 27$), Belize, and Cristobal Island ($n = 11$), Bocas del Toro, Panama. A few *P. bipinnata* from the Bahamas (San Salvador) and Colombia (Bancos de Salmedina, Cartagena), as well as a sequence of *Gorgonia mariae*, were chosen as outgroups. However, there was no a priori information on the genetic distance between western and eastern Caribbean populations. Total DNA was extracted using a cetyltrimethylammonium bromide (CTAB), proteinase K, phenol–chloroform–isoamyl alcohol extraction method (Coffroth et al., 1992); DNA was resuspended and conserved in TE buffer at −70°C; DNA quality was checked in agarose (1%) electrophoresis at 80 V for 30 min. Using the best DNA extraction quality, primers 5.8s 5′-AGCATGTCTGTCTGAGTGTTGG-3′ and 28s 5′-GGGTAATCTTGCCTGATCTGAG-3′, designed by Aguilar and Sánchez (2007), were used for the ITS2 amplification. Conditions for polymerase chain reaction (PCR) were as follows: an initial denaturizing step of 2 min at 94°C; followed by 35 cycles of 30 s at 94°C, 30 s at 56.8°C, and 1 min at 72°C; and a final extension step of 2 min at 72°C; using 1 unit Taq polymerase (Invitrogen), 3.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates (dNTPs; Biorad Mix), 0.15 μM primers (each), and 4 μL DNA (dilution 1/50) in 20 μL as the final volume. The amplification was standardized with an efficiency of 95%. PCR reactions were screened in denaturing gradient gel electrophoresis (DGGE) containing 8% polyacrylamide, 1 × TAE buffer, and a linear urea-formamide denaturing gradient from 45% to 80%. The gels were pre-run at 60°C and 90 V for 30 min, followed by electrophoresis at 60°C and 90 V for 13 h. Gels were stained with ethidium bromide during 15 min and visualized using a BIORAD Chemidoc system. DGGE separates DNA fragments not only by the fragment size but also by the DNA sequence, where GC-richer sequences migrate further independently of small differences in size (Figure 1). All reactions were conducted without a CG-clamp in the primers, which is a 40 bp GC-rich sequence added before the 5′-primer that adds an additional denaturing domain allowing further migration of the DNA before denaturing (LaJeunesse and Pinzon, 2007). In the case of gorgonian corals, there was no need for the GC-clamp owing to the great migration in the DGGE of gorgonian ITS2 sequences, which avoided the problems involved with PCR reaction tailed primers. Bands visualized in the DGGE gel were excised using sterilized micropipette tips in the Bio-Rad Chemidoc system and placed in 0.5 mL tubes with 100 μL sterilized double distilled water. The tubes were incubated in a shaker at room temperature for 24 h at 150 rpm. Each band extract was collected in a 0.5 mL tube and the DNA was precipitated with 300 μL cold absolute ethanol; tubes were placed at −20°C for 24 h and then centrifuged at 13,000 rpm for 30 min; the supernatant was discarded,

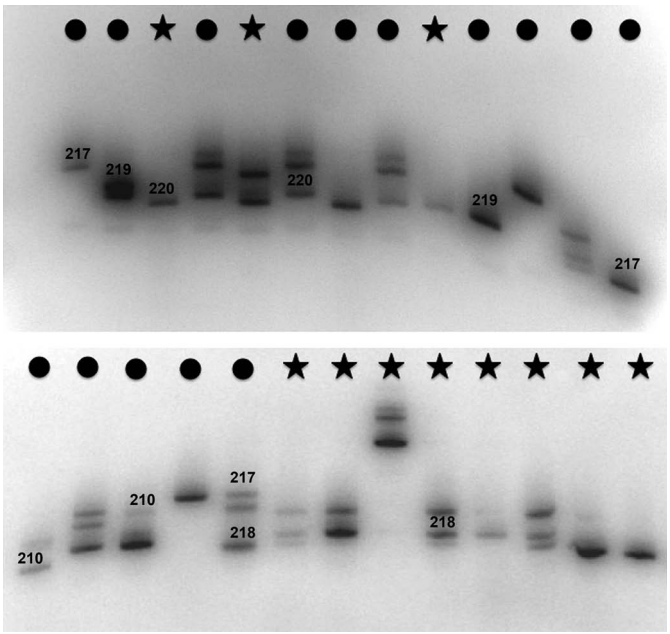

FIGURE 1. Runs (2) of internal transcribed spacer 2 (ITS2) denaturing gradient gel electrophoresis (DGGE) banding patterns from *Pseudopterogorgia bipinnata* colonies from Panama (Bocas del Toro; stars) and Belize (Carrie Bow Cay; circles). Numbers correspond to the sequence size when available. The gels have a common artifact in the form of a "smile" (more accentuated in the upper gel), where lateral wells tend to migrate slightly further because of the pressure acting on the gel edges.

and the pellet was dried and resuspended in 15 μL sterilized double distilled water.

Reamplification of bands was conducted using PCR as just described, using the same set of primers, except that DNA was used without dilution and the annealing temperature was raised a few degrees to increase specificity. Purification of PCR products for sequencing was performed by the Exo-SAP (Exonuclease 1 and shrimp alkaline phosphatase) method using 1 unit Exonuclease, 0.2 units shrimp alkaline phosphatase, and 2 μL SAP buffer 10× per 20 μL in a 0.2 mL tube. Reactions were held at 37°C for 1 h and at 80°C for 15 min. Sequencing reactions were performed with the BigDye 3.1 system according to the manufacturer's instructions (Applied Biosystems) and sequenced in a capillary electrophoresis automated sequencer (ABI310). Each sample was sequenced with forward and reverse primers. The consensus sequences were obtained by assembling the two complementary electrophenograms in Sequencer 4.7 software.

Secondary structures of all sequences were obtained by reconstructing by comparison via Pairwise Alignment (Bioedit) with previously reported structures in octocorals (Aguilar and Sánchez, 2007). The sequences were then submitted with a few constraints and restrictions in MFOLD at a default temperature of 37°C (Zuker, 2003). Constraints force bases to be double stranded whereas restrictions cause them to be single stranded, which are chosen depending on the sequence homology between the sequences with known structure against each problem sequence without known structure. A good example for a constraint are the two complementary sequences that make a stem; an example of a restriction is a string of free nucleotides between helices or any kind of loop. The structure chosen was the one with the greater negative free energy but conserving the ring model known for ITS2. The obtained secondary structures were used to construct a matrix for cladistic analysis as described by Aguilar and Sánchez (2007). Phylogenetic analyses included maximum parsimony and maximum likelihood as well a Bayesian inference for a combined sequence-molecular morphometric analysis (see details in Grajales et al., 2007).

RESULTS

Denaturing gradient gel electrophoresis (DGGE) analysis revealed that most individuals from Belize and Panama contained intragenomic variants of ITS2 (see Figure 1). There were as many as three different bands per individual that were similar or nearly equal in length because of their closeness in the DGGE gel (Figure 2). Some banding patterns were identical for individuals from both Belize and Panama, which indicated exact ITS2 copies, although some patterns unique to each population were also observed (see Figure 1). Great effort was made to obtain sequences from most bands, but not all of them were successfully recovered. The sequences had an average GC content of 55.6%, which afforded the great migration of intragenomic ITS2 variants in the DGGE. The sequences from *P. bipinnata* had more than 85.6% of sequence similarity, contrasting with just 48% with respect to *G. mariae*.

Predicted secondary structures from all excised bands exhibited functional structures with the conserved six helicoidal ring model previously reported for octocorals (Aguilar and Sánchez, 2007), but great variability was observed in the length and complexity of each stem and spacer (Figures 2, 3). Intragenomic differences were frequently discrete changes that did not affect the predicted secondary

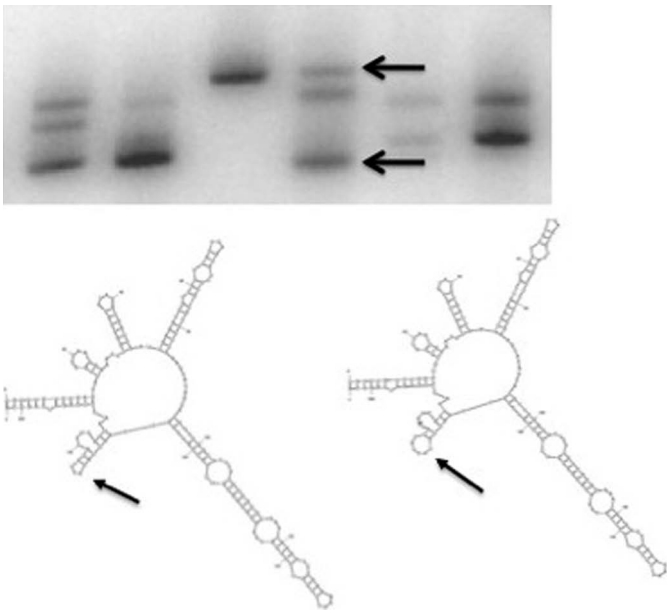

FIGURE 2. Two different intragenomic ITS2 variants from an individual colony of *Pseudopterogorgia bipinnata* from Belize. The variants were excised from the two bands indicated by arrows in the DGGE gel above, reamplified, and sequenced. The arrows below show the differences between the two predicted RNA secondary structures corresponding to one INDEL (insertion or deletion) only.

structures (see Figure 2). The ITS2 in *P. bipinnata* from Panama and Belize varied from 212 to 224 nucleotides (Figure 3). In general, the stems 2, 3, and 6 were shorter than the stems 4 and 5, with stem 5 being the longest. Multiple internal loops were frequent in stems 3, 4, and 5, with more nucleotides (nt) on stem 5, where up to six internal loops were observed (Figure 3). The spacers were often short, ranging from 1 to 4 nt. Spacer '1i' showed a conserved sequence, UG, with little variation across individuals, while spacer '4i' was the longest, with 4 to 12 nt and a conserved core sequence (AGUNCAGC) observed in most of individuals (Figure 3). Phylogenetic results from sequence and alignments or combined data sets, including 11 excised bands from individuals from Belize and 3 from Panama, showed little divergence between Panama and Belize despite the long distance with respect to a few individuals from Bahamas and Colombia (Figure 4). Very modest support was found within individuals from Panama or Belize, and no particular grouping could be discerned (data not shown). In addition, no particular features of the ITS2 secondary structure as seen with helix 5, which showed the largest number of characters, were supporting any particular clade or group of individuals (Figure 4).

DISCUSSION

The intragenomic ITS2 variation in *Pseudopterogorgia bipinnata* individuals involved functional copies, as corroborated by reconstructing their predicted RNA secondary structures. Given the low divergence among the ITS2 sequences recovered from DGGE gels, intragenomic variation was restricted to a few mutations that did not compromise the functionality of the ITS2 secondary structure. Despite frequent intragenomic ITS2 variation in *P. bipinnata*, predicted RNA secondary structures exhibited no signs of including pseudogenes or structural degeneration. Having in mind that the ITS2 secondary structure has a major role in the maturation of the ribosomal RNA (Coté and Peculis, 2001), little tolerance of changes is expected as a result of the restrictions imposed by the ITS2 splicing machinery (Van Nues et al., 1995; Coleman, 2003); this means purifying selection is acting on secondary structural constraints (Coté and Peculis, 2001) or concerted evolution mechanisms are acting similarly (Liao, 2000; but see Nei and Rooney, 2005; Harpke and Peterson, 2006). Similarly, compensatory base changes (CBC), occurring at the stem regions, are very unlikely to occur at the intraspecific level (Müller et al., 2007). Thus, it is expected that only variants or alleles carrying only minor changes occur, which was evident with the functionality of co-occurring secondary structures found at the intraspecific level.

ITS intragenomic variation has been also observed in scleractinian corals. Van Oppen et al. (2001) examined diverse nuclear and mitochondrial DNA sequences, concluding that paraphyly from intragenomic ITS copies could be explained by extensive introgressive hybridization and reticulate evolution. Similarly, Marquez et al. (2003) found the presence of ribosomal pseudogenes as a possible consequence of multiple hybridization events. However, Vollmer and Palumbi (2004) examined the multiple copies of the Caribbean *Acropora* species and concluded that there is no proper way to evaluate if the intragenomic shared variation of genes such as ITS1 and ITS2 was the result of incomplete lineage sorting or recent hybridization processes. Nonetheless, all the studies mentioned studied the intragenomic variation of ITS using the DGGE technique, and it is clear that traditional cloning methods overestimate the intragenomic diversity (LaJeunesse and Pinzon, 2007).

Regardless of the limited number of individuals analyzed in this study, the method used here, excising bands from DGGE gels for further amplification and sequencing, probed its reliability to separate intragenomic variants up to one nucleotide difference (see Figure 2). DGGE is a

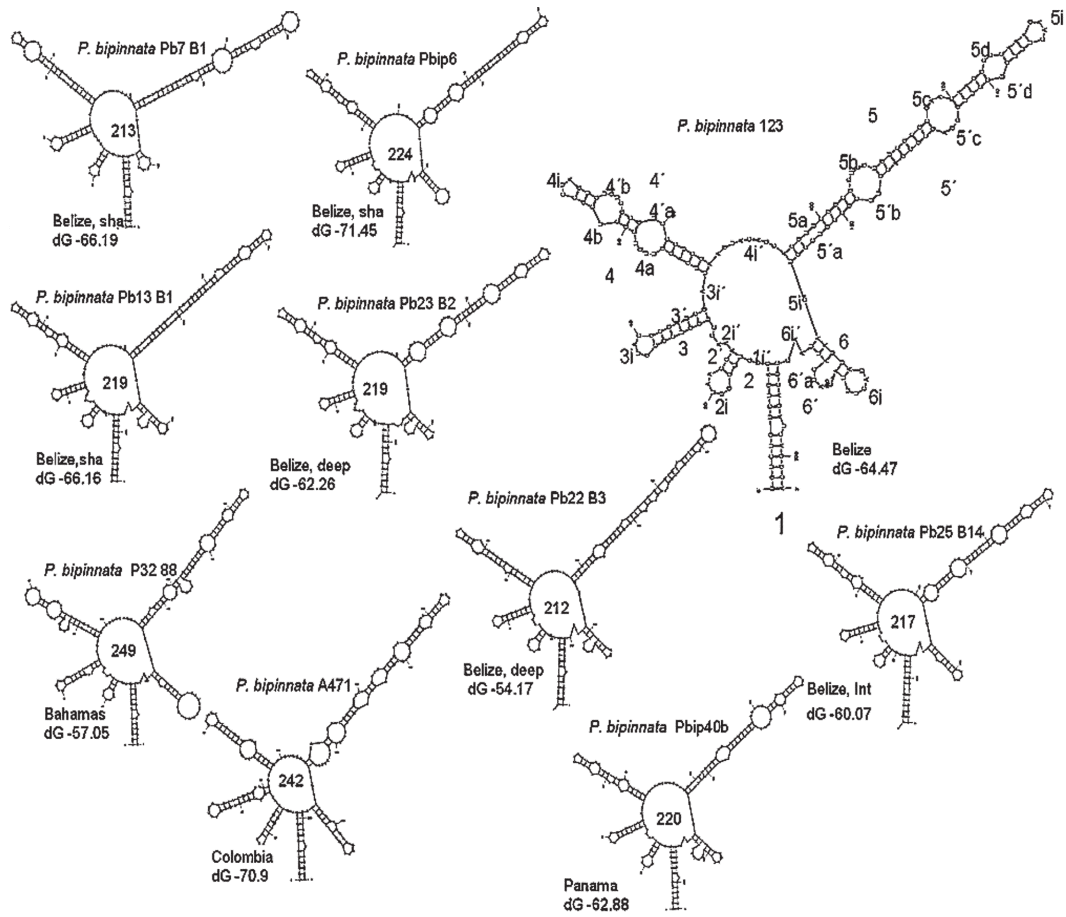

FIGURE 3. Predicted ITS2 RNA secondary structures in *Pseudopterogorgia (P.) bipinnata*. The upper right structure shows the characters for the molecular morphometrics analysis used in the combined Bayesian inference analysis. The number within the ring structure refers to the total number of nucleotides at each structure.

method useful to detect the most prevalent intragenomic variants of ribosomal genes, whereas traditional methods to screen intragenomic variation such as cloning miscalculate the codominance of the different copies (LaJeunesse and Pinzon, 2007; Thornhill et al., 2007). There are two main approaches for depicting the nature of intragenomic ITS2 variants in octocorals. One method is to study in detail the genetics of the different ITS2 variants by crossing individuals with different intragenomic patterns, which can provide inheritance information and linkage disequilibrium configurations. An alternative method includes techniques such as reverse transcription (RT)-PCR and quantitative real-time PCR, which can offer more accurate information on the functionality of the different intragenomic ITS2 copies. The RT-PCR technique can filter copies that are not expressed in the cell, and quantitative PCR can quantify the amount of ITS transcripts from each particular copy. These methods could also test if the intensity of bands in DGGE actually corresponds to the number of copies of a particular intragenomic variant.

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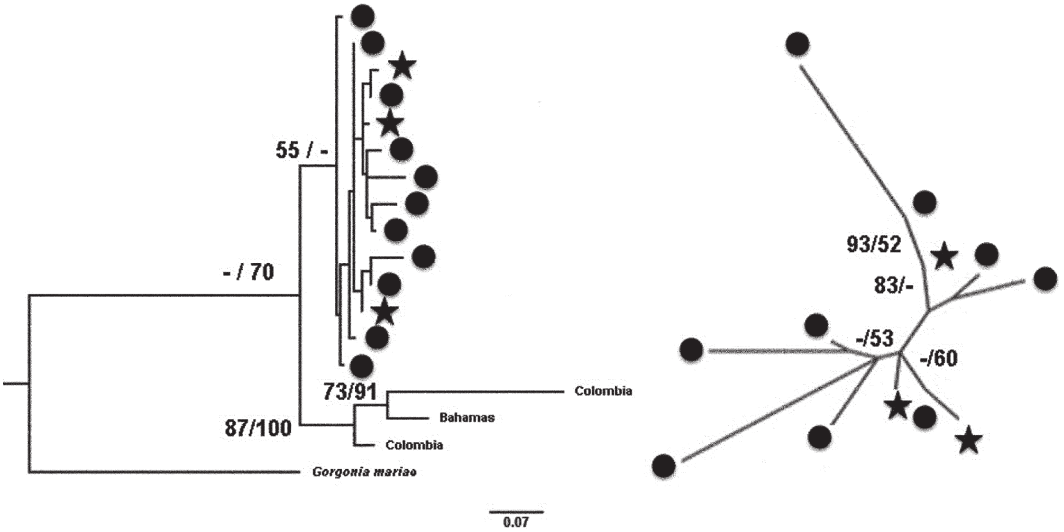

FIGURE 4. Maximum-likelihood phylograms show above-node support from the combined sequence-molecular morphometrics Bayesian analysis (left) and maximum-parsimony bootstrapping (1000 replicates, right): *Pseudopterogorgia bipinnata* colonies from Panama (Bocas del Toro; stars) and Belize (Carrie Bow Cay; circles). The tree at the right is a radial representation of a set of terminal branches corresponding to Panama and Belize sequences pruned from the left tree.

Cairns, BIOMMAR colleagues, and the Smithsonian Station at Carrie Bow Cay, Belize. The Minister of Environment, Household and Territorial Development of Colombia granted access to genetic resources to JAS for the DNA analyses included in this paper (Contract 007, resolution 634; 14 March 2007). This work is contribution number 841 of the Caribbean Coral Reef Ecosystems Program (CCRE), Smithsonian Institution, supported in part by the Hunterdon Oceanographic Research Fund.

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Obvious Invaders and Overlooked Infauna: Unexpected Constituents of the Decapod Crustacean Fauna at Twin Cays, Belize

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ABSTRACT. Decapod crustaceans in the vicinity of Carrie Bow Cay and Twin Cays, Belize, have been under study for more than 25 years. Large collections have been assembled, and new species have been discovered. The effort has included photographic documentation of coloration, yielding characters of value in identification of problematic tropical taxa. Measurements of diversity have been markedly enhanced by extraction corer (yabby pump) sampling in shallow subtidal sediments, especially at Twin Cays. This technique revealed species, genera, and families of thalassinidean decapods not previously known from the region. Studies continue on the ecological roles of these burrowers, dominant bioturbators in seagrass beds where they produce conspicuous mounds of sediment and constitute a major infaunal biomass at Twin Cays. By contrast, familiar large reptant decapods typically dominate shallow rocky substrates. Within the past four years, however, the nonindigenous portunid crab *Charybdis hellerii* has extensively invaded large portions of hard substrates at Twin Cays. In 2007, it was found to dominate cavities under coral heads in survey areas along the northeastern and southwestern shorelines, possibly displacing populations of large *Mithrax*, *Menippe*, *Callinectes*, and *Panulirus* previously found there in abundance.

INTRODUCTION

Fieldwork centered on Carrie Bow Cay and surrounding habitats, including a variety of settings at Twin Cays. The effort continues work by the first author in collaboration with the late Ray Manning in 1983, as well as work by the late Brian Kensley during the 1980s and early 1990s (Kensley, 1981, 1996). Early efforts produced abundant grass-bed and reef-crest species generally identifiable with known Caribbean taxa, along with small cryptic forms obtained by cutting open sponges, breaking rubble, poisoning in situ, or using several narcotants to drive out small decapods from rubble isolated in containers. Rich collections that have accumulated in the the Smithsonian Institution's National Museum of Natural History were fixed in formalin, limiting their value in genetic analyses. Efforts in 2002 and 2007 shifted emphasis to varied intertidal and subtidal habitats of Twin Cays and to resampling the regional fauna to obtain alcohol-fixed materials for molecular genetic analyses.

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Concerted effort has been made to photographically document coloration of fresh specimens, given the value of color in the identifications of tropical species and the long-term goal of producing a guidebook for the regional decapod fauna (DLF and RL, in progress). More than 260 decapod species have been enumerated in our collections from the Carrie Bow Cay region, some yet to be named. Under U.S. National Science Foundation AToL "Decapod Tree of Life" support, molecular and morphological systematic studies are under way concerning alpheid and other caridean shrimps, paguroid hermit crabs, thalassinidean shrimps, and panopeid, portunid, grapsoid, pinnotherid, and majoid crabs, as well as family-level relationships among all major decapod groups. Work incorporating porcelain crab collections from the region has been published (Rodríguez et al., 2005, 2006) as has work by other investigators on some alpheid shrimp groups (Duffy, 1996; Duffy and Macdonald, 1999; Duffy et al., 2000, 2002; Macdonald et al., 2006; Ríos and Duffy, 2007). Previous collections of upogebiid thalassinidean shrimp from Belize were included in Williams (1993). Several descriptions of new species from our Belize collections have also appeared (Goy and Felder, 1988; Manning and Felder, 1996; Felder and Manning, 1997), but many species remain to be described. The second author has been involved in several ecological studies of the infaunal decapods of the region (Dworschak and Ott, 1993; Abed-Navandi and Dworschak, 2005; Dworschak et al., 2006).

Our protracted field sampling program has in some cases allowed us to observe apparent changes in community composition. In a striking example, shallow subtidal habitats at Twin Cays have been recently invaded by the nonindigenous swimming crab *Charybdis hellerii* (A. Milne-Edwards, 1867), previously unreported from Belize. Recurrent trips have also provided opportunities for shallow subtidal sampling and burrow-casting of fossorial infauna in turtle grass (*Thalassia*) beds along shorelines of Twin Cays, revealing unexpected thalassinidean diversity. A brief account of these latest efforts is our present focus, preliminary to more comprehensive treatment of the full decapod assemblage.

MATERIALS AND METHODS

Sampling included the breaking of dead coral and conch shell rubble, netting, extraction of sediments, and sorting through hard-surface fouling organisms, but sampling of large crabs such as *Charybdis hellerii* (Brachyura) and its macrocrustacean associates was a targeted effort. These decapods were captured from under pieces of dead subtidal coral and debris that were lifted while snorkeling over and adjacent to seagrass (*Thalassia*) beds in water 1–2 m deep. Sampling of most thalassinideans and related decapod burrowers was accomplished with a suction extractor (yabby pump) and bag-sieve while wading, snorkeling, or SCUBA diving in water 0.5–4 m deep. In addition to collections of *Glypturus acanthochirus* (Callianassidae) by suction extractor, some specimens of this species were obtained with "weighted line" traps (de Vaugelas, 1985). Specimens of *Axiopsis serratifrons* (Axiidae) were obtained by baiting animals to the apertures of their burrows, where they were captured by cutting off the burrow or by spearing the specimens. Casts of burrows were made as described by Dworschak and Ott (1993). Specimens were immobilized by immersion in chilled seawater or by narcotization with clove oil before photography. Photographs of specimens immersed in a pan of seawater were made with a Fuji Fine Pix S1Pro digital camera equipped with a 60 mm macrolens while the subject was lighted by a combination of direct and reflected sunlight or high-intensity 5000°K fluorescent photographic lamps. All specimens were subsequently preserved in several exchanges of 95% nondenatured ethanol and then stored in 75% nondenatured ethanol. Photographic voucher specimens were archived in the Zoological Collections of the University of Louisiana at Lafayette (ULLZ), and most other materials were deposited in the Smithsonian Institution–National Museum of Natural History (USNM). Some collections by the second author (especially thalassinideans) were deposited in the Naturhistorisches Museum in Wien, Austria (NHMW) and the Muséum National d'Histoire Naturelle, Paris, France (MNHN). For figured specimens, size is indicated as carapace width (cw) or carapace length (cl).

RESULTS AND DISCUSSION

Invasion by *Charybdis hellerii*

Large bottom debris (waterlogged wood, discarded building materials, dead coral heads) typically provides cover for large reptants such as spiny lobsters (*Panulirus* spp.), swimming crabs (*Callinectes* spp.), stone crabs (*Menippe* spp.), and large spider crabs (*Mithrax* spp.), especially in shallow well-lighted waters. Sampling of these environments at both Carrie Bow Cay and Twin Cays in October 2002 revealed no large decapods other than these genera. That same year, however, a small specimen of the

nonindigenous portunid crab *Charybdis hellerii* was found in an empty conch shell on the inshore side of Carrie Bow Cay, the first such occurrence recorded in our sampling program.

In April 2007, sampling under large pieces of cover at Twin Cays was undertaken to obtain fresh materials of the aforementioned resident genera for genetic analyses. Initial sampling centered in the vicinity of the "Fisheries Camp" on the southeastern end of Twin Cays, where a storm had scattered sheets of metal building siding from the shoreline to depths of nearly 2 m. Inspections beneath 20 such sheets across this entire range of depths revealed none of the target species but at least seven variously sized individuals of the nonindigenous swimming crab *C. hellerii*.

Sampling was thereafter shifted to dead coral heads scattered among turtle grass beds on the northeast side of Twin Cays. A crude survey was there undertaken for coral heads in 1–1.5 m depths, each head roughly 0.5–0.7 m in diameter and separated from one another by roughly 6–15 m. Of the 25 coral heads inspected, 13 were uninhabited by large reptant decapods, 8 harbored large specimens of *C. hellerii* (Figure 1b), and four harbored only *Menippe nodifrons* Stimpson, 1859 (Figure 1a). Large single individuals of *C. hellerii* were found under 6 of the 25 heads that were lifted, a mating pair of *C. hellerii* was found under a single head, and a specimen of *C. hellerii* together with a large specimen of *M. nodifrons* was found under another head. No specimens of *Mithrax* spp., *Callinectes* spp.,

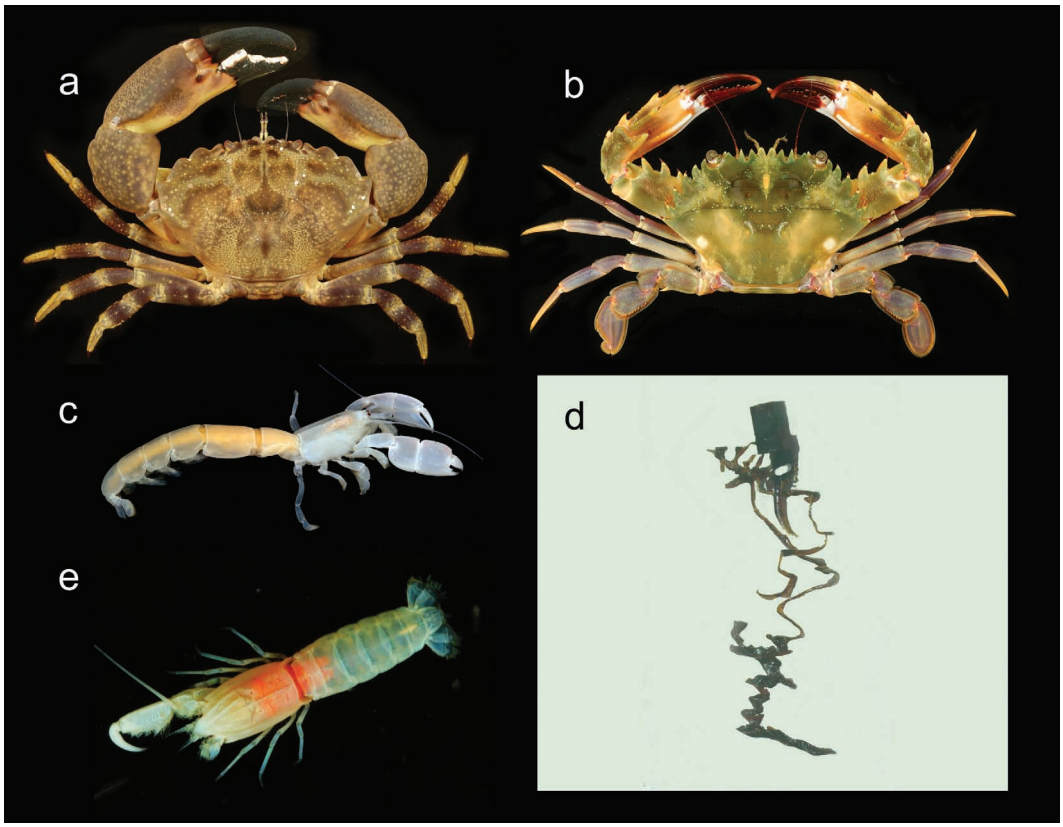

FIGURE 1. a, Stone crab *Menippe nodifrons*, male, 69.7 mm carapace width (cw), Twin Cays, Belize 10 April 2007, ULLZ 8991. b, Invasive Indo-Pacific swimming crab *Charybdis hellerii*, male, 75.3 mm cw, Twin Cays, Belize, 10 April 2007, ULLZ 8990. c, Callianassid *Eucalliax* sp., female, 8.3 mm carapace length (cl), South Water Cay, 22 October 2002, ULLZ 9230. d, Polyester resin burrow cast from Twin Cays, probably assignable to *Axianassa australis*, cast length 85 cm, made by PCD, Twin Cays, Belize, August 1989, NHMW 24001. e, Laomediid *Naushonia* sp. female, 5.8 mm cl, Carrie Bow Cay, Belize, 3 April 2007, ULLZ 8895. ULLZ, University of Louisiana at Lafayette; NHMW, Naturhistorisches Museum Wien. Photographs a–c and e by DLF; photograph d by PCD.

or *Panulirus* spp. were observed, despite these taxa being commonly found in such settings during 1983 and 2002.

Small or immature specimens of *Charybdis hellerii* are easily confused with *Cronius ruber* (Lamarck, 1818) and to a lesser extent with *Achelous tumidulus* Stimpson, 1871, both of which also occur in Belize and adjacent waters of the Caribbean, Gulf of Mexico, and other areas of the warm temperate Atlantic. This similarity led us to initially question the identity of the single small specimen collected in 2002, but it was confirmed to be *Charybdis hellerii* by 16S mtDNA sequence analysis by comparing to other sequence data for the species (Robles et al., 2007; Mantelatto et al., 2009). Widely used diagnostic morphological characters that apply well to full-sized adults do not readily facilitate identification of juveniles among these three species, and records of subadults could easily be in error if based on presently limited descriptions. At the very least, *A. tumidulus* differs from both *C. ruber* and *Charybdis hellerii* by lacking a striking posterior or posterodistal meral spine on the fifth pereiopod (swimming leg) in all crab stages. *Cronius ruber* and *Charybdis hellerii*, however, share a strongly spined fifth pereiopod, albeit with the spine usually occupying a relatively more distal position and being less posteriorly directed on the merus of *Cronius ruber*. The relative position of the spine is, however, difficult to distinguish in small juveniles. These two species also share the presence of small spinules bordering the posterior margin of the fifth pereiopod propodus, although these spinules are of relatively larger size in *Charybdis hellerii*. This characteristic is readily evident in adults, where setation obscures small acute granules along the margins of the propodus in *Cronius ruber*, which are unlikely to be confused with the well-formed adult spinules of *Charybdis hellerii* (see Figure 1b). In juveniles of *Cronius ruber*, microspination of this propodal margin is relatively stronger than in adults, and distinction from juveniles of *Charybdis hellerii* is somewhat subjective, especially if one lacks comparative specimens of similar size. No feature in the carapace of early crab stages (Dineen et al., 2001: fig. 24) appears to separate small individuals of these species.

Recent observations have revealed an ongoing invasion of *C. hellerii* into coastal western Atlantic locations, and its documented distribution must now include Belize along with Brazil, Venezuela, Colombia, Cuba, the Yucatán shelf of Mexico, both coasts of Florida, and other northern Atlantic U.S. coastal habitats through at least the Carolinas (Campos and Türkay, 1989; Gómez and Martínez-Iglesias, 1990; Hernández and Bolaños, 1995; Lemaitre, 1995; Calado, 1996; Mantelatto and Dias, 1999; Dineen et al., 2001; Mantelatto and Garcia, 2001; Mantelatto et al., 2007; Robles et al., 2007; McMillen-Jackson, 2008; Felder et al., 2009). Clearly, the foregoing chronology of reports reveals continued western Atlantic range expansion for *C. hellerii*, although the potential trophic impacts of this invader remain poorly documented. The first author has on two occasions observed individuals of *C. hellerii* in the Indian River Lagoon, Florida, feeding (inside shallow cavities of hard substrates that they occupied) on soft-shelled, postmolt individuals of native species of large decapods (one *Callinectes*, one *Panulirus*), and in another instance feeding on small mussels. As in the present report, all such observations and inferences of this invader's potential competitive and predatory impacts in the western Atlantic remain very limited and anecdotal, but they serve to justify a call for controlled experimental studies.

Thalassinideans

Our collections of cryptic burrowing thalassinideans from various habitats in the vicinity of Carrie Bow and Twin Cays, along with the few previously reported records, include at least 17 species representing the families Callianassidae, Laomediidae, Thomassiniidae, Axianassidae, Axiidae, and Upogebiidae. The species of these often overlooked groups are presented in the following list, with collection sites indicated as TC (Twin Cays), CB (Carrie Bow Cay), SW (South Water Cay), and SL (shorelines near Dangriga); catalogue numbers are shown for archived specimens.

Infraorder Thalassinidea *sensu lato*

Callianassidae (Ghost Shrimps)

Corallianassa longiventris (A. Milne-Edwards, 1870)—TC, CB: NHMW 6774, 6775, 15352–15355; ULLZ 4228–4230, 6083, 8997.

Eucalliax sp.—TC, SW: ULLZ 9230.

Glypturus acanthochirus Stimpson, 1866—TC, CB: NHMW 6765–6770, 15338–15342; MNHN Th 1181, Th 1185; ULLZ 8993–8995, 9233; USNM 266241–266244.

Lepidophthalmus richardi Felder and Manning, 1997—SL [near river mouths]: NHMW 15343–15349: ULLZ 3577, 5186–5188, 8992; USNM 277777–277779.

Neocallichirus grandimana (Gibbes, 1850)—TC, CB, SW: NHMW 6796–6799, 15356–15367; MNHN Th

1182–1184; ULLZ 8998, 9235–9237, 9239–9241, 9243, 9244.

Neocallichirus maryae Karasawa, 2004—TC: ULLZ 9234, 9238.

Laomediidae

Naushonia sp.—CB: ULLZ 8895, 8915.

Axianassidae

Axianassa australis Rodrigues and Shimizu, 1992—TC [identified by burrow cast]: NHMW 24001.

Thomassiniidae

Mictaxius thalassicola Kensley and Heard, 1991—TC: ULLZ 9246.

Upogebiidae (Mud Shrimps)

Pomatogebia operculata (Schmitt, 1924—CB: ULLZ 9231.

Upogebia acanthura (Coêlho, 1973)—?CB: USNM 251246.

Upogebia omissa Gomes Corrêa, 1968—TC, SL: ULLZ 5165.

Upogebia sp.—CB: ULLZ 9232.

Axiidae (Lobster Shrimps)

Axiopsis serratifrons (A. Milne-Edwards, 1873)—CB: NHMW 6771–6773, 15350–15351: ULLZ 4232, 4233, 5827, 8996; USNM 18905, 18907, 18908.

Coralaxius nodulosus (Meinert, 1877)—CB: USNM 170856, 171764–171766, 243431–243434.

Paraxiopsis hispida Kensley, 1996—CB: USNM 211462.

Paraxiopsis spinipleura Kensley, 1996—CB: USNM 211451.

Sediments in lower intertidal to subtidal seagrass beds at Twin Cays are densely populated by *Neocallichirus grandimana*, *Glypturus acanthochirus*, *Corallianassa longiventris*, *Neocallichirus maryae*, *Mictaxius thalassicola*, and *Eucalliax* sp., often burrowing more than 1 m into sediments. Dworschak and Ott (1993) previously analyzed burrow morphologies and distributions for three of these species, as well as for *Axiopsis serratifrons* and two species of pistol shrimp. Their food sources were investigated by stable isotope studies (Abed-Navandi and Dworschak, 2005). Among the species from Twin Cays, *M. thalassicola* has not previously been reported from the northern Caribbean, and *Eucalliax* sp. (Figure 1c) represents an undescribed taxon presently known only from Belize.

The newly reported *Neocallichirus maryae* is a replacement name for the more familiar *N. rathbunae* (Schmitt, 1935), which proved to be a junior primary homonym of a different fossil species (Karasawa, 2004). Although Sakai (2005) placed *N. raymanningi* Blanco Rambla and Lemaitre, 1999, in synonymy with *N. rathbunae* (Schmitt, 1935), and *N. raymanningi* would thus predate recent establishment of *N. maryae*, we do not accept the presently limited evidence for this synonymy.

Ejecta from burrows of these thalassinideans dominates bottom topography in intertidal to shallow subtidal seagrass beds of this area, but along intertidal muddy shorelines at Twin Cays, especially those immediately adjacent to mangroves, it appears that the axianassid *Axianassa australis* also occurs. As no specimens have been captured, this can be deduced only from highly characteristic ejecta patterns and spiraled burrow casts (see Dworschak and Rodrigues, 1997; Felder, 2001), the latter obtained by the second author in 1989 (Figure 1d).

Neocallichirus grandimana appears to be the most widely distributed callianassid among sites sampled in the vicinity, inhabiting both vegetated and nonvegetated sediments. Together with *Eucalliax* sp., it densely populates sparsely vegetated calcareous sands of shallow shoals bordering South Water Cay in addition to sites at Twin Cays. At South Water Cay, upper reaches of its burrows are commonly inhabited by *Processa* sp. and early juvenile stages of *Callinectes* sp., the latter being uniquely pigmented an opaque bluish-black. *Glypturus acanthochirus* and *Corallianassa longiventris* range into deeper grass beds, where they appear to draw grass blades into their burrows. Distributions of all the collected thalassinideans depend on sediment characteristics, depths, vegetation, and water quality, whereas characteristic burrow architectures are both diagnostic of species and suggestive of ecological adaptations (Dworschak and Ott, 1993, Abed-Navandi and Dworschak, 2005; Dworschak et al., 2006). Less conspicuous evidence of sediment ejecta characterizes areas among seagrasses that are burrowed primarily by nonthalassinidean decapods such as the *Alpheus* spp. reported by Dworschak and Ott (1993). Surface features of these burrows can be all

but indistinguishable from those made by what appear to be several species of *Upogebia*, including *U. omissa*.

The assemblage of upogebiids in the Carrie Bow Cay region remains poorly understood. It appears that *Upogebia omissa* ranges widely here, from the shoreline along the mainland to offshore cays, and the first author has identified specimens taken as "pests" from commercial penaeid shrimp farms on the mainland. General treatment of western Atlantic upogebiids by Williams (1993) included records of *U. acanthura* from a patch reef southwest of Carrie Bow Cay and *U. brasiliensis* Holthuis, 1956 from more distant shoreline areas of Belize, although our collections have produced no additional specimens. Two other species listed by Williams (1993) from nearby coastal environments of Quintana Roo (*U. corallifora* Williams and Scott, 1989 and *U. vasquezi* Ngoc-Ho, 1989) could also be expected in Belize, although we have yet to find them. Specimens of this genus from coralline rubble just off the reef crest at Carrie Bow Cay (ULLZ 9232) and other uncatalogued specimens from Twin Cays (in areas also burrowed by alpheid shrimp) cannot confidently be assigned to known species and warrant further study. Generally found in deeper subtidal habitats (Felder et al., in press), the upogebiid *Pomatogebia operculata* ranges into waters as shallow as 2 m depth off Carrie Bow Cay and likely occurs elsewhere between cays in appropriate deeper calcareous rubble habitats; these have been collected by breaking open highly eroded pieces of coralline rubble to expose the muddy interstices and cavities occupied by this upogebiid.

Axiids are also found in association with rubble and reef structures of outer cays, as, for example, at Carrie Bow. The widely distributed *Coralaxius nodulosus*, a small-sized species inhabiting cavities in subtidal coralline rubble from the fore-reef (see also Kensley, 1994), is routinely found along with the upogebiid *Pomatogebia operculata* in interstices of broken rubble retrieved from depths greater than 2 m. By contrast, the large and strongly armed *Axiopsis serratifrons* is widely distributed between pieces of coarse coral rubble in back-reef flats of Carrie Bow (0.5–2 m depths), there positioned to ambush prey from its somewhat concealed burrow aperture. In addition, two new species of *Paraxiopsis* described by Kensley (1996) both range into reef habitats of Carrie Bow Cay. Although *P. spinipleura* was originally found there in shallow (1.5 m) back-reef rubble, we have not encountered additional specimens. We have also not found additional materials of *P. hispidus*, previously collected at the reef drop-off in depths greater than 20 m.

A remarkable thalassinidean find at Carrie Bow was the April 2007 discovery of a laomediid assignable to *Naushonia* sp. (Figure 1e). Two specimens were captured, both from cavities of empty conch shells in shallow (<1.5 m) subtidal waters. These individuals appear to also represent an undescribed species of a rarely encountered genus in the northern Caribbean region. To date known only from Carrie Bow Cay, they are currently being described.

The thalassinidean fauna of the general region also includes an abundant nearshore species, *Lepidophthalmus richardi*, adapted to euryhaline waters and muddy sand shorelines of the Stann Creek District (Felder and Manning, 1997). This species has not been found in habitats immediately associated with Twin Cays or Carrie Bow Cay, despite intensive search.

These collections have allowed us to update and expand the burrow distribution schemes for Belize given by Dworschak and Ott (1993). We herewith add additional taxa and habitat distributions (Figure 2) to underscore the overlooked diversity of infaunal macrocrustaceans, some of which are dominant bioturbators.

Note Added in Press

Additional sampling in Belize was conducted in February 2009. Observations in shallow waters at Twin Cays confirmed that populations of *Charybdis helleri* remained as seen in 2007. Further sampling for thalassinideans supported accounts on the preceding pages, with noteworthy additions. Sampling among shoreline mangrove roots at Twin Cays produced the first specimens of the Axianassidae, representing new records for *Axianassa intermedia* Schmitt, 1924. Five such specimens were extracted by yabby pump from beneath a surface area of no more than 0.25 m^2 at low tide, but less productive adjacent sampling suggested heterogeneous patterning. Given the small size of these specimens, we question whether this species accounts for burrows provisionally attributed to *A. australis* on the basis of castings mentioned on the preceding pages. From these same habitats at Twin Cays, the first specimen of the callianassid *Biffarius fragilis* (Biffar, 1970) was captured, along with a specimen of the same *Naushonia* sp. reported from Carrie Bow Cay on preceding pages. Finally, the first specimen of the family Callianideidae, *Callianidea laevicauda* Grill, 1959, was taken from intertidal rubble of the exposed reef crest at Carrie Bow Cay. These latest efforts confirm presence of at least one species of the family Axianassidae, add a seventh thalassinidean family to our report, and bring the documented number of thalassinidean species in our survey to at least 19.

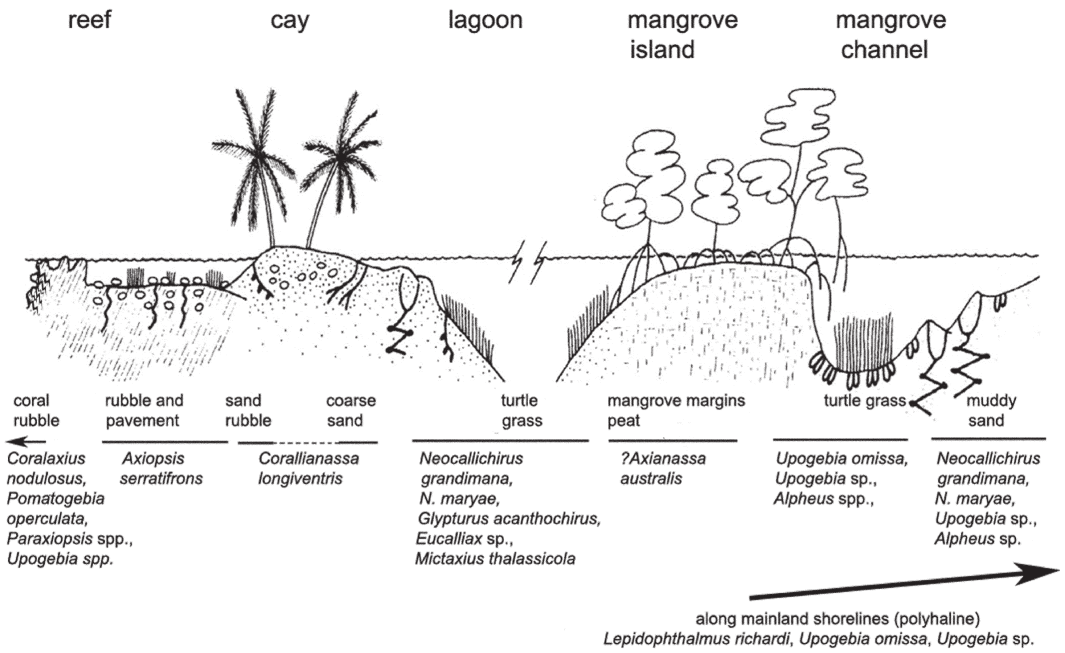

FIGURE 2. Schematic of thalassinidean distributions in channel and back-reef environments near Carrie Bow Cay and Twin Cays, Belize. Modified from Dworschak and Ott (1993:fig. 9).

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Imposex in One of the World's Busiest Shipping Zones

Carter Li and Rachel Collin

ABSTRACT. Tributyltin pollution from antifouling paint is well known to disrupt the endocrine system in female marine gastropods. The masculinization of females, including the aberrant growth of a penis and vas deferens and occlusion of the capsule gland, has been reported primarily in neogastropods and is particularly well documented in muricids. Compared to temperate areas, few studies of imposex have been undertaken in the tropics, and there are few studies in general on non-neogastropods. Here we report a high frequency of imposex near the Pacific mouth of the Panama Canal in two species of muricids and two species of calyptraeids. The frequency of imposex declined rapidly with distance away from the canal, and several species appeared to be mostly normal less than 10 km from the entrance. This is the first report of imposex in *Acanthais brevidentata*, *Thaisella kiosquiformis*, *Bostrycapulus calyptraeformis*, *Crepidula* cf. *nivea*, and *Anachis fluctuata*. Because imposex has not previously been reported for the Calyptraeidae, a family of protandrous gastropods, a laboratory study was conducted to verify that imposex was not simply retention of the penis after sex change. The 2007 ratification of the International Maritime Organization's convention on antifouling systems should reduce the levels of TBT worldwide, but the persistence of this compound in sediments suggests that imposex may continue to be a problem at the mouth of the canal as routine dredging and large tides frequently resuspend sediment.

INTRODUCTION

Tributyltin (TBT) is well known to be a highly effective antifouling agent, used primarily on ship hulls, but it has numerous detrimental effects on a wide variety of non-target taxa. Despite having demonstrable effects on molluscan shell growth (Alzieu et al., 1981), embryological development of fish and marine invertebrates (Hano et al., 2007; Inoue et al., 2006), neurulation in ascidians (Dolcemascolo et al., 2005), and testosterone metabolism in mysids (Verslycke et al., 2003), the most well studied and widespread effect is the disruption of the endocrine system in marine gastropods. Exposure to very low levels (as little as 0.5 ng/L) of TBT causes the masculinization of females, including the aberrant growth of a penis and occlusion of the capsule gland (Gibbs and Bryan, 1996). This condition is referred to as imposex, and severe cases can lead to reproductive failure. For example, an extreme case of population decline as a result of imposex has been demonstrated for *Nucella lapillus* in southwest England (Bryan et al., 1986; Gibbs and Bryan, 1986).

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In a recent review, Shi et al. (2005) reported that imposex has been recorded in 170 species of gastropods from 28 families. The vast majority, 134 species, are neogastropods. Among the non-neogastropod, caenogastropod families, ampullarids, rissioids, cypreaids, cymatids, and tonnids all contain several species for which imposex has been reported (Shi et al., 2005). Although the taxonomic coverage is wide, much of the basic information on imposex in relation to TBT pollution is centered on muricids, buccinids, and conids (Fioroni et al., 1991; Shi et al., 2005). On a worldwide scale, it is necessary to extend the scope of studies to include more tropical forms and locations (Ellis and Pattisina, 1990) to get a global picture of the effects of TBT pollution on gastropods.

The Panama Canal is one of the world's busiest shipping zones, and commercial transport through the canal represents about 5% of world trade. About 14,000 vessels pass through the Canal annually (statistics available from the Autoridad del Canal de Panama web site http://www.pancanal.com/), and the most common shipping route is between the east coast of North America and Asia. Most of the shipping traffic is composed of large, oceangoing vessels, which have not previously been subject to restrictions on the use of tributyltin antifouling paint. The entrance to the Canal, on the Pacific coast adjacent to Panama City, was the site of Rodman Naval Base (1943–1999), and is currently the site of the container port of Balboa and a shipyard. The anchorage for the canal commonly has more than 30 vessels waiting to transit the Canal. The substrate in this area is primarily a mix of rocky debris and sandy mud in the intertidal and fine mud in the subtidal. With the consistently high levels of shipping traffic, frequent dredging, and muddy substrate (which is known to retain TBT for years, as reviewed in de Mora, 1996), the local levels of TBT and, therefore, imposex are expected to be higher around the entrance to the Canal than they are along the open coast. We conducted a survey of four common intertidal gastropod species around the mouth of the canal to document the levels and geographic extent of imposex in this area.

MATERIALS AND METHODS

Gastropods were collected between February and April 2005 from four sites along the Pacific coast of Panama at varying distances from the mouth of the Panama Canal (Figure 1). The site closest to the mouth of the canal consisted of rocky outcrops near Farfan beach (8.93°N, 79.58°W) and the Bridge of the Americas. Progressively further away to the west were Isla Venado (8.91°N, 79.63°W), Chumical (8.5°N, 79.66°W), and Bique (8.90°N, 79.66°W). In November 2007 additional samples were collected from Punta Culebra (8.91°N, 79.53°W), which faces the entrance to the Canal and is at the edge of the Canal anchorage.

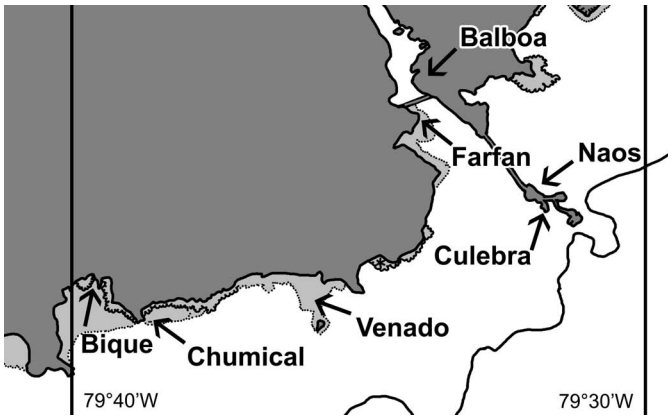

FIGURE 1. Map of the study area at the entrance to the Panama Canal. Arrows indicate the sites of sample collection and locations mentioned in the text.

We collected four species, which were clearly identifiable and abundant at two or more of the sites. Efforts were made to collect the same species from all sites, but because of the habitat heterogeneity in the area, we were not able to collect sufficient numbers of females for statistical analyses for several sites. Adequate samples were collected for the muricids *Acanthais brevidentata* (Wood, 1828) from Farfan and Chumical and *Thaisella kiosquiformis* (Duclos, 1832) from Farfan and Bique, and the calyptraeids *Bostrycapulus calyptraeformis* (Deshayes, 1830) and *Crepidula* cf. *nivea* from Farfan, Venado, and Chumical (Table 1).

Shell length was measured with vernier calipers, and live snails were extracted from their shells. The reproductive system was immediately examined under a stereomicroscope, and the sex was determined based on characteristics of the gonad and presence or absence of seminal receptacles and seminar vesicles. If the sex was not easily identified, sex was verified by examining gametes from a smear of gonad. The length of the penis (if present) was measured using an ocular micrometer on a stereomicroscope.

TABLE 1. Frequency of imposex in four gastropod species at sites arranged here from nearest to furthest from the Panama Canal entrance. Frequency at each site was compared to that at Farfan (the site at the entrance to the Canal) using a Fisher's exact test. A one-tailed test was used, but two-tailed results did not differ; * $P = 0.001$; ** $P = 0.0001$; *** $P = < 0.0001$.

	Site				
Species	**Farfan**	**Culebra**	**Venado**	**Chumical**	**Bique**
Muricids					
Thaisella kiosquiformis	29/53	–	–	–	13/52*
Acanthais brevidentata	8/32	–	–	0/57**	–
Calyptraeids					
Bostrycapulus calyptraeformis	60/63	22/43[a]***	2/79***	1/122***	–
Crepidula cf. *nivea*	87/90	19/22	–	0/99***	–

[a] Significantly different from Venado and Chumical <0.0001.

Significant differences in the frequency of imposex between the entrance to the Canal and more distant sites were tested for using Fisher's exact test. Analysis of covariance (ANCOVA) was used to examine the relationship between penis length in male and imposex females, with shell length as a covariate for samples collected from Venado and Farfan. Because samples from Culebra were preserved in ethanol before examination, the penis length from these samples could not be directly compared to the others that were measured fresh.

Experiments to determine if imposex develops in adult snails after exposure to ambient water levels of TBT were conducted at STRI's Naos Marine Laboratories, only a few hundred meters from the Culebra site. *Anachis fluctuata* (Sowerby, 1832) and *Bostrycapulus calyptraeformis* were both collected from Isla Venado, an area with low levels of imposex, and maintained in the laboratory. Sixty adult *Anachis fluctuata* were kept in a 100 L fiberglass tank in the outside seawater system and fed frozen commercial clams once a week. After five months the animals were killed and levels of imposex were determined as already described. *Bostrycapulus calpytraeformis* were collected as small males. They were maintained in pairs in the laboratory in 350 mL plastic cups. The water was changed every other day and the animals were fed 10 mL *Isochrysis galbana* culture every day. Animals were measured every four weeks, and their sexual state was recorded on the basis of external features. The experiment was terminated after 400 days. Both species were cultured using the same source of seawater (from the side of Isla Naos away from the Canal entrance), and neither was exposed to local sediment other than that which settled out of the seawater.

RESULTS

Field Collections

Imposex was detected in all four species. In the two muricids, the imposex was almost always in the early stages with limited penis development and no indication of any occlusion of the capsule gland. We never observed imposex that was so far advanced that the females were found to retain eggs or that an obvious vas deferens had developed. Imposex in the calyptraeids was more developed; penes were large in many specimens and could easily be confused with a normal male penis. Several imposex females of *Bostrycapulus calyptreaformis* and *Crepidula* cf. *nivea* were observed brooding egg capsules, showing that imposex females were not sterile. Near the entrance of the Canal the frequency of imposex ranged from 25% to 50% in muricids and was greater than 80% in calyptraeids. The number of females collected for each species at each site and the frequency of imposex are given in Table 1. In all cases the frequency of imposex was significantly higher near the entrance to the Canal than at farther sites (Table 1).

Acanthais brevidentata: Because there were no imposex individuals in Bique and because animals from that site were significantly larger (mean = 28.9 mm) than from Farfan (mean = 26.9 mm; $P < 0.001$), comparisons of imposex females with normal males and females were conducted for data collected from Farfan only. Imposex females were significantly larger (length = 30.1 mm) than non-imposex females (length = 26.4 mm; $P < 0.02$). ANCOVA showed that there were significant effects of shell length ($P = 0.01$) and imposex ($P < 0.001$) on

penis length as well as a significant interaction effect ($P < 0.04$). Penes of imposex females were smaller than those of normal males, and male penis length increased with shell length, although imposex penis length was not associated with shell length (Figure 2).

Thaisella kiosquiformis: Animals from Bique and Farfan did not differ in size, nor did the sexes differ in size. Imposex females were also the same size as non-imposex females. The average size for all categories was 26–27 mm. Data from Bique and Farfan were combined for the analysis. ANCOVA showed that there were significant effects of shell length ($P < 0.0001$) and imposex ($P < 0.0001$) on penis length as well as a significant interaction effect ($P = 0.003$). Penes of imposex females were smaller than those of normal males, and penis length increased with shell length in both sexes (Figure 2). There was a significant incidence of imposex at Bique, despite it being the site furthest from the Canal. We attribute this relatively high frequency of imposex to this site's prox-

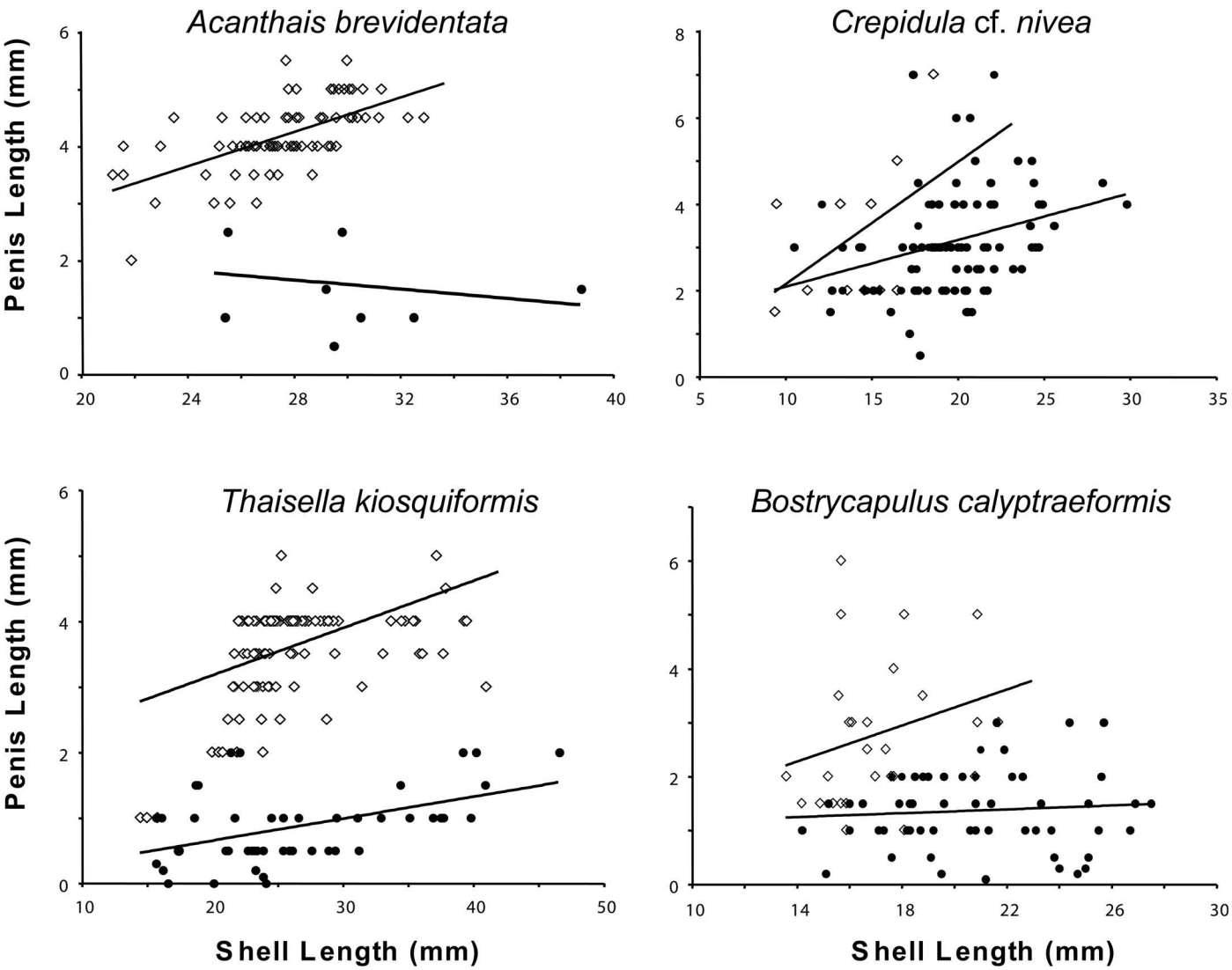

FIGURE 2. Relationship between shell length and penis length for males (white diamonds) and imposex females (black dots) of *Acanthais brevidentata*, *Thaisella kiosquiformis*, *Crepidula* cf. *nivea*, and *Bostrycapulus calyptraeformis*.

imity to a dry dock facility slightly further to the west in Vacamonte.

Crepidula cf. *nivea*: Calyptraeids are protandrous hermaphrodites (Collin, 2006) and the small animals are almost always males; therefore, we did not make as much effort to collect the smallest animals as we did in the other species. The size of females differed significantly between the sites (16.1 mm for Chumical vs. 19.6 mm for Farfan; $P < 0.0001$). Imposex females were larger than non-imposex females. Because all females at Chumical were normal and virtually all in Farfan had imposex, this size difference cannot be distinguished from a site effect on size. An ANCOVA showed that shell length had a significant effect on penis length ($P = 0.03$), that imposex females and males did not differ in penis length as there was considerable penis growth in the imposex females, and that there was no significant interaction between imposex status and shell length (see Figure 2). Although there were significant levels of imposex in samples from Culebra, in all these cases the penis was very small; they were not much more developed than a small bump at the base of the tentacle, whereas those from Farfan were often as long as or longer than the tentacles.

Bostrycapulus calyptraeformis: The average size of females differed significantly among the three sites (17.5 mm at Chumical; 16.5 mm at Venado; 20.6 at Farfan; $P < 0.01$). Again, because nearly all the females in Farfan had imposex but no imposex was detected in the other locations, the larger size of imposex females may have been a site effect. ANCOVA analysis of animals from Farfan showed that there was a significant effect of imposex on penis length ($P < 0.001$), and imposex females had smaller penes than males. Shell length and the interaction between shell length and imposex had no significant effect on penis length. The level of imposex in animals from Culebra was again very rudimentary, with penes little more than a nub at the base of the tentacle.

In summary, all four species showed significant higher rates of imposex near the entrance of the canal. By 20 km away, rates were generally of the order of 1%–2%. In the two muricids and one of the calyptraeids, the penes of imposex females were smaller than those of similar-sized males. In the two muricids and the other calyptraeid, shell length was a significant covariate of penis length, and in two species the penis length of imposex females increased with shell length.

Laboratory Experiments

After five months in the laboratory, 2 of 29 female *Anachis fluctuata* had developed penes, indicating that this species can develop imposex. However, this was not statistically significantly different from the frequency of imposex in the field in Venado ($P = 0.09$; Table 2). No comparisons to the entrance to the Canal could be made because this species could not be found there.

Of the 60 *Bostrycapulus calyptraeformis* that were raised in the laboratory, the largest animals in 6 of the 30 cups retained penes throughout the experiment and did not change to become female. In the remaining 24 cups, the larger of the 2 animals lost the penis, indicating sex change from male to female. Of these 24 animals, 10 lost the penis and then subsequently regained it 1 to 3 months after sex change. In many cases the penis was not as long or thick as a normal male penis, but they were fairly large, and casual observers would be likely to categorize such animals as males (Figure 3). The largest animals in the remaining 14 cups underwent transition to normal females and did not develop imposex before the end of the experiment. The smaller of the 2 animals in each cup was not examined, as they usually remain male in the presence of the larger animals (Collin et al., 2005).

TABLE 2. Frequency of imposex from field-collected and laboratory-reared snails.

Species	Laboratory	Venado	Chumical	Fisher's exact test, *P* value
Anachis fluctuata	2/29	1/133	–	$P = 0.091$
Bostrycapulus calyptraeformis	10/24	2/79	1/122	$P < 0.0001, < 0.0001$

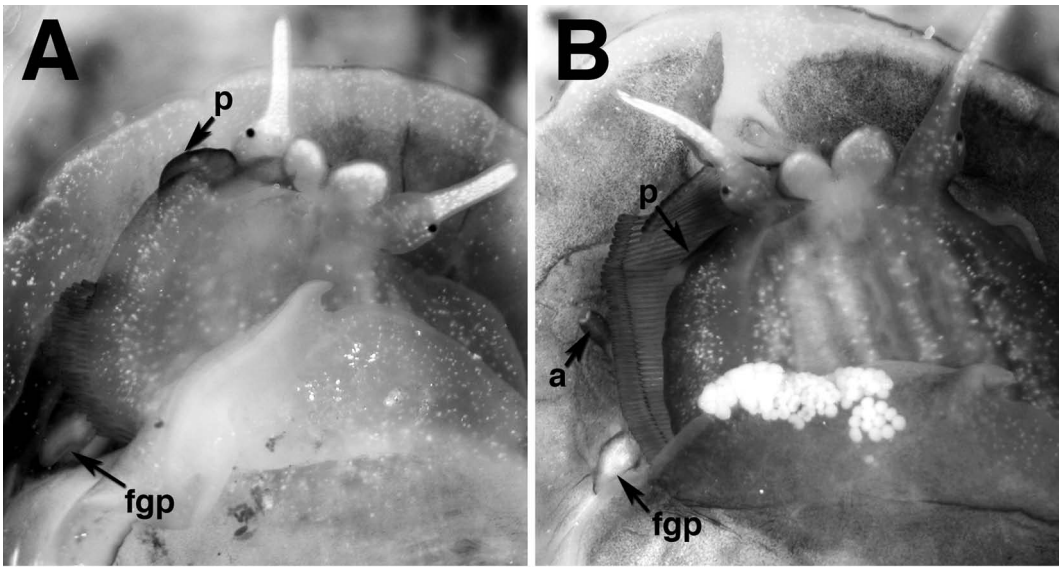

FIGURE 3. A, B. Photographs of two individuals with well-developed imposex in *Crepidula* cf. *nivea* from Farfan, with penis (p) and female genital papilla (fgp) indicated in each image. B. The female genital papilla can be confused with the anus (a), which is usually obscured by the gills; brooded eggs are visible as the light mass in this image.

DISCUSSION

Imposex was found in all snail species examined from the mouth of the Panama Canal, and in all cases the frequency and severity of imposex declined with distance away from the Canal. The frequency of imposex differed among the species examined, with calyptraeids more likely to display imposex than the muricids. Calyptraeids showed higher frequencies of imposex at the mouth of the Canal, and the penes of imposex females were much more fully developed then we ever observed in muricids. Species differences in sensitivity both to TBT (Wilson et al., 1993; Tan, 1999) and to its bioaccumulation (Liu et al., 1997) have been demonstrated in other surveys. Liu et al. (1997) found that imposex was much more severe in *Thais* species than *Morula*, despite similar organotin burdens, and suggested a genus-specific susceptibility to organotin pollution with the ranking order of *Nucella*, *Thais*, and *Morula*. The differences in habitat (high on the intertidal versus low on the intertidal), diet, and physiology have been suggested as causes of interspecific differences in imposex (Tan, 1999). If TBT were primarily waterborne either in solution as *bis*(tributyltin) oxide or adsorbed by suspended solids (de Mora, 1996) at our study sites, it is possible that filter-feeding calyptraeids would be exposed to more TBT, by filtering large volumes of water, than would other gastropods. Suspended particles may have TBT adhered to them and may be captured in the mucous net and ingested during filter feeding, thus increasing the exposure of calyptraeids relative to the muricids. These scenarios are not in agreement with a number of laboratory studies (Bryan et al., 1989; see Gibbs and Bryan, 1996, for review) that show that TBT accumulates more rapidly from the diet than from the ambient water and which suggest that carnivores could accumulate more TBT from their diet than would herbivores. However, controlled experimental comparisons of bioaccumulation between carnivorous and suspension-feeding gastropods have not been made, and the effects of suspended solids have not been examined.

Another factor that can influence the expression of imposex is age. Because extended exposure to TBT is necessary to elicit imposex, those species that are longer lived or slower growing may be more likely to have high levels of TBT and thus exhibit imposex. Studies have also shown that juvenile snails are more sensitive to TBT than are adults (Gibbs and Bryan, 1996). Our data for *Acanthais brevidentata*, showing that females with imposex are larger than normal females, are consistent with either increase in imposex development with long-term exposure or recent reductions in TBT levels. However, *Thaisella kiosquiformis* did not show this pattern. Few data on the

age or lifespans of tropical gastropods are available and so this possibility is difficult to evaluate. However, Panamanian calyptraeids grown in the laboratory generally reach maturity at sizes similar to animals that matured in the field, in less than a year (Collin et al., 2005, and personal observation), and it seems unlikely that TBT in the sediment, which has a half-life of years, would have changed drastically in such a short interval.

Imposex has not been previously reported in calyptraeid gastropods. Because animals normally change from males to females and transitional animals may sometimes retain a penis while also showing well-developed female reproductive structures, it is possible that imposex individuals have previously been misidentified as undergoing the normal transition between the male and females phases. Here we found, in sites with low expected TBT exposure, that there are virtually no individuals that display both male and female characteristics at the same time. In addition, our laboratory studies show that during sex change the penis can be reabsorbed and that the penes of imposex individuals can grow following this reabsorption. These results show that the abundant large females with penes collected at the entrance to the Canal are indeed imposex females and not transitional individuals that have yet to lose the penis.

Numerous studies have shown a tight relationship between levels of TBT in the environment, levels of TBT in gastropod tissues, and the frequency of imposex within species (Gibbs et al., 1987; Horiguchi et al., 1994; Minchin et al., 1997; Ruiz et al., 1998). However, the relationships between sites, species, and the different types of triorganotins are not always simple (Ide et al., 1997). Imposex has also been shown to be a more sensitive way to detect TBT than many chemical detection methods, and imposex has been used as a bio-indicator when TBT levels are too low for easy analytical detection (Gibbs and Bryan, 1996). Despite an extensive literature on the relationship between TBT and imposex, one study (Nias et al., 1993) indicates imposex could result from exposure to paint matrix or copper. However, this result has not been pursued or elaborated. Although we could not measure levels of TBT directly at the sites around the Canal, it can, in the light of this literature, be inferred with some level of confidence that the exposure of animals to TBT is higher at the entrance to the Canal than it is in the surrounding areas. Despite the high levels of shipping and presumably high levels of TBT leaching into the surrounding water, the development of imposex was not so severe as has been reported for areas with high shipping traffic in Europe and Asia, and TBT does not have an extreme impact on reproduction by occluding the pallial oviduct or splitting the bursa copulatrix and capsule gland, as has been reported from these regions (Oehlmann et al., 1996; Shi et al., 2005). Less obvious effects on reproduction were not directly evaluated in this study. The high amount of flushing in the area, from large volumes of discharge from the Canal and the 6 m tides, may help to prevent local buildup of high concentrations of TBT in this partially enclosed area.

In 2002 the International Maritime Organization adopted a Convention on Antifouling Systems (AFS) that called for a global prohibition of the application of organotin compounds as biocides in antifouling systems on ships by 1 January 2003 and a complete prohibition by 1 January 2008. However, the prohibition was only to be implemented 12 months after 25 states representing 25% of the world's merchant shipping tonnage ratified it. In September 2007 this quota was met when Panama ratified the convention, and therefore these regulations went into effect in September 2008. As the AFS convention applies to ships flagged in, operated by, or docking in states that have ratified it, the convention should significantly reduce the exposure of Panama's marine habitats to TBT pollution in the coming years. This regulation is especially important because the planned expansion of the Canal in 2014 will significantly increase shipping traffic along both the Pacific and Caribbean coasts of Panama.

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Shorefishes of the Tropical Eastern Pacific Online Information System

D. Ross Robertson

ABSTRACT. Shorefishes of the Tropical Eastern Pacific Online Information System (SFTEP) version 1, 2008, provides an online electronic identification guide and information system for the known fauna of shorefishes found in the Tropical Eastern Pacific. SFTEP allows users (i) to identify all shorefishes known from the Tropical Eastern Pacific (TEP) (1,287 species in version 1) and (ii) to analyze and conduct biogeographic research on the composition of that fish fauna at varying spatial scales. Tools for identification emphasize the use of color photographs, along with descriptive text that highlights key morphological features; allow comparison of similar species; facilitate identification of unfamiliar species using information on location and fish morphology (shape, color pattern, and color); and incorporate interactive keys to members of two species-rich families (Gobiidae, Sciaenidae) that have many similar-looking species. To accommodate nonspecialist users, scientific jargon is minimized; the interface is intuitive and user-friendly, and searches for species can be made using common names. The Research Engine, which provides information about the composition of local faunas and the regional fauna, allows users to compare geographic ranges of multiple taxa, to construct faunal lists of taxonomic and functional groups of species for single and paired sites, and, at varying spatial scales, to determine local endemism and to display region-wide patterns of species richness of different taxa and functional groups of fishes. The system is accessible online at www.stri.org/sftep.

INTRODUCTION

Shorefishes of the Tropical Eastern Pacific Online Information System (SFTEP), version 1, 2008, provides an online electronic identification guide and information system for the known fauna of shorefishes found in the Tropical Eastern Pacific (TEP). This version represents the latest iteration of a series that began with the 1994 English-language printed identification guide of the same name (Allen and Robertson, 1994). That book was followed by a Spanish-language printed edition in 1998 (Allen and Robertson, 1998). Both these works were succeeded by a dual-language CD-based information system in 2002 (Robertson and Allen, 2002), which was revised and expanded in 2006 (Robertson and Allen, 2006).

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SYSTEM FEATURES

Dual Language Interfaces

The system incorporates separate, full-capability English- and Spanish-language interfaces.

Aids to Visual Impairment

The system incorporates two types of aids:

1. Variable map-color formats are available. Users can select various color schemes designed to accommodate different patterns of color blindness, including monochrome or color with the ability to select colors.
2. Page layout structure accommodates variation in font size. Two page layouts are possible—landscape and portrait. Page structure is stable over a threefold range in font size.

SYSTEM MODULES

Home

The home page provides an overview of the capabilities of the system and access to all major modules through buttons and/or tabs that act as shortcuts (Figure 1). In addition there are links to several modules not accessible from other parts of the system: to the **Copyright notice**, a switch to change between **English** and **Spanish** interfaces, and to the websites of the **Smithsonian Tropical Research Institute** and **Coeus,** the company that programmed the system.

Each of the authors and major contributors of information directly related to the construction of SFTEP has an individual contributor page, accessible from the **Contributors** button and from a link at the top of any screen. In addition, the major contributors of information presented on each family are noted on each family page.

General Information

General information about Shorefishes of the Tropical Eastern Pacific Online Information System (SFTEP) is shown in Figure 2; this module includes three sections.

Introduction

The "Introduction" to the TEP and its shorefish fauna provides background information on the oceanography of the region and its marine habitats (geographic and temporal variation in climate, rainfall and salinity, primary production and coastal upwelling systems, ocean current systems, influences of the El Niño cycle, shoreline habitats and rocky and coral reefs in the region); a history of taxonomic fish guides, major modern guides, global online resources, systematic ordering of the fishes, and the scientific and common names of fishes); the ecology of TEP shorefishes (species that occur in the upper 100 m of the water column over the continental shelf or within ~50 km of the shore), their use of different environments and habitats, their depth-distribution patterns, their dietary groupings, and their modes of reproduction; and the zoogeography of the fauna—studies of the region's zoogeography, resident versus vagrant species, relationships of the fauna to the faunas of other areas, distribution of the fauna in different climate zones, the geography of variation in species richness and local endemism throughout the region, and biogeographic subdivisions of the TEP.

Features & User Guide

The "Features & User Guide" section describes system features, providing information available on taxon pages, databases on biological and zoogeographic characteristics, information used to identify fishes, an interactive glossary of ichthyological terms, the functioning of the zoogeographic research engine (comparison of taxon ranges, assembly of faunal lists, determination of local endemism, assembly of maps of species richness and sampling intensity, assembly of lists of species from predefined parts of the TEP), the functioning of the interactive library, the database of images, and credits to contributors.

Acknowledgments

The "Acknowledgments" section recognizes support from STRI, funding, government permissions, logistical support, assistance collecting fishes, identification of specimens and reviews of section, databases, Spanish translations, images and illustrations, database management, and digital image preparation.

The Fishes

A Page for Each Species, Genus, and Family

Information on the members of the fauna is provided through interlinked species, genus and family pages. Genera and species are ordered alphabetically within each family, with families being arranged in "phylogenetic" order. "The Fishes" module provides access to **Species**, **Genera**, and **Families** pages by browsing within each taxo-

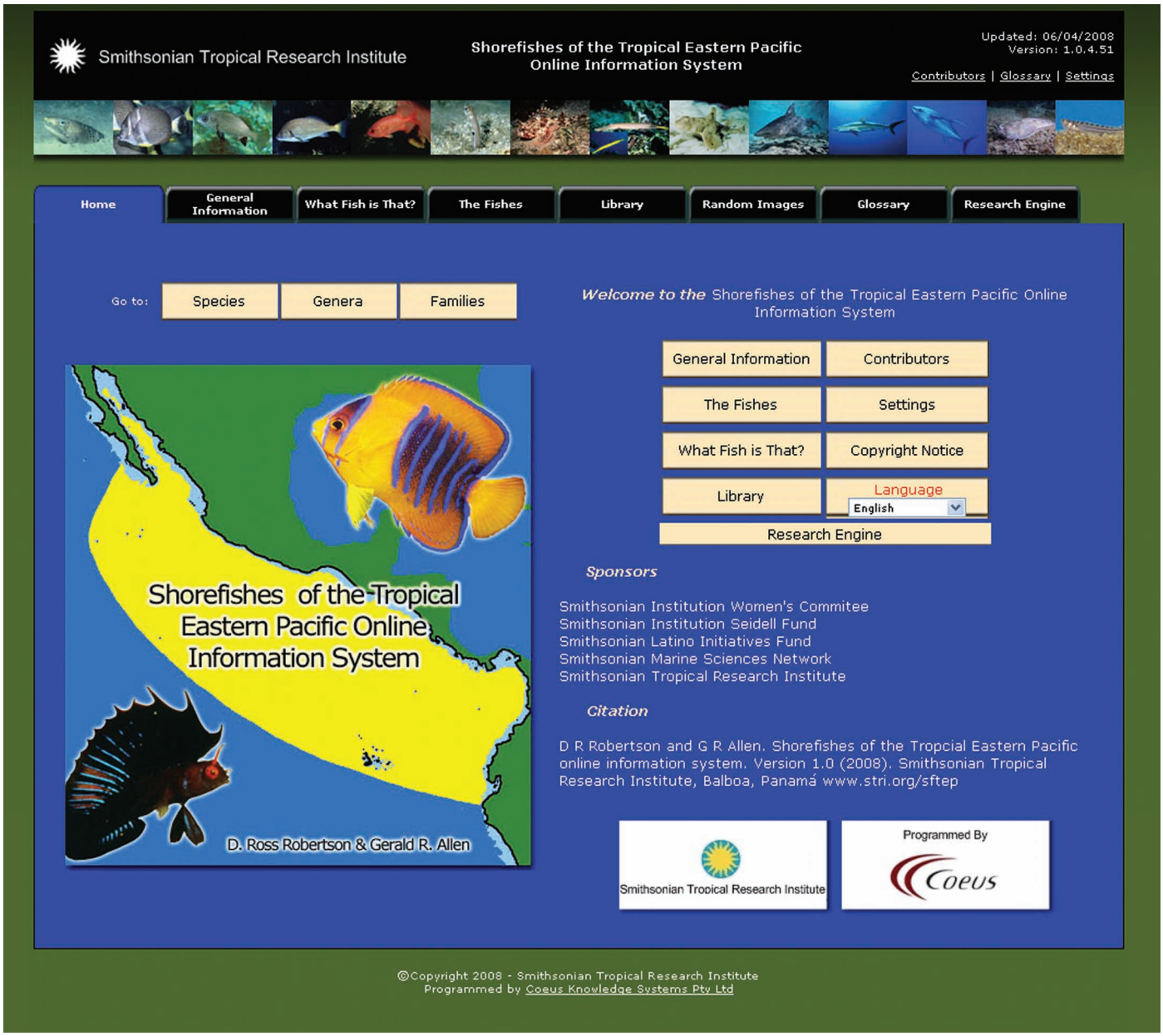

FIGURE 1. Opening screen and "Home" module.

nomic level, browsing from within a **Systematic Tree** (with optional alphabetic or systematic ordering, and optional use of common or scientific names), browsing from within a **Book Mode** (species within genera within families), or user-selection of level and taxon from pull-down lists.

Family and genus pages include a brief introduction to systematics, biology, global geographic distribution, and an estimate of the number of genera and species worldwide and present within the TEP; a text description of distinguishing morphological features—*black text* indicates the least distinctive features for identification purposes, *red text* indicates important features, and *red text with yellow high-lighting* shows the most important features (see Figure 3); a database map of the taxon's range limits distribution in the TEP (assembled from the distributional maps of component species) and a list of component genera and species with links to their pages; an image of a representative species that has a key feature

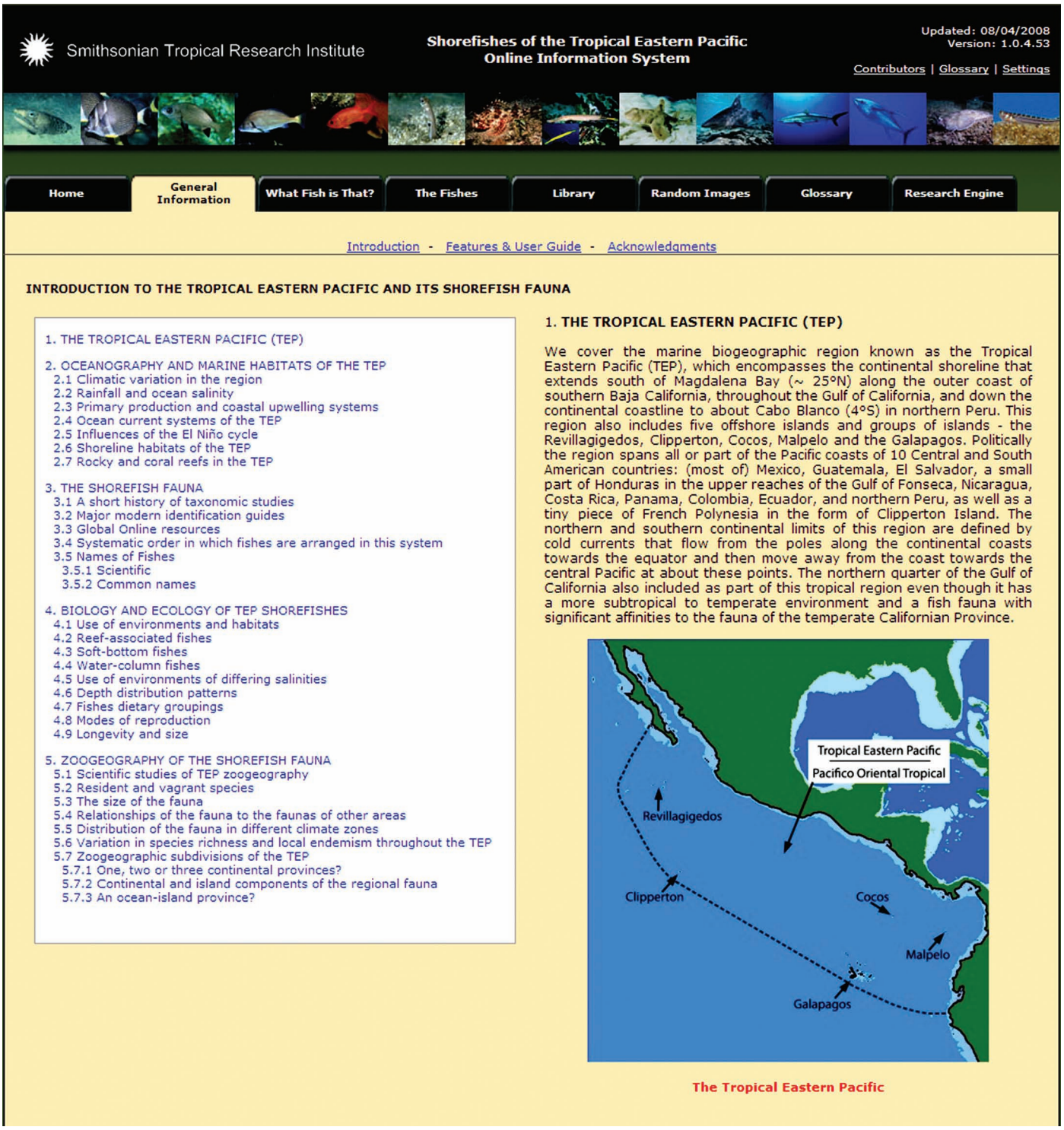

FIGURE 2. Opening screen from the "General Information" module.

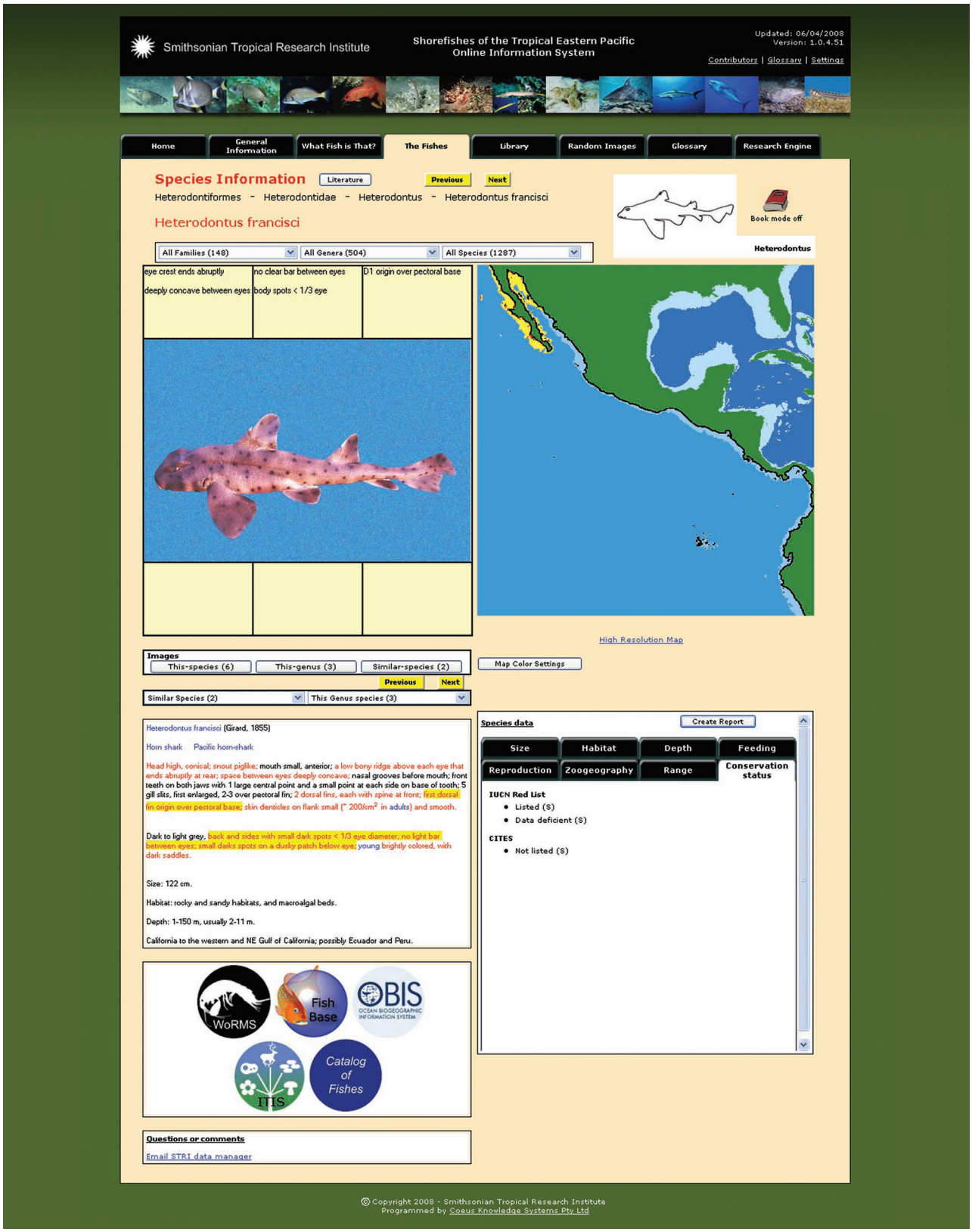

FIGURE 3. Example of a species page.

overlay indicating diagnostic features of the taxon that distinguish it from similar taxa; and comparisons with similar taxa. To assist in distinguishing look-alike fishes, each taxon page includes a button-link that allows the user to compare images (with key feature overlays) of such taxa. Each page also includes a list of designated similar fishes (at the same taxonomic level), with links to their taxon pages.

Species data pages are similar to genus and family pages but also include multiple images (e.g., juvenile, female, male, color morphs, specific morphological characteristics) and access to a downloadable list of species zoogeographic and ecological attribute data. For example, the **Zoogeography** tab includes **Global endemism**, a species global-scale distribution and its occurrence outside the TEP; **Regional endemism**, distributions of species within the TEP, including TEP endemics (species that occur only in the region or have the great bulk of their distributions within it), temperate eastern Pacific endemics (whose distributions are primarily to the north and south of the TEP, in the Californian and Peruvian provinces), eastern Pacific non-endemics (species that have populations outside the eastern Pacific, for instance circumtropical species). Categories relating to the distributions of species within the TEP include the occurrence of endemic and non-endemic species at offshore islands and/or the continental shore, whether TEP endemics are endemic to the offshore islands (and which islands) or to the mainland, and to which of the three mainland provinces (or combinations thereof) each continental TEP endemic is restricted. Attributes for **Climate zone** and **Residency** (whether the species appears to be a resident or a vagrant in the region) are also included.

Other species ecological attributes that are presented include the following:

- the known maximum total length of each species;
- a species' maximum and minimum depth of occurrence;
- the salinity of environment(s) in which a species occurs;
- the specific habitat(s) a species uses (as well as habitat categories as defined by FishBase, see www.FishBase.org);
- whether a species is restricted to inshore waters or occurs in offshore, oceanic conditions;
- the position in the water column at which a species lives (e.g., bottom, surface);
- a species' feeding group (e.g., carnivore);
- items in a species' diet (e.g., fishes, pelagic crustaceans, microalgae);
- a species' reproductive mode (e.g., different types of eggs, live birth); and
- a species' CITES and IUCN REDLIST status.

When information is available (e.g., for diet) for a species itself, an "S" is given after the value in the database. In cases for which such species-level information is lacking, the page displays information for the genus (indicated by "G"), or for the family ("F") if there is no information for the genus.

Taxon pages includes direct links to external websites concerning the same taxon in the following external online sources: William Eschmeyer's Catalog of Fishes (www.calacademy.org/research/icthyology/catalog), which provides comprehensive up-to-date data on the systematics of fishes; FishBase (www.fishbase.org), which covers a variety of aspects of the biology of fishes; ITIS, the International Taxonomic Information System (http://www.itis.gov) and WoRMS, the World Register of Marine Species (http://www.marinespecies.org), both of which focus on scientific names of fishes; and OBIS, the International Biogeographic Information System (http://www.iobis.org), which aggregates geo-referenced databases of collection records of fishes.

What Fish Is That?

This module facilitates identification of unknown fishes using four distinct tools (Figure 4).

Find a Fish

This tool allows users who are not scientifically trained to identify an unfamiliar fish by choosing among the following in any order or combination, with the ability to back-up steps: **Where was it?**—select location and size of area in question on a database map—and combinations of **Body Shape**, **Color Pattern**, and **Colors**. Each step narrows the list of possibilities, with each species on the possibilities list linked to its image, and hence to its species page.

Identification Keys Search

Illustrated dichotomous keys are provided for the genera and species in the two families with the largest number of species: Gobiidae (88 species in 27 genera) and Sciaenidae (82 species in 26 genera). Search results link to species pages.

Compare Images of Fishes

This function allows simultaneous comparison of images of any two to six families, genera, or species selected. The feature enables users to compare "apples" with "oranges," whereas the comparison of designated similar taxa on taxon pages limits users to comparing only "apples." Resultant images are linked to taxon pages.

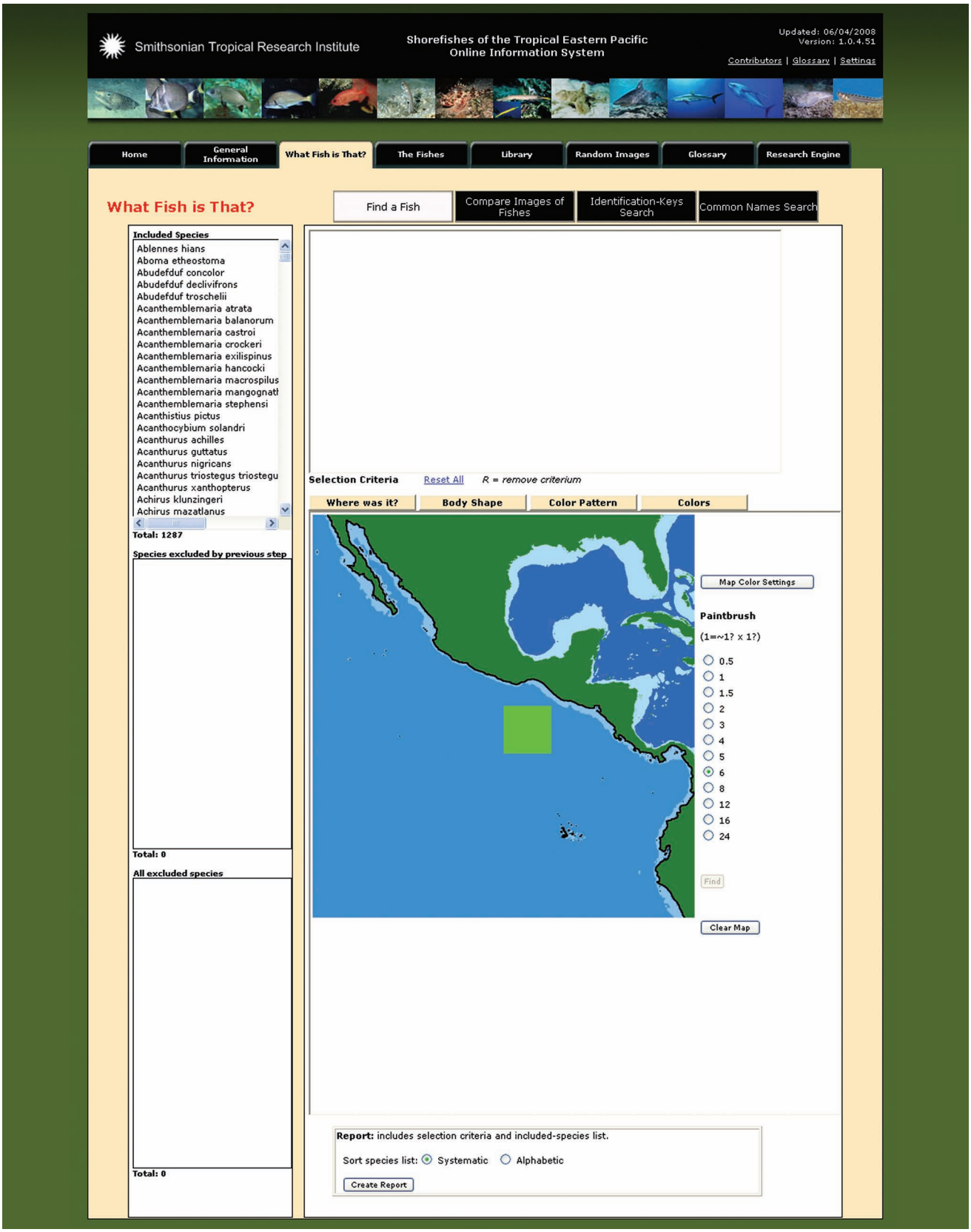

FIGURE 4. Screen capture from the "What Fish Is That?" module.

Common Names Search

Searches can be made for families, genera, and species from pull-down lists of common names, with results linked to taxon pages. The systematic tree or taxonomic hierarchy (see "The Fishes" module) also functions with the use of either common names or scientific names. The use of common names in this hierarchy helps users who are not scientifically trained to appreciate the relationships among fishes.

Glossary

An interactive glossary of taxonomic terms is provided that uses a combination of images and text to explain basic terms relating primarily to morphological characteristics that are used in the identification of fishes. In addition the usage of scientific jargon has been reduced as much as possible throughout the taxon pages by using simple descriptive phrases from everyday English to replace technical terms.

Research Engine

This module provides a variety of types of zoogeographical data and the ability to generate maps and site-specific species-lists based on complex queries constructed by the user (Figure 5).

Taxon Range Maps

This feature provides overlaid displays of the regional ranges of up to three taxa (species, genera, families, or a mixture thereof). In addition, maps can be generated of the geographic distribution of all species-range centroids (both paint and point data) and of all geo-referenced sampling points in the system's database.

Species Richness Displays

This feature provides maps with color-coded overlays of patterns of variation in species richness throughout the region. Those patterns include richness of individual families and richness of species in specified "functional groups" (e.g., species sharing one or more biogeographic and ecological attribute). Richness displays can indicate either absolute richness (number of species) or relative richness (number of species as a percentage of the local fauna). A display of relative sampling intensity indicates the number of species recorded at minimally one site within each unit of area (1° of latitude × 1° of longitude) as a percentage of the number of species whose ranges encompass that unit.

Species – List Assembly

Family and genus lists can be constructed for single locations. Species lists for "functional groups" of fishes for a particular location can be constructed using any combination of biogeographic and ecological attributes. Species lists include both single-location lists and lists of species found or not found at two locations. The spatial scale of a location in such a search varies from a single island to an area of variable shape and size, to the entire TEP or map. Locations are defined by the user employing a library of approximately 300 preformed templates that include geographic entities (e.g., shoreline, continental shelf, named gulfs), habitat features (e.g., mangroves, rocky shores, upwelling areas), islands (individual and archipelagos), biogeographic entities (provinces of the TEP), political areas (Exclusive Economic Zones and parts thereof), and marine reserves (individual reserves, combine country reserves). In addition, quadrants of varying sizes (12 groups ranging from 0.5° latitude × 0.5° longitude to 24° × 24°) used by the map of the **Find a Fish** tool in the "What Fish Is That?" module provide approximately 5,000 additional [square] templates.

Unconfirmed/Confirmed Occurrences

Single-area species lists indicate both likely occurrences (species whose ranges include the selected area) and confirmed occurrences (species with at least one collection record in the same area).

Local Endemism Indicator

This feature provides a list of species found only within one or two template areas, and nowhere else on the system's map.

List and Map Exports

Lists and Maps produced by searches are exportable/printable. Lists may be arranged alphabetically or systematically (genera and species arranged alphabetically within families arranged in systematic order).

Species Range Maps and Range Data

A database map on each taxon page incorporates two types of data: a two-dimensional painted representation of the geographic range based on museum and literature records of occurrence and range maps, and our own field surveys in Mexico, El Salvador, Costa Rica, Panama, Colombia, Ecuador, the Revillagigedos, Clipperton, Cocos,

FIGURE 5. Screen capture from the "Research Engine" module.

Malpelo, and the Galapagos; and points indicating site records from museum collections, the scientific literature, and our own field surveys. Geographic-range statistics derived from these two offsets of data and presented on species pages include: latitudinal and longitudinal limits, ranges and midpoints based on paint data, and, separately, site records; data on range characteristics derived from paint data have been adjusted in species that occur in the eastern Pacific beyond the limits of the system's base map; habitat area, based on number of painted pixels in the species range map; and separate range-area polygons with centroids based on painted data and site record data.

Continental Ranges

Comprehensive faunal lists exist for few locations, and large sections of the coastline and continental platform of the TEP remain poorly sampled, a situation that will not be rectified in the near future. Hence the range of most species on the continental shelf is derived from data on the northern and southern limits of occurrence. Thus painted areas on taxon page maps represent the potential range and potential habitat area, and a species is assumed to be present *in appropriate habitat* anywhere between those limits. Exceptions include species that are known to have wide gaps in their distributions, such as some well-known anti-tropical species. Those gaps are represented in the range maps of such species.

Habitat Area Calculations

Maps constructed for the determination of habitat areas incorporate information on habitat usage and depth range as well as the extent of the geographic range. Continental areas of range maps were modified to exclude large areas of habitat that was inappropriate for the particular species; for example, shorelines composed primarily of sand and mud were excluded from ranges of reef-fishes and rocky shores were excluded from ranges of fishes living on beaches or in lagoons and mangroves. The depth ranges of individual species were also taken into account: ranges of demersal species restricted to very shallow water (less than ~20 m depth) are indicated by lines that follow coastlines. For habitat area calculations of such species, the coastal strip of habitat was taken to be 1 km wide. Ranges of coastal species found in deeper water on the continental and insular shelves are divided into three groups: those occurring down to ~60 m have maps that span the inner continental shelf; species that are limited to depths below ~60 m occur on the outer shelf; and the third group has depth ranges (and maps) that span the entire shelf. Maps for pelagic species variously include parts of the shelf and/or open ocean, depending on the biology of the species.

Mercator Projection Distortions and Adjustments to Habitat Area Calculations

Mercator projection maps, such as that used in this system, incorporate distortions of both latitude and longitude that affect estimation of habitat area. In such projections lines of longitude are shown as parallel rather than converging with increasing latitude, and lines of latitude diverge with increasing latitude instead of remaining a fixed distance apart. When calculating the habitat area for each species those two distortions were taken into account by making appropriate adjustments to the areas of individual pixels in different latitudinal bands. Range polygon areas were calculated independently using the GIS (Geographic Information System) ArcInfo system.

Cleanup of the Geo-Referenced Records Database

Both the scientific literature and databases from museums inevitably include erroneous records as a consequence of misidentification of fishes and sites, as well as bookkeeping errors. In addition museum specimens of demersal species include not only individuals collected in demersal habitats but also larvae collected in the open sea far from adult habitat, and, in some cases, far from the known adult geographic range (Robertson, 2008). Records from the multisource database of ~67,000 collection site records included here that were adjacent to the currently known limits of the geographic range were used to adjust (by expanding) those ranges. However, we removed from the systems database those "suspect" records that were well outside the known habitat and geographic ranges of the "adult" phase of each species, based on extensive records from other sources, the biology of the species, and expert determinations of ranges. This cleanup process reduced the size of the database by approximately 6%. Points outside the current range were retained for some species that, because of overfishing, have had their historical ranges reduced. For example, historically the mackerel *Scomberomus concolor*, which currently occurs only in the northern Gulf of California, occurred throughout that gulf and also off California, USA (B. B. Collette, National Marine Fisheries Service Systematics Laboratory, personal communication, 2008).

Library

The library database (Figure 6) includes 1,143 citations. The citation for its original description is included for each species, along with citations of revisions of genera and families. Other citations include local and larger scale

FIGURE 6. Screen capture from "Library" module.

lists of species; identification guides to species; and publications about the biology and zoogeography of species inside and outside the TEP. Each citation is linked to the species it discusses (and hence to appropriate genera and families).

Exportable lists can be generated for:

- Citations linked to individual families, genera, and species.
- Species linked to a particular citation.
- Citations linked to a particular author, date, or source journal.
- The entire bibliography arranged alphabetically by author name.

Random Images

The image database incorporates 2,927 images. These include 2,346 color photographs that cover 83% of the fauna (1,068 of 1,237 species). In comparison, the 1994 book from which this system was developed included color images of 683 species and treated only 768 species.

This module presents color images in a randomized order.

Digital Manipulation of Images

The user should assume that **all** illustrations used in this system have been digitally manipulated to some extent, to increase their utility as identification aids. Such manipulation includes cropping, image sharpening, changes in lighting and contrast relationships of different parts of individual subjects, changes of subject-to-background contrast, changes of background to enhance subject visibility, the (occasional) combination of multiple images of fishes in a montage to provide examples of variation in color patterns within the same image, and minor repairs to fin membranes and removal of body blemishes (scratches, minor cuts, blood spots) that resulted from capture handling.

Image Credits

All images are accompanied by a relevant ownership credit, copyright notice, and usage notice. Each image is accompanied by a link to either the owner's e-mail contact or website.

Acknowledgments

Funding throughout the development of this system (1990–2008) was provided the following Smithsonian Institution (SI) sources: the SI Women's Committee, the SI Seidell Fund, the SI Latino Initiatives Fund, the SI Marine Science Network, and the Smithsonian Tropical Research Institute.

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Nephasoma pellucidum: A Model Species for Sipunculan Development?

Anja Schulze and Mary E. Rice

ABSTRACT. Recent developments in metazoan phylogeny, especially with regard to the position of the Sipuncula in the annelid clade, have sparked a renewed interest in sipunculan development. If Sipuncula are annelids, they must have secondarily lost segmentation. By comparison with segmented annelids, they could provide important clues for the evolution of segmentation. A sipunculan model species is needed to examine fundamental developmental processes. Here we describe the development of *Nephasoma pellucidum* and explore its potential as a model species for sipunculan development. Like other sipunculans, *N. pellucidum* produces eggs with a thick, porous, multilayered egg envelope. Cleavage in *N. pellucidum* is spiral, holoblastic, and unequal. The species shows the most common, and likely ancestral, developmental mode in the group. Its life cycle includes a lecithotrophic trochophore and a planktotrophic pelagosphera larva. The trochophore is enclosed in the egg envelope, with cilia growing through the envelope's pores. The trochophore larva metamorphoses into the pelagosphera larva at approximately 60 h. Pelagosphera larvae reached metamorphic competence at about five weeks. Metamorphosis to the juvenile was induced by supplying sediment that had been inhabited previously by conspecific adults. Juveniles were observed for several weeks. We conclude that *N. pellucidum* is a good model species for sipunculan development, although rearing conditions in the laboratory still need to be optimized.

INTRODUCTION

During the past two decades, our understanding of metazoan relationships has changed radically, starting with the first use of ribosomal RNA sequences for phylogenetic analysis (Field et al., 1988). Many taxa for which evolutionary origins have long been mysterious or controversial can now be placed with more certainty into the metazoan tree of life (Dunn et al., 2008; Halanych, 2004). Among those, two groups that were long regarded as distinct phyla have been absorbed into the Annelida: the Echiura, or spoon worms, and the Siboglinidae, previously called Pogonophora and Vestimentifera (McHugh, 1997; Rouse and Fauchald, 1997).

The Sipuncula, commonly known as peanut worms or star worms, have had a complex taxonomic history but now appear to be following the same route as the echiurans and siboglinids. Nearly 50 years after Hyman (1959) affirmed phylum status for the group, recent authors place them into the annelid clade, based on

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phylogenetic analyses of mitochondrial gene order (Bleidorn et al., 2005; Boore and Staton, 2002), sequence data from several genes (Struck et al., 2007), and expressed sequence tags (Dunn et al., 2008). Although there is a growing consensus on the annelid affinities of sipunculans, it remains to be determined which of the incredibly diverse annelids is the sister group to the Sipuncula. With a simple body, consisting of a trunk and a retractable introvert with an array of tentacles at the anterior end, they show little similarity to any other polychaete group. In the molecular analyses, support for a sister group relationship with any other polychaete taxon is low. The monophyly of the Sipuncula is uncontested, and solid hypotheses of within-group relationships have been published (Maxmen et al., 2003; Schulze et al., 2005, 2007; Staton, 2003).

Sipuncula are an interesting case in the field of "EvoDevo," or the interface of evolution and development. Embryonic and larval characters have often been cited as support for phylogenetic hypotheses. Rice (1985) listed several similarities between sipunculan and annelid development, such as the larval prototroch and metatroch and the retention of the egg envelope to form the larval cuticle. She also noted that in some sipunculan larvae the ventral nervous system develops in paired cords, similar to most polychaetes. On the other hand, Scheltema (1993), comparing embryos and larvae of annelids, mollusks, and sipunculans, argued that sipunculan development shows more similarity with that of mollusks. The development of all three taxa includes spirally cleaving embryos and a trochophore larva. A long-held view is that annelid and mollusk embryos can be distinguished at the 64-cell stage by the arrangement of the micromeres around the animal pole: they form either an "annelid cross" or a "molluskan cross." Reproducing Gerould's (1906) drawing of the embryo of *Golfingia vulgaris* with a molluskan cross, Scheltema concluded that sipunculans and mollusks were sister groups. However, Maslakova et al. (2004) showed that the annelid and molluskan crosses are far from universal within the respective taxa and probably hold no phylogenetic significance.

The primary reason why few past researchers have recognized sipunculans and echiurans as annelids is that adults of both taxa show no sign of segmentation, either externally or internally. It took advanced techniques in immunohistochemistry and confocal laser scanning microscopy to demonstrate segmentation in the nervous system of echiuran larvae (Hessling and Westheide, 2002). Similar techniques initially failed to show segmentation in sipunculan larvae (Wanninger et al., 2005) but a recent study showed a segmental mode of neural patterning in the early pelagosphera stage (Kristof et al., 2008).

If the Sipuncula fall into the annelids, they must have secondarily lost segmentation in the later larval stages and the adult. If no morphological segmentation is evident, what happened with the molecular pathways responsible for segment formation in other annelids? By comparison with other species, sipunculans are valuable for the identification of the genetic and cellular basis of segment formation in annelids.

The recent developments in metazoan phylogeny have thus sparked a renewed interest in sipunculan development. A model species is needed to study fundamental developmental processes. A good model species has to be readily available, be easy and inexpensive to maintain in the laboratory, lend itself to a variety of examination techniques, and be representative for its taxonomic group. Here we describe the development of *Nephasoma pellucidum* and explore its potential as a model species. *N. pellucidum* is a relatively common species that inhabits cracks and crevices in hard substrates in shallow warm waters. The species exhibits the most common developmental mode within the Sipuncula, which includes a lecithotrophic trochophore stage and a planktotrophic pelagosphera larva (Rice 1967, 1975a, 1975b, 1976, 1989). We have accumulated these data between 1972 and 1984 and, more recently, between 2003 and 2006.

MATERIALS AND METHODS

Specimens of *Nephasoma pellucidum* were collected from numerous localities offshore from Fort Pierce, Florida, extending from Capron Shoal and Pierce Shoal 4 and 6 miles, respectively, southeast of the Fort Pierce Inlet to the Sebastian Pinnacles approximately 32 miles north of the Inlet. At the Pinnacles, worms inhabited rubble of oculinid coral at depths of 70 to 100 m, whereas on the more southern shoals they occurred in depths of 9 to 15 m in rubble composed of mollusk shells, sand dollar tests, and rocks. Occasionally specimens were found in the Fort Pierce Inlet in intertidal clumps of oyster shells. The worms were carefully removed from the rubble with hammer and chisel. Multiple adults from each collection were kept in glass dishes in approximately 200 mL seawater at room temperature. Spawning occurred in the lab, generally after changing the water. Whenever eggs were observed in the culture dishes, they were pipetted into a clean dish and observed for development. Larval cultures were kept until the larvae either died or metamorphosed. Water was changed at least every two days. The larvae were periodically fed with unicellular algae or diatoms (*Isochrysis*,

Dunaliella, or *Nanochloropsis*). To induce metamorphosis, larvae were pipetted into dishes with muddy sediment previously inhabited by conspecific adults.

For scanning electron microscopy (SEM) and transmission electron microscopy (TEM), specimens were fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer (Millonig, 1964) for at least 1 h and up to several days at 4°C. Fixation was followed by three washes in a 1:1 mixture of Millonig's phosphate buffer and 0.6 M sodium chloride and postfixation in 1% osmium tetroxide (1:1:2 mix of 4% OsO_4 : Millonig's buffer : 0.75 M NaCl). Samples were then dehydrated in an ethanol series up to 100%. For SEM, they were critical point dried and mounted on SEM stubs using double-sticky tape and viewed in either a Nova Scan or a JEOL 6400 Visions scanning electron microscope. Images were either scanned from negatives or stored digitally. For TEM, the dehydrated specimens were transferred to propylene oxide and subsequently embedded in Epon resin and sectioned. Thin sections were stained with uranyl acetate and lead citrate and viewed in a JEOL 100CX transmission electron microscope.

RESULTS

Gametes

The spermatozoan of *Nephasoma pellucidum* is of the primitive type according to Franzén's classification (Franzén, 1958). The nuclear region is rounded and capped by a doughnut-shaped acrosome with a central nipple-like protuberance. The head, including nucleus and acrosome, measures 1.5 × 1.7 μm. Posterior to the nucleus, four mitochondrial spheres are arranged in a circle, from the center of which extends the flagellum (Figure 1A).

The egg at the time of spawning is spherical, measuring 105 μm in diameter (Figures 1B, 2A). In direct light the surface appears opalescent, and the color is pale gray. The egg envelope, up to 6 μm in thickness, is multilayered and perforated by numerous pores (Figure 3).

Spawning

As in most sipunculans, sexes are separate; eggs and sperm are spawned freely via the nephridipores into the surrounding water where fertilization occurs. From data accumulated on spawning in the laboratory, two spawning peaks are evident: one in the spring (April–May) and the other in the fall (September–November). Observations of spawning were carried out on animals in the laboratory, usually for a period of one month after collection from the field: 139 spawnings were recorded over a period of 8 years (1972–1980), and spawning occurred every month of the year except January. Although a few animals were observed to spawn after maintenance in the laboratory for as long as 18 months, 88% of the spawnings were recorded within 30 days of collection.

Cleavage

The eggs at spawning may be arrested in the first meiotic metaphase, or they may possess an intact germinal vesicle. In the latter case, the germinal vesicle breaks down soon (within at least 30 min) after spawning, regardless of whether the egg is fertilized. Within 40 min after fertilization (23°C), the first polar body is formed (Figure 2B), and at 55 min the second polar body makes its appearance. The first cleavage, occurring at 90 min, is unequal, the CD cell exceeding the AB cell in size (Figure 2C). The next three cleavages occur at approximately half-hour intervals, and the 16 cell stage is attained within 3 h after fertilization. The third cleavage, from 4 to 8 cells, is spiral and unequal. The A, B, and C cells, all approximately the same size, divide simultaneously, preceding the initiation of division of the larger D cell by about 1 min and completing their divisions 5 min before that of the D cell. In the 8 cell stage, the micromeres and macromeres of the A, B, and C quadrants are approximately the same size, the C sometimes being slightly larger, but all are smaller than the d cell which, in turn, is smaller than the D cell.

After the first few cleavages, the divisions are more frequent, and by 7 h after fertilization the egg has developed into an early blastula; cilia from the prototrochal cells protrude through the pores of the egg envelope and the embryo begins to rotate slowly on the bottom of the container. By 16 h the embryos are swimming throughout the dish, no longer confined to the bottom. At this time the stomodaeal invagination is evident, and the embryos show the first signs of positive phototropism (Figure 2D).

Trochophore: Morphology and Metamorphosis to the Pelagosphera

By 48 h the embryo has reached the stage of trochophore. The shape has changed from spherical to oval, as a result of a slight posttrochal elongation (see Figure 1C). A pair of dorsolateral red eyespots is present in the pretrochal hemisphere. Prototrochal cavities are evident to the inner side of the prototroch cells, and the gut is differentiated into three regions: esophagus, stomach, and intestine.

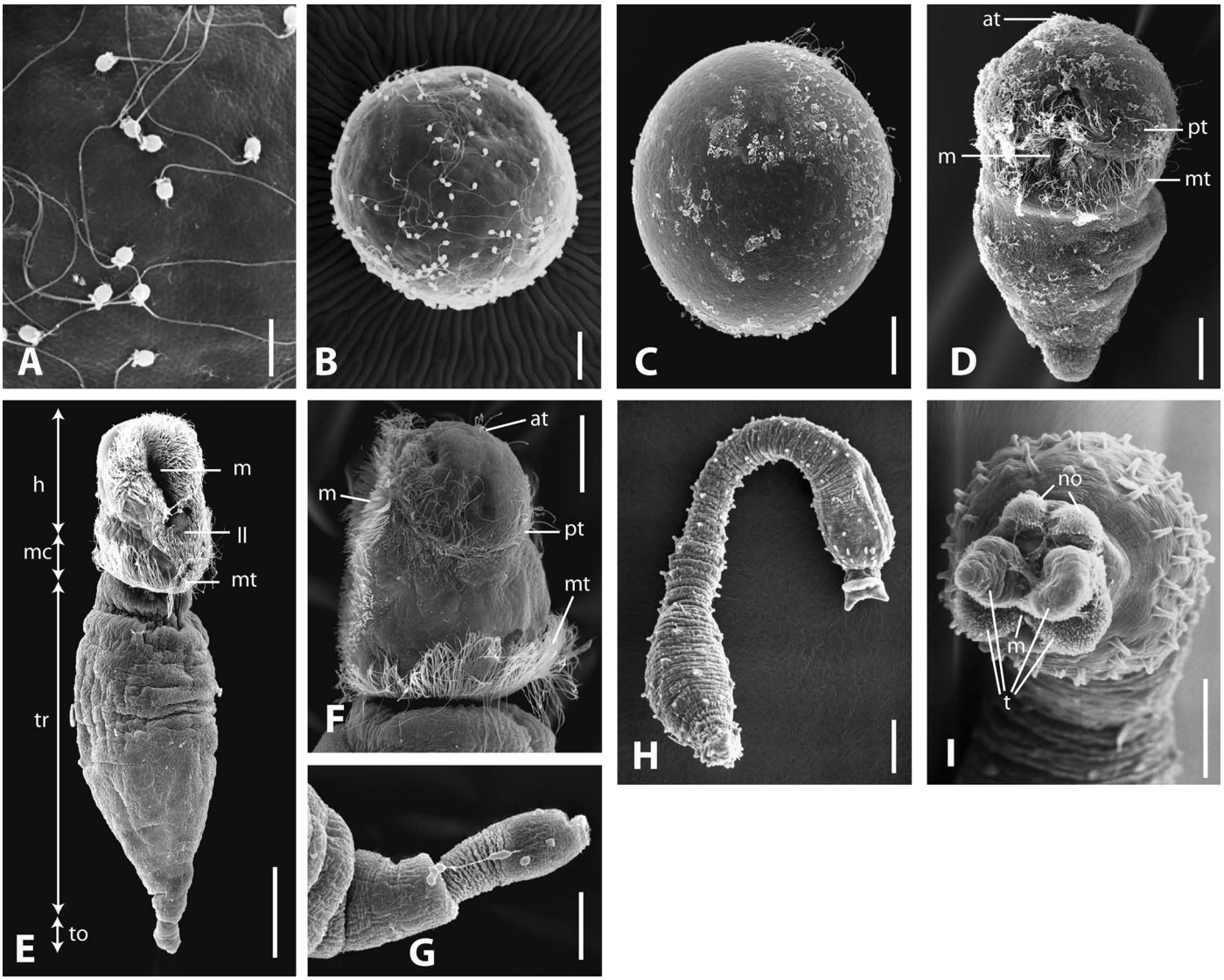
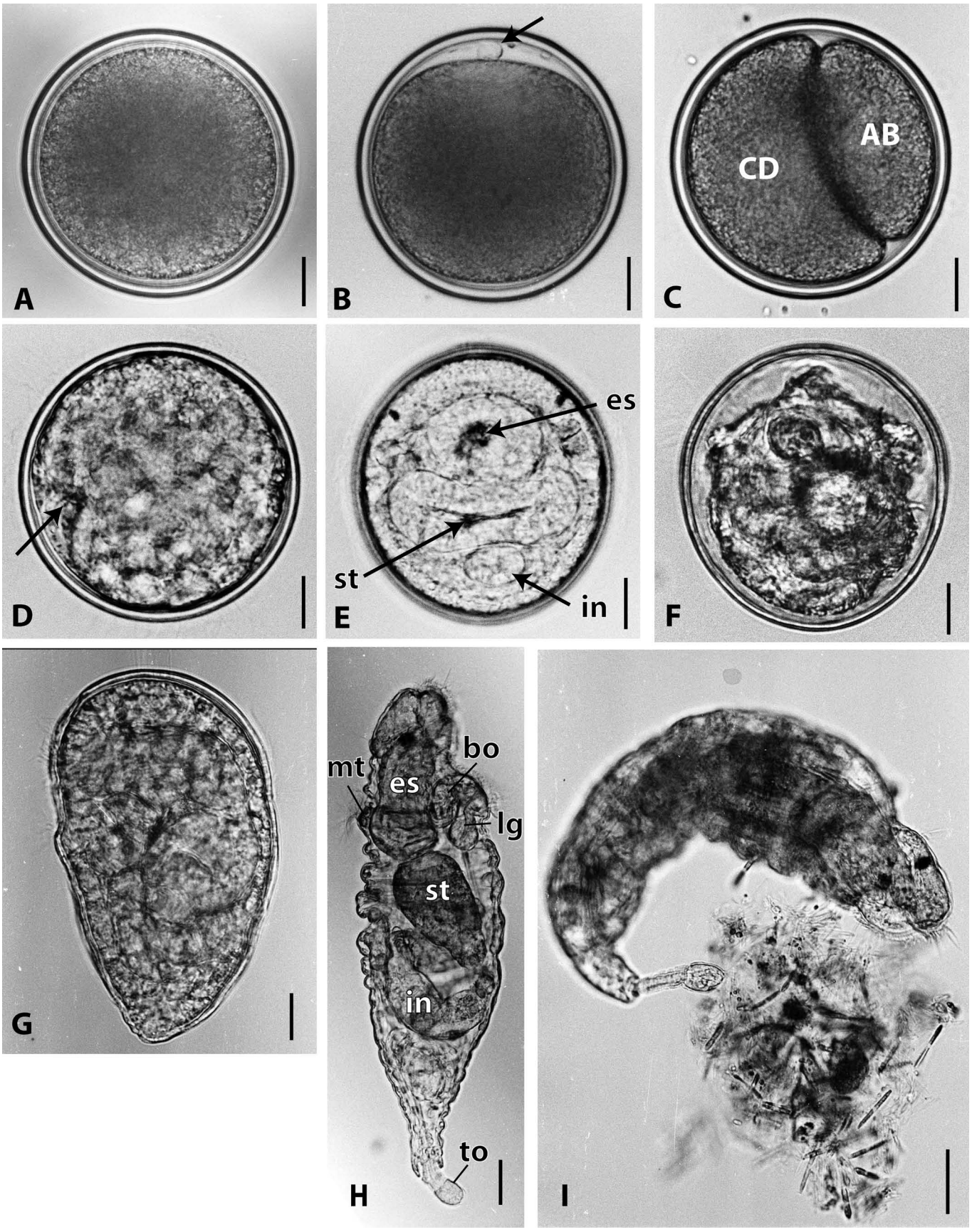


FIGURE 1. Scanning electron micrographs showing development of *Nephasoma pellucidum* (scale bar lengths here in parentheses). A. Sperm on the surface of the egg (5 μm). B. Egg with sperm on surface (20 μm). C. Trochophore larva; note cilia extending through egg envelope (20 μm). D. Early pelagosphera larva (20 μm). E. Fully formed pelagosphera larva, ventral view (50 μm). F. Head of a pelagosphera larva, lateral view (20 μm). G. Terminal organ of the pelagosphera larva (10 μm). H. Metamorphosed juvenile (100 μm). I. Tip of juvenile introvert with tentacle buds and lobes of nuchal organ (50 μm). Abbreviations: at = apical tuft; h = head; ll = lower lip; m = mouth; mc = metatrochal collar; mt = metatroch; no = nuchal organ; pt = prototroch; t = tentacles; to = terminal organ; tr = trunk. (Images A, B from Rice, 1989: fig. 4E,F; used with permission)

FIGURE 2. (*facing page*) Light micrographs showing development of *Nephasoma pellucidum* (scale bar lengths here in parentheses). A. Unfertilized egg (20 μm). B. Egg with polar body (arrow) (20 μm). C. Two-cell stage; note size difference between CD and AB blastomeres (20 μm). D. Blastula stage; beginning invagination of stomodaeum (arrow) (20 μm). E. Early trochophore; note eyespots at anterior end (top) (20 μm). F. Trochophore shortly before metamorphosis to pelagosphera (20 μm). G. Early pelagosphera in the process of elongation (20 μm). H. Fully metamorphosed pelagosphera larva (20 μm). I. Feeding pelagosphera, 10 d old (50 μm). Abbreviations: bo = buccal organ; es = esophagus; in = intestine; lg = lip gland; mt = metatroch; st = stomach; to = terminal organ.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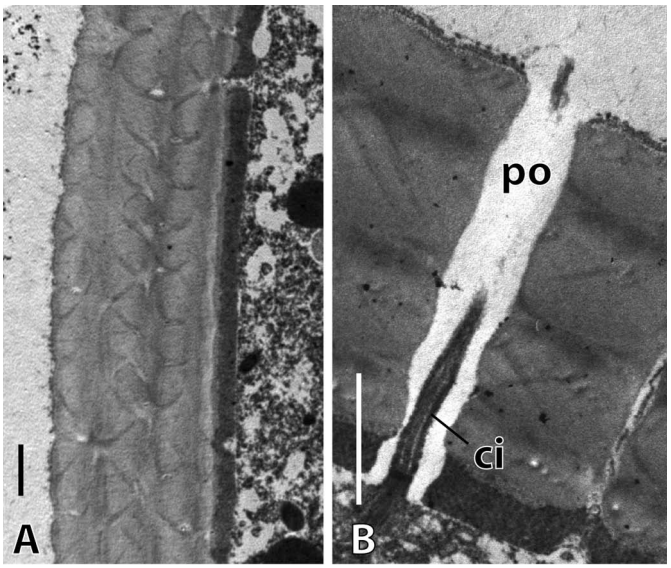

FIGURE 3. Transmission electron micrographs showing egg envelopes of *Nephasoma pellucidum* (scale bars = 1 μm). A. Section through multilayered egg envelope. B. Cilium growing through pore in egg envelope of trochophore larva. Abbreviations: ci = cilium; po = pore.

The trochophore is lecithotrophic, being completely enclosed by the egg envelope (Figure 2E,F).

Metamorphosis of the trochophore to the pelagosphera larva occurs at 60 to 65 h (23°C) and extends over a period of 6 to 8 h. The body elongates from 140 μm to 250 μm, by an increase in the length of the posttrochal hemisphere. The lumen of the gut is completed, and mouth and anus break through the overlying egg envelope (Figures 1D, 2G). The ventral ciliated surfaces of the head and the lower lip are formed apparently by an evagination and expansion of the anterior stomodaeum. Larval organs of the lower lip become functional: the buccal organ is protrusible, and the pore of the lip glands opens (Figure 2H). The metatrochal cilia project from a prominent metatrochal collar posterior to the mouth and lower lip. As the retractor muscles become functional, the entire pretrochal body is retractable into the posterior or posttrochal region of the larva. The coelom is considerably expanded, and the posttrochal body is capable of great extension and contraction. Whereas the posttrochal egg envelope is transformed into the larval cuticle, the pretrochal egg envelope is gradually sloughed off, leaving a thin cuticle covering the head. The terminal organ appears first as an evagination of the posterior extremity of the trunk and within a few hours differentiates into a more discrete elongate structure (40 μm) that is retractable into the trunk and provides a temporary attachment for the larva to the substratum (Figure 1G).

Pelagosphera: Morphology, Behavior, and Metamorphosis to the Juvenile

Four regions of the body can be distinguished: head, metatrochal collar, trunk, and terminal organ (see Figures 1E,F, 2H). The terminal organ is well developed with an unusually long neck, terminated by a bulbous posterior expansion (Figure 1G). The terminal organ of a 10-day larva may be extended to a length one-third that of the entire larva.

For approximately two weeks after metamorphosis, the majority of larvae are attached by their terminal organs; some continue to swim, or else attach and swim intermittently. At two weeks there is a high rate of mortality and, in the absence of substratum, most larvae die within two months; the maximum survival time of larvae reared in culture dishes is 103 days. Surviving larvae of one month of age attain a maximum size of 1.2 mm. At this age the body proportions have changed, the head being relatively smaller than in the younger stages. The external body wall is smooth, glistening in reflected light, and through the relatively transparent body wall the gut is apparent as an elongate dark yellow stomach and a lighter yellow recurved intestine, ending at the dorsal anus in the anterior trunk. Usually larvae are still attached by the terminal organ at these later stages, although some may lie on the bottom, relatively quiescent. Swimming occurs only rarely, although metatrochal cilia are still present.

Attached larvae are observed to feed on the substratum surrounding their points of attachment (Figure 2I). The body may be bent downward so that the ventral surface of the head is applied to the bottom of the dish, or the body may be stretched out from the point of attachment parallel to the substratum. In culture dishes in which there is an algal growth covering the bottom, the area surrounding the attached larva is often bare, indicative of larval grazing activity. The area of attachment is often marked by clumps of feces on which the larva may graze and ingest. Occasionally larvae release themselves from the attachment and swim or move along the bottom to a new site. Free larvae sometimes move with head applied to the substratum and posterior end directed upward, either exploring or feeding on the bottom. Frequently the terminal organ is placed in or near the mouth. Older larvae detach and move to new locations less often than younger larvae. A larval behavior, common to all sipunculans but of unknown function, is placement of the terminal organ in or near the mouth.

Metamorphosis of larvae reared in culture dishes could be induced at the age of 5 to 6 weeks by exposure to a fine, muddy sediment that had been occupied previously by adults. Attempts to induce metamorphosis before this age were not successful. Before metamorphosis, larvae buried themselves in the sediment and in 3 d underwent metamorphic changes to the juvenile stage.

The process of metamorphosis is initiated by the loss of the metatrochal cilia, reduction in the size of the lower lip, narrowing of the head, and elongation of the pretrochal body. At the end of 3 d, both posttrochal and pretrochal regions of the body are narrowed and elongated, the metatrochal collar is reduced, the terminal organ and lip are partly regressed, the mouth moves to a terminal position, and dorsal to the mouth a pair of developing tentacular lobes is apparent (Figure 1H,I). These morphological modifications, along with the behavioral changes of initiation of burrowing and cessation of swimming, mark the beginning of the juvenile stage. Regions of the body of the juvenile are reduced from the four found in the larva to two: (1) the broader and longer posterior trunk, formed from the posttrochal larva, and (2) the more narrow anterior introvert, which is terminated by mouth and developing tentacles and formed from the pretrochal larva. Similar to the pretrochal larval body, the introvert of the juvenile is retractable into the trunk.

The most immediate modifications are found in the head and metatrochal regions. As the mouth becomes terminal, the dorsal surface of the head is foreshortened. The ventral lip is lost, but ciliation of the ventral surface of the head persists to surround the mouth and the ventral surface of the developing dorsal lobes. On the dorsal head two heavily ciliated patches that will give rise to the paired nuchal organ have moved further anteriorly as the head foreshortens. In the 7- to 9-day-old juveniles the buccal organ is no longer apparent. One to four rings of simple hooks appear in the region of the former metatrochal band. Papillae, already apparent in older larvae, are more prominent and numerous. Scattered among the hooks, the papillae are dome shaped and, as seen in scanning electron micrographs, have central pores from which several cilia protrude. Papillae of similar structure, but somewhat larger, cover the entire trunk (Figure 1H). A vestigial terminal organ may persist for one or two weeks. Within two to four weeks a second pair of rudimentary tentacles appear ventral to the mouth.

The body wall of the juvenile thickens, losing its transparency. Externally circular constrictions, also seen in late larval stages, are more prominent. Juveniles of one week also show longitudinal "folds" of the body wall, resulting in a checkered appearance of the integument in some regions.

DISCUSSION

Nephasoma pellucidum is one of the few sipunculan species in which the life cycle has been observed from spawning to juvenile stage. Other species are *Siphonosoma cumanense* (Rice, 1988), *Thysanocardia nigra* (Rice, 1967), *Themiste pyroides* (Rice, 1967), *Themiste lageniformis* (Pilger, 1987), *Themiste alutacea* (Rice, 1975c), *Phascolion strombus* (Åkesson, 1958; Wanninger et al., 2005), and *Phascolion cryptum* (Rice, 1975c). Most of these species show abbreviated development, either omitting both the trochophore and pelagosphera stage, or omitting the pelagosphera stage, or having a lecithotrophic pelagosphera (Rice, 1976). The oceanic, planktotrophic pelagosphera larvae of many aspidosiphonid and phascolosomatid larvae have been recovered in plankton tows; however, their complete life cycles are unknown (Rice, 1981; Hall and Scheltema, 1975).

We argue that a model species should show the ancestral developmental mode for the taxon. Cutler (1994) concluded that indirect development with a planktotrophic pelagosphera was ancestral in Sipuncula. The most recent phylogenetic analyses (Schulze et al., 2007; Schulze and Rice, 2009) seem to confirm this view. The genera *Sipunculus* and *Siphonosoma*, which to our present knowledge only contain species with planktotrophic pelagosphera larvae, represent the two basal clades in both analyses. The remaining three major clades have species of *Phascolosoma* and *Apionsoma* as their basal branches, two additional genera in which abbreviated development is unknown.

Of the species listed above, only the life cycle of *Siphonosoma cumanense* includes a planktotrophic pelagosphera as *N. pellucidum* does. *Siphonosoma cumanense* is a large, sand-burrowing species. Like other sipunculans, it survives well under laboratory conditions, when supplied with sediment and adequate aeration. However, its potential for use as a model species is limited by two factors. First, even though the species has a wide geographic distribution, it is rarely found in significant numbers, and the establishment of a viable population would require major efforts. Second, larvae do not seem to be competent to metamorphose until about 8 weeks old (Rice, 1988).

Nephasoma pellucidum is geographically widespread, mostly in shallow warm waters, although it does not seem to be as abundant in most places as at our collecting station

near Fort Pierce, Florida. Collection of a significant number of individuals can be time consuming because they have to be carefully removed from the cracks and crevices of rubble; the removal process can damage the animals, often causing their death. After successful retrieval, however, adults are easy to maintain in laboratory conditions. Removed from their shelter, they survive in simple glass bowls without aeration or food supplement for at least a year, feeding only on the biofilm at the bottom of the dish. We assume that, left in their shelter or in sediment, with proper aeration and occasional food supply, they would survive for years. This assumption is based on the longevity of other sipunculans: individuals of *Apionsoma misakianum* have been kept in holding tanks at the Smithsonian Marine Station for nearly 30 years.

Nephasoma pellucidum spawns frequently during the warmer months of the year. Spawning can be induced by changing the seawater in the dish, although this procedure does not reliably yield the desired results, leaving some uncertainty as to when the spawning occurs. Embryos and larvae are easy to observe with different microscopic techniques. Mortality before the first metamorphosis, from trochophore to pelagosphera, is minor. The pelagosphera larvae are more transparent than other sipunculan larvae, facilitating observation by light and confocal laser scanning microscopy. In contrast to the pelagosphera larvae of some other sipunculan species, they relax relatively well when temporarily cooled to 4°C and treated with menthol, magnesium chloride, or 10% ethanol, leaving their head and terminal organ exposed.

The increase in mortality during the prolonged pelagosphera phase presents some difficulties. By the time metamorphic competence is reached, the percentage of surviving larvae is low. A further reduction in numbers occurs at metamorphosis, because not all larvae respond to the settlement cue, that is, adult-conditioned sediment. Therefore, postmetamorphic juveniles are only rarely observed. Common metamorphosis-inducing agents such as potassium chloride, cesium chloride, gamma-aminobutyric acid, 3,4-dihydroxy-L-phenylalanine (L-dopa), and isobutylmethylxanthine (Bryan et al., 1997; Morse et al., 1979; Yool et al., 1986) seem to have no effect on metamorphosis in *N. pellucidum*.

As a conclusion, among the sipunculans for which development has been studied, *N. pellucidum* is a good candidate for a model species. Rearing larvae through metamorphosis still presents some difficulties, and future work should focus on optimizing the conditions. Recently the cold-water species *Phascolosoma agassizii* from the Sea of Japan, which also has a planktotrophic pelagosphera, has been reared through metamorphosis (A. S. Maiorova and A. V. Adrianov, Institute of Marine Biology, Far East Branch of the Russian Academy of Sciences, Russia, personal communication) and might be another appropriate candidate for a model species, even though metamorphosis could never be observed in individuals of the same species collected in the Pacific Northwest and reared at the University of Washington Friday Harbor Laboratories.

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Mitochondrial Phylogeography of the Intertidal Isopod *Excirolana braziliensis* on the Two Sides of the Isthmus of Panama

Renate Sponer and Harilaos A. Lessios

ABSTRACT. The intertidal isopod *Excirolana braziliensis* Richardson possesses limited means of dispersal; there is no larval stage, and adults remain sedentary under the sand. It is represented on the two coasts of Panama by three morphs, two in the Pacific (P and C′) and one in the Atlantic (C). Previous work has quantified morphometric differences between the morphs, found that there are multiple allozyme differences between them, and produced indirect evidence that they are reproductively isolated from each other. Here we report comparisons of 345 bp of 12S and 678 bp of cytochrome oxidase I (COI) mitochondrial DNA (mtDNA) from three populations of each morph. The mtDNA sequences from the three morphs are reciprocally monophyletic, strengthening the case for recognizing them as separate species. As in morphology and isozymes, the C morph and the C′ morph are sister clades, and the P morph is an outgroup. In contrast to what was previously supposed, the C and C′ morphs neither are the result of a recent introduction from one ocean to the other, nor were separated at the final stages of the completion of the Isthmus of Panama three million years ago, but rather are anciently separated sister clades that now exist on separate shores. Patterns of mitochondrial gene flow between populations of the same morph vary. The C and C′ morphs show large genetic differences between local populations, as would be expected from an organism with such limited vagility. In the P morph, on the other hand, populations from localities 5 km apart are identical in mitochondrial DNA, even though they differ in one allozyme locus, suggesting the possibility of sex-biased migration.

INTRODUCTION

Many marine organisms are capable of dispersing over large distances at some point of their life cycle. The population genetics of such organisms usually reveal a genetic neighborhood size in the order of thousands or tens of thousands of kilometers. Some marine species, however, provide a contrast to this picture of wide dispersal in that they lack any means of transferring their genes by either vagile adults or free-swimming larvae, yet have wide geographic ranges. How much gene flow may occur between noncontiguous populations of such species and whether species cohesion is maintained in the face of limited vagility is of special interest to population genetics. The tropical isopod *Excirolana braziliensis* is an example of a species apparently spread over the tropical seas of Americas, even though it lacks the means of maintaining genetic contact between distant populations.

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Excirolana braziliensis Richardson is a common isopod of intertidal beaches on both sides of the Americas, from the Pacific coast of Mexico (30°N) to S. Chile (40°S) and from the northern Caribbean (31°N) to Uruguay (25°S) (Cardoso and Defeo, 2003). It is a small (approximately 3–4 mm in length), dioecious species, which reaches its highest abundance just above the high-tide mark, where it lives buried in the sand during low tide and rises to the water column at high tide to feed on live and dead fishes and invertebrates (Brusca and Iverson, 1985). *E. braziliensis* has very limited means of dispersal. The female carries broods of 4 to 17 offspring per reproductive event. Young are released directly into the adult habitat (Klapow, 1970). The frequency of reproduction is such that a population may turn over every four months (Brusca and Iverson, 1985). In Panama, recruitment occurs throughout the year (Dexter, 1977). Dispersal in *E. braziliensis* may occur as a result of feeding events, during which individuals of this genus have been observed attached to fish or other prey items for several minutes (Brusca, 1980). This behavior may represent the only means of transport of this organism between beaches because free-swimming individuals are likely to be eaten by fish.

Weinberg and Starczak (1988, 1989) reported the existence of three morphological variants of *E. braziliensis* from Panama. Two similar and presumably closely related types, termed C and C′, are found on the Caribbean and Pacific coasts, respectively. The third type (P) is morphologically distinct from C and C′; its distribution overlaps with that of C′ throughout most of its range (Weinberg and Starczak, 1989). In general, Pacific beaches contain only one of the two morphotypes. Nevertheless, 2 of 43 beaches sampled by Weinberg and Starczack (1989) were found to contain C′ and P morphs in approximately equal numbers. The geographic patterns of morphotype distribution and genetic composition remain stable over time, but there are occasional complete replacements of entire beaches by a different morph, presumably as the result of extirpation and subsequent recolonization (Lessios et al., 1994). Morphological and genetic divergence (based on allozyme data) between morphs are highly correlated and large enough to suggest that the P morph constitutes a distinct species (Lessios and Weinberg, 1994). The allozyme data are also consistent with the hypothesis that the C and C′ morphotypes are geminate species that resulted from the rise of the Panamanian Isthmus three million years ago (Lessios and Weinberg, 1994).

Allozyme analyses indicate that the three morphotypes of *E. braziliensis* are probably reproductively isolated, because they form few hybrids even when they co-occur at the same beach. Even within morphotypes, gene flow between populations from different beaches is low, as deduced from the predominance of distinct alleles in one or more loci, even among beaches situated less than 5 km apart. However, dispersal (as measured by individuals homozygous for alleles that otherwise occur on a different beach) is rather high, suggesting that some form of reproductive isolation prevents them from mating with individuals from the local population (Lessios and Weinberg, 1993).

The purpose of the present study is to investigate the phylogenetic and phylogeographic relationships within and between the three morphotypes of *Excirolana braziliensis* using sequences of mitochondrial DNA (mtDNA). Specifically, we are addressing the following questions: (1) Does mtDNA show patterns of genetic divergence, phylogeny, and geographic distribution congruent with those suggested by isozymes and morphology? (2) When did the three lineages diverge? (3) What are the patterns of population genetic structure? Do mtDNA data show similar levels of gene flow as isozymes within and between morphotypes? (4) What processes can explain mtDNA discrepancies between patterns from mtDNA and allozyme markers?

MATERIALS AND METHODS

Sample Collection

Excirolana braziliensis were obtained from nine locations along the Pacific and Caribbean coasts of Panama (Figure 1). Each of the three morphotypes was represented in our collections by three populations. Isopods were collected on beaches during low tide. The top 10 cm of sand at haphazard locations above the high-tide mark were sifted through a 500 μm sieve, and isopods were placed in plastic bags with wet sand. The collected isopods were brought alive to the laboratory and frozen at −80°C. The majority of samples used in this study were from collections made in 1988, the same collections used to assay isozymes (Lessios and Weinberg, 1993, 1994). Additional individuals were collected in 1998 from Isla Culebra. Specimens of *Excirolana mayana* were also collected at Isla Culebra to be used as outgroups.

DNA Extraction, Polymerase Chain Reaction, and mtDNA Sequencing

Genomic DNA was extracted using a standard phenol/chloroform protocol (Sambrook et al., 1989) with ethanol precipitation. For amplification and sequencing

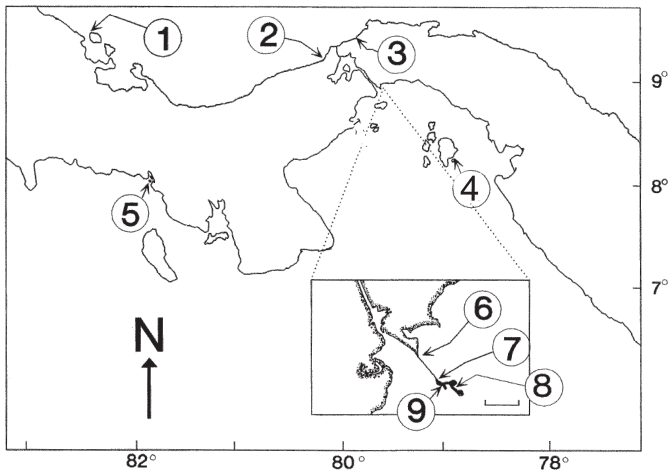

FIGURE 1. Map of Panama, indicating localities in which *Excirolana braziliensis* was sampled (sample size in parentheses). 1, Bocas del Toro (14); 2, Shimmey Beach (10); 3, Maria Chiquita (9); 4, Isla Santelmo (18); 5, Isla Adentro (15); 6, Causeway (6); 7, Lab (8); 8, Perico (10); 9, Isla Culebra (14).

of 345 base pairs (bp) of the 12S mtDNA gene, we used the universal primers 12Sa and 12Sb (Simon et al., 1994). A 678 bp fragment of cytochrome oxidase I (COI) was amplified and sequenced with combinations of the forward primers BWBK (5′-GAG CTC CAG ATA TAG CAT TCC-3′) and ISO-F1 (5′-CYC TTT TAT TAG GRA GGG GG-3′) and the reverse primers BWBJ (5′-CAA TAC CTG TGA GTC CTC CTA-3′) and ISO-R2 (5′-ACR GCA ATA ATT ATG GTA GC-3′). The following conditions were used for polymerase chain reaction (PCR): initial denaturation for 2 min at 94°C, then 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 50°–53°C, extension for 1 min at 72°C, and final extension for 10 min at 72°C. PCR products were cleaned for sequencing using silica gel purification columns. Cycle sequencing was carried out in both directions, with the ABI PRISM d-Rhodamine Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystematics). Sequences were obtained on an ABI 377 automated sequencer and verified and aligned by eye in the program Sequencher (Gene Codes Corporation). 12S was sequenced in 104 individuals; a subset of 22 individuals was also successfully sampled for COI, whereas the rest failed to amplify for this locus.

Phylogenetic Analysis

For phylogenetic analysis, identical haplotypes from multiple individuals were collapsed. We applied the program Modeltest 3.0 (Posada and Crandall, 1998) to calculate the goodness of fit of various models of DNA evolution. The selected model for the 12S data was that of Tamura and Nei (1993), with equal base frequencies, a gamma distribution with a shape parameter of 0.443, and the following substitution rates: [A—C] = 1.00; [A—G] = 6.00; [A—T] = 1.00; [C—G] = 1.00; [C—T] = 11.71; and [G—T] = 1.00. The selected model for the COI sequence was a transversional model (TVM + I; Posada and Crandall, 2001), with a proportion of 0.69 of invariable sites and the following substitution rates: [A—C] = 8908.76; [A—G] = 258211.81; [A—T] = 26092.37; [C—G] = 0.0001; [C—T] = 258211.82, and [G—T] = 1.00. A partition homogeneity test, executed in version 4.0b10 of PAUP* (Swofford, 2000), indicated that phylogenetic signals in the COI and 12S data were not significantly different ($P = 0.256$). The best fitting model for the combined 12S and COI data was HKY (Hasegawa et al., 1985) with a transition/transversion ratio of 11.46 and a gamma distribution shape parameter of 0.782. Employing these parameters, we ran phylogenetic analyses for 12S and COI separately and with the two DNA regions concatenated. We used the BioNJ algorithm (Gascuel, 1997) and heuristic searches in maximum parsimony and maximum likelihood with PAUP* (Swofford, 2000). Bootstrap confidence values for distance and likelihood trees were calculated in 5,000 and 500 iterations, respectively. Bayesian phylogeny inference was carried out in the program MrBayes v.3.04b (Huelsenbeck and Ronquist, 2001). Bayesian analyses on the COI and the combined data sets were run for 800,000 generations, of which the first 20,000 (2,000 trees) were discarded. For 12S, 2,760,000 generations were run, and 67,500 (6,750 trees) were discarded. Convergence of chains was determined by average standard deviations of split frequencies less than 0.01 and by potential scale reduction factors approximately equal to 1.0. The trees were rooted on sequences of *Excirolana mayana*. Clock-like evolution of sequences was tested with likelihood ratio tests. The tests were carried out in PAUP* 4.0b10 by calculating the difference in log-likelihood of the neighbor-joining trees (see above) with and without the enforcement of a molecular clock and comparing the likelihood ratios to the χ^2 distribution.

Geographic Distribution of Genetic Variation within Morphs

Genealogical relationships of haplotypes within species may be better represented by networks than trees, as

ancestral haplotypes may still be present in the population (Crandall and Templeton, 1993; Posada and Crandall, 2001). We calculated unrooted parsimony haplotype networks based on 12S for each of the three morphotypes separately, using the computer program TCS (Clement et al., 2000). In this method the parsimony limit (the maximum number of differences among haplotypes as a result of single substitutions) is calculated with 95% statistical confidence, and haplotypes are connected in order of increasing number of substitutions. To investigate the population genetic structure within each morphotype, we applied analysis of molecular variance (AMOVA; Excoffier et al., 1992) to the 12S data. Genetic variation for this analysis was assessed based on the Kimura (1980) two-parameter distance between haplotypes. The significance of fixation indices was tested by 10,000 rearrangements of haplotypes between populations. Calculations were carried out in version 2000 of the computer program ARLEQUIN (Schneider et al., 2000).

RESULTS

Descriptive Statistics and Phylogenetic Analysis

Although we sampled many more individuals of *Excirolana braziliensis* for 12S than for COI, trees based on the former DNA region (Figure 2) displayed less resolution than the combined analysis of both genes together. Despite this, all analyses of the 12S segment alone resulted in three distinct lineages, which correspond to the previously described C, C′, and P morphs. The 12S sequences of each morph were monophyletic in all analyses. The node joining C and C′ was well supported by maximum-parsimony analysis but fairly weakly supported by neighbor-joining, maximum-likelihood, and Bayesian analysis. The three main lineages were present in more than one beach, but each beach contained representatives of only one lineage. Although Santelmo had previously been found to contain a mixture of C′ and P morphotypes and the allozymes corresponding to these morphs (Weinberg and Starczak, 1989; Lessios and Weinberg, 1994), all nineteen 12S sequences from Santelmo, differing from each other by a maximum of three substitutions, belonged to the C′ morph. The tree based on fewer sequences of COI (not shown) and the tree based on the combined data (Figure 3) were well resolved and gave strong support to the expected grouping of the C and C′ lineages as sister groups, irrespective of the type of phylogenetic algorithm used.

The 12S Tamura and Nei average distances ranged between 11% and 18% between morphs (Table 1). Within morphs, distances varied between 0% and 2.3%. For COI, average distances (TMV) among lineages were 17.4%–26.1% and within lineages 0%–1.5%. There were five amino acid changes in the COI fragment, of which four were substitutions of nonpolar for nonpolar residues (Met/Ileu; Val/Ileu) and one was a nonpolar for a polar residue (Ala/Thr). Three of the changes differentiate the C/C′ and P lineage; one groups C and P, versus C′, and one is shared between C′ and P, compared to C. Likelihood ratio tests failed to reject the hypothesis of clock-like evolution of either the 12S or the COI sequences ($P > 0.05$).

Genetic Variation within Morphs

Parsimony haplotype networks showed that populations of the C and C′ morphs, but not the P morph, were genetically structured (Figure 4). The most common and (presumably) ancestral haplotype of the P morph was shared by all three populations. Two derived haplotypes were also shared, one between all populations and the other between two populations. In the C′ morph two haplotypes, including the ancestral one, were shared between Santelmo and Isla Culebra. Although Isla Adentro contained three haplotypes not found in any other population, the majority of specimens from this island were of a single haplotype, leading to a low haplotype diversity compared to other populations ($H = 0.14$). The population at Bocas del Toro (C morph), was characterized by high nucleotide diversity compared to other populations ($\pi = 0.0077 \pm 0.0045$). Haplotypes from Bocas del Toro were differentiated from Maria Chiquita and Shimmey Beach by one to eight substitutions whereas the latter two populations shared the ancestral haplotype.

In the C morph, AMOVA (Table 2) found that 67.44% of genetic variance was partitioned among populations; population pairwise F_{ST} comparisons (Table 3) showed that all populations of this morph were significantly differentiated from each other. In the C′ morph, 35.62% of the variance was the result of differences between populations. The population at Adentro had significantly high F_{ST} values when compared to both Santelmo and Culebra, whereas the latter two were not significantly different from each other (Table 3). In the P morph, all the genetic variance was contained within populations, a result in stark contrast with high levels of population subdivision seen in the other two morphs.

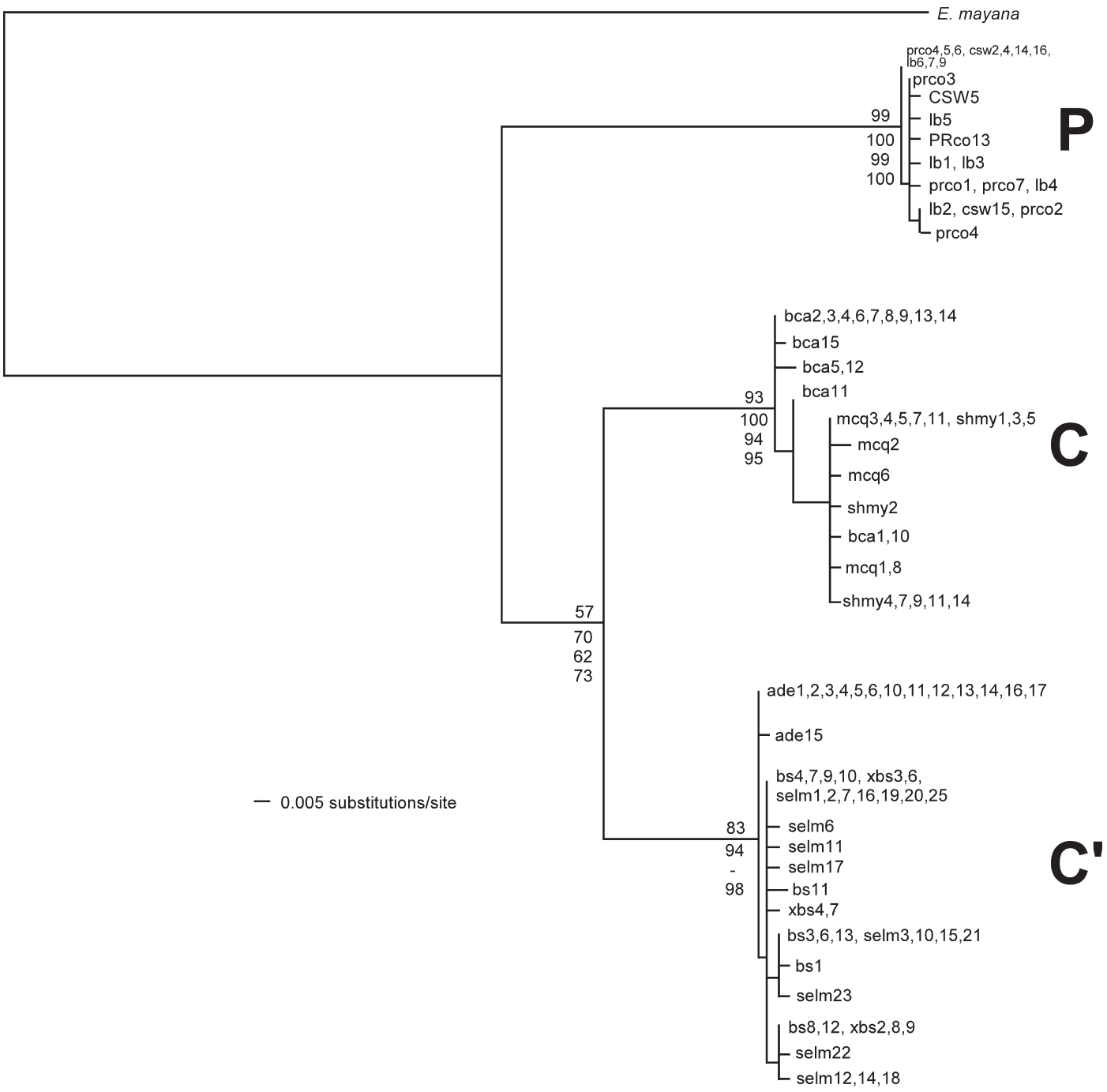

FIGURE 2. 12S mitochondrial DNA (mtDNA) maximum-likelihood bootstrapped consensus tree relating three morphotypes (C, C′, and P) of *Excirolana braziliensis*. Numbers above branches indicate maximum-likelihood bootstrap confidence values; numbers below branches refer to posterior probabilities (Bayesian analysis), neighbor-joining bootstrap support, and maximum-parsimony bootstrap support, respectively, from top to bottom. Support values <50% are not shown. Locality codes of specimens: prco = Perico; csw = Causeway; lb = Lab; bca = Bocas del Toro; mcq = Maria Chiquita; shmy = Shimmey Beach; ade = Isla Adentro; bs = Isla Culebra; selm = Santelmo; xbs = Isla Culebra (xbs specimens were collected in 1998; all other samples were collected in 1988). See Figure 1 for the position of each locality.

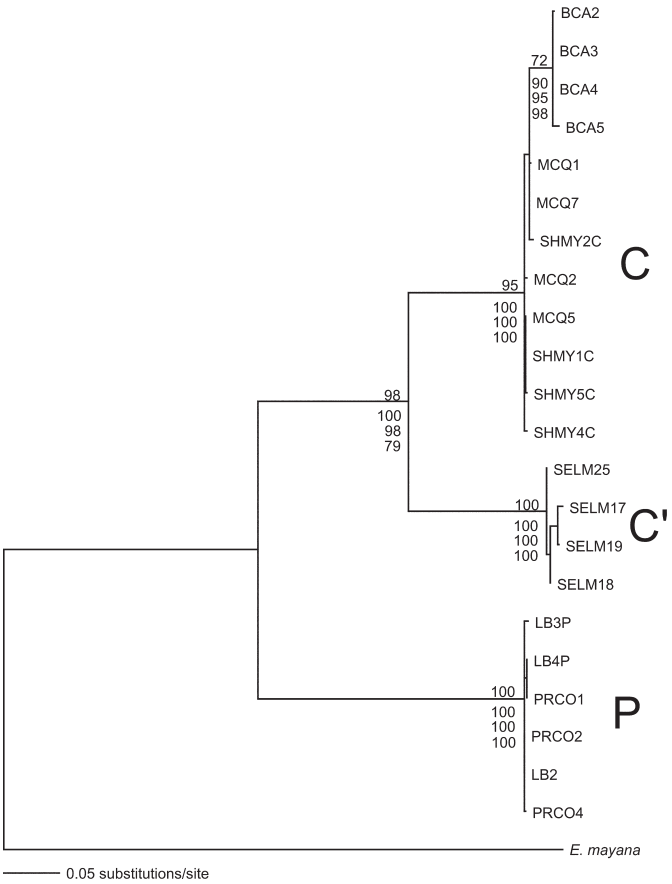

FIGURE 3. Combined 12S/cytochrome oxidase I (COI) mtDNA maximum-likelihood bootstrapped consensus tree relating three major lineages (C, C′, and P) of *Excirolana braziliensis* in Panama. Numbers above branches indicate maximum-likelihood bootstrap confidence values, numbers below branches refer to posterior probabilities (Bayesian analysis), neighbor-joining bootstrap support, and maximum-parsimony bootstrap support, respectively, from top to bottom. Localities: BCA = Bocas del Toro; MCQ = Maria Chiquita; SHMY = Shimmey Beach; SELM = Santelmo; LB = Lab; PRCO = Perico.

DISCUSSION

The mtDNA data presented here confirm the results from analysis of both morphology (Weinberg and Starczak, 1988, 1989; Lessios and Weinberg, 1994) and allozymes (Lessios and Weinberg, 1994) that *Excirolana braziliensis* populations from the Pacific and Caribbean coasts of Panama consist of three distinct lineages. Allozymes suggest that these lineages are reproductively isolated (Lessios and Weinberg, 1993) and should, therefore, be considered separate species. Although mtDNA data agree with morphological and allozyme data on the grouping of the C and C′ lineages as sister groups with respect to P, the relative magnitude of the measures of differentiation in the three sets of characters is different. Mahalanobis generalized distance from morphometric characters and Nei's D from allozymes indicate that the P morphotype is three times more distant from C and C′ than the latter are from each other (Lessios and Weinberg, 1994). Mitochondrial DNA, on the other hand, gives a P/(C, C′) distance that is only 1.2 to 1.3 times higher than that between C/C′. It is clear that each type of character evolves at a different rate.

TABLE 1. Genetic distances within (along diagonal) and between (below diagonal) morphs of *Excirolana braziliensis*, for 12S (Tamura and Nei, 1993) and cytochrome oxidase I (COI) mitochondrial DNA (mtDNA) (in parentheses; TVM + I [Posada and Crandall, 2001]).

Morph	C	C′	P
C	2.25 (1.52)	–	–
C′	11.03 (17.38)	1.36 (0.49)	–
P	16.21 (24.32)	17.84 (26.12)	1.19 (0.84)

A review of molecular divergence across the Isthmus of Panama in 34 lineages likely to have been separated by the final closure of the Isthmus of Panama (Lessios, 2008) has shown that during 3 million years of independent evolution (Coates and Obando, 1996; Coates et al., 2005), crustacean COI has accumulated genetic distances ranging from 4.1% to 8.7% (Knowlton and Weigt, 1998; Schubart et al., 1998; Williams et al., 2001; Morrison et al., 2004) and 12S ranging from 2% to 3% (Robles et al., 2007). Based on these calibrations, and given the differences we determined in COI and 12S, the divergence of the P morph from the two C morphs occurred between 9 and 25 million years ago and that of C from the C′ morph between 6 and 17 million years ago. Thus, in contrast to what was surmised by Lessios and Weinberg (1994) on the basis of isozymes, mtDNA data do not support the idea that the C and C′ morphotypes were isolated at the final stages of the closure of the Panamanian Isthmus, 3 million years ago, but rather that their populations were separated well before the final closure. On the basis of molecular divergence, this appears to be also the case in 73 other amphi-isthmian sister lineages of crustaceans, sea urchins, fishes, and mollusks (Lessios, 2008).

The combination of large mitochondrial differences and evidence for reproductive isolation from allozyme data (Lessios and Weinberg, 1993, 1994; Lessios, 1998) rules out the hypothesis that C′ merely represents a recently

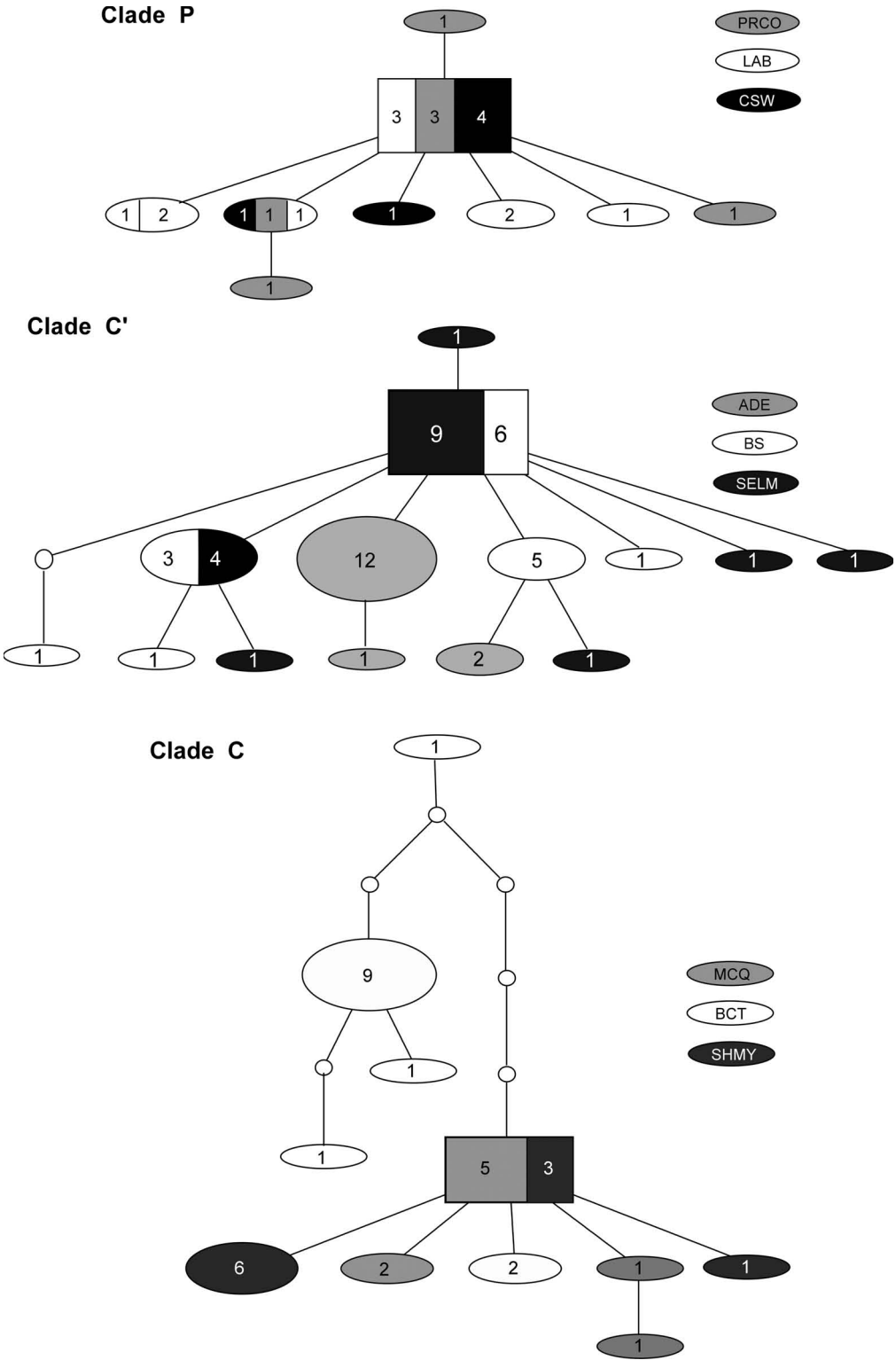

FIGURE 4. Parsimony network of 12S mtDNA haplotypes of the three morphs (clades) of *Excirolana braziliensis*. Each large oval represents a unique haplotype, boxes represent ancestral haplotypes, and small ovals indicate hypothetical, intermediate haplotypes not observed in the populations. The size of each shape represents the frequency of each haplotype. Numbers within each symbol indicate the number of individuals bearing each haplotype. Localities: Prco = Perico; Lab = Lab; CSW = Causeway; ADE = Isla Adentro; BS = Isla Culebra; SELM = Santelmo; MCQ = Maria Chiquita; BCT = Bocas del Toro; SHMY = Shimmy Beach.

TABLE 2. Analysis of molecular variance (AMOVA) of Panamanian populations of *Excirolana braziliensis* based on 12S mtDNA sequences. Partitioning of genetic variance within and between populations (beaches) was estimated for each morph (clade) separately. The significance of fixation indices was tested by 10,000 permutations.

	Variance (%)			
Morph	Between populations	Within populations	Φ_{CT}	*P*
C	67.44	32.56	0.67	<0.001
C′	35.62	64.38	0.36	<0.001
P	−2.39	102.39	−0.02	>0.05

TABLE 3. *Excirolana braziliensis* population pairwise F_{ST} values from 12S mtDNA sequences. Bold values are significant at the $P < 0.01$ level.

	P Morph	
Locality	Lab	Perico
Perico	−0.02	–
Causeway	−0.02	−0.04
	C Morph	
Locality	Maria Chiquita	Bocas del Toro
Bocas del Toro	**0.69**	–
Shimmey Beach	**0.27**	**0.71**
	C′ Morph	
Locality	Isla Adentro	Isla Culebra
Isla Culebra	**0.58**	–
San Telmo	**0.50**	−0.02

introduced population of C from the Caribbean into the Pacific, as had been suggested by Weinberg and Starczak (1988, 1989) and strengthens the case that each of the three lineages represents a distinct species.

Population Structure and Dispersal

Populations of the C and C′ morphs were characterized by population subdivision, as illustrated by high F_{ST} estimates (overall values of 0.67 and 0.36, respectively), whereas samples from different localities of the P morph can be considered as belonging to the same genetic population ($F_{ST} = -0.02$). Populations from Isla Adentro (C′ morph) and from Bocas del Toro (C morph) stand out for their lack of alleles shared with individuals from other localities. Maria Chiquita and Shimmey Beach (C morph) also have significantly different allele frequencies, whereas the populations at Santelmo and Isla Culebra, as well as at Perico, Lab and Causeway, are not significantly differentiated. The two populations most divergent from others in the same morph, Adentro and Bocas del Toro, are also the most geographically distant from other localities containing individuals of their respective morphs, raising the possibility that dispersal to and from these localities is restricted as a result of physical distance. With only three populations per morph, statistical verification of a correlation between geographic and genetic distances is not meaningful.

We observed several differences in the degree of population subdivision when comparing mtDNA and allozyme markers (Lessios and Weinberg, 1994). Based upon mtDNA sequence, the populations at Bocas del Toro and Shimmey Beach were the most different of all ($F_{ST} = 0.71$), whereas their allozyme allele frequencies were rather similar ($F_{ST} = 0.097$, as calculated from data in Lessios and Weinberg, 1993). On the whole, mtDNA data suggest a higher divergence between the morphs, but a lesser degree of subdivision between populations of the same morph, compared to data on allozymes. These results support Lessios and Weinberg's (1993) findings that dispersal among populations is much higher compared to gene flow, because even individuals of the same morph show some sort of reproductive isolation. According to their estimates, up to 2.5% of individuals in a locality consist of new immigrants that do not inject their genes into the host population, indicating that some form of reproductive isolation exists between populations of the same morph, even at the scale of a few kilometers. The data from Santelmo are interesting in this connection: This is the only locality in which two morphs, P and C′, coexist (Lessios and Weinberg, 1993, 1994). The number of hybrids between them, as judged by allozymes, is lower than would be expected from random mating (Lessios and Weinberg, 1993), but hybrids do exist. However, all 19 mitochondrial haplotypes from this locality belong to the mtDNA clade that corresponds to the C′ morph, despite having been sampled from the same collections as the allozymes. Barring the unlikely possibility of a sampling accident, this finding indicates that some individuals with a P nuclear genotype, as manifested in morphology and isozymes, actually carry

a C′ mitochondrial DNA. This, in turn, suggests that hybridization between the morphs, when it occurs, is successful in only in one direction, that is, only if the mother belongs to the C′ clade.

In conclusion, *E. braziliensis* in Panama consists of at least three lineages (C, C′, and P), which diverged well before the final closure of the Isthmus and warrant separate species status. Populations that are more than 30 km distant from each other (C, C′) are genetically divergent, whereas those at less than 5 km (P) are panmictic in mtDNA, even though they are different in at least one allozyme locus (Lessios and Weinberg, 1994). It remains to be seen whether population structure is a result of isolation by physical factors or whether the three species have inherently different dispersal potential, and whether the higher degree of gene flow in mtDNA relative to isozymes is the result of sex-biased migration.

Acknowledgments

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Stability and Change in the Indian River Area Bryozoan Fauna over a Twenty-Four Year Period

Judith E. Winston

ABSTRACT. Two surveys describe changes and stability in bryozoan assemblages at sites in the temperate to tropical transition zone of the Florida Atlantic coast over a 24-year interval in which seawater temperatures increased. Results of a monthly survey of the Indian River Area bryozoan fauna carried out in 1974–1975 as part of a postdoctoral fellowship at the Smithsonian Marine Station were published in 1982. The existence of this baseline work made it possible to resurvey some of the same areas during 1998–1999 to determine whether the bryozoan communities at three of the sites in the original study had changed or remained stable. Results showed that most of the species that had been abundant at a site still occurred at that site 24 years later, indicating a high degree of stability. However, there were some important changes. Temperate species such as *Hippoporina verrilli*, *Cryptosula pallasiana*, and *Bugula stolonifera*, which had been abundant in 1974, were rare or absent in 1998. Those species were replaced by Caribbean species, such as *Exechonella antillea* and *Caulibugula armata*. Although local seawater temperatures during the time period were not available, the Fort Pierce air temperature records indicated that despite the year-to-year variability in both minimum and maximum temperatures over the seasons, mean winter air temperatures maintained a slow increase from 1974 to 1999.

INTRODUCTION

Most ecological research projects are carried out over a very short time period, the length of a research grant or dissertation project, a few years at most, and once the researcher moves on to new studies these research efforts are seldom repeated. Long-term studies are essential to document effects of climate change in communities over time, but the number of such publications for marine communities is extremely low compared with the number documenting the effects of climate change in terrestrial systems (e.g., Richardson and Poloczanska, 2008). This paper describes a repeated survey of coastal and lagoon sites conducted 24 years after the original survey was completed.

In 1974 and 1975, as part of my research as a postdoctoral fellow at the Smithsonian Marine Station, I carried out monthly surveys of the bryozoan fauna at five intertidal sites in the Indian River Lagoon region, both in the lagoon itself and on the coasts of North and South Hutchinson Islands. Descriptions of the species found at these sites, together with descriptions of species taken in one-time

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collections at 18 additional localities in the region and notes on their distribution and ecology, were published in a taxonomic paper, "Marine Bryozoans (Ectoprocta) of the Indian River Area (Florida)" (Winston, 1982). Over the years I returned to the area many times to study various aspects of the biology and ecology of the bryozoans of the region. The apparent persistence of species at particular sites year after year led me to believe that bryozoan communities in the area might be very stable. Yet, the patchiness and limited extent of the hard substrata available for settlement, combined with the fact that certain species were found consistently at only a single site, made me wonder about the potential effect of a man-made or natural disturbance. If a site were to be destroyed, would that mean the regional extinction of the bryozoan species uniquely found there, or did they, in fact, have additional refuges at other sites in the area? To begin to answer these questions, 24 years after the first study, I resurveyed three of the original sites over a one year period in 1998–1999 to learn how stable was the species composition and to look for additions or losses of species at each site.

STUDY AREA

The Indian River Lagoon system, including Mosquito Lagoon, extends along about a third of the Atlantic coast of Florida, from Ponce de Leon Inlet to Jupiter Inlet, a distance of 295 km. Its western boundary is the Florida mainland, while a barrier island complex broached by several inlets forms its eastern boundary. The Indian River Lagoon proper is a shallow microtidal lagoon 195 km in length. It is believed to have the highest biodiversity of any estuarine system in North America, perhaps in part because of its location at the transition between two biogeographic provinces, the warm temperate Carolinean and the tropical Caribbean (Swain et al., 1995).

METHODS

The samples taken in the original survey had been gathered at first only to acquire living colonies of as many species as possible for behavioral and morphological studies (Winston, 1978). As I became interested in the life histories of the species involved, I began collecting at the most convenient and interesting sites in the south central part of the Indian River Lagoon area on an approximately monthly schedule from the fall of 1974 through the summer of 1975. The sites studied were the inner breakwater at Sebastian Inlet, the Johnson House seagrass bed at Harbor Branch Oceanographic Institution at Link Port, the North Beach breakwater at Fort Pierce Inlet, Walton Rocks, South Hutchinson Island, and Seminole Shores, South Hutchinson Island. Collections from those sites were taken in all seasons, an important consideration in the seasonal environment of the Indian River Lagoon region. For bryozoans, as for many organisms inhabiting the area, the highest diversity is achieved and the greatest amount of reproduction, recruitment, and growth of colonies of most species take place during the cooler months (Winston, 1982, 1995). However, tropical species are more apt to be present or active in summer. It was not possible to return to Florida monthly in 1998–1999, but for the best comparison to 1974–1975, the sampling dates were selected to span the seasons and thus reflect the known seasonality of the bryozoan fauna.

Collections were made quarterly (in November 1998, and February, April, and July 1999) at four sites: two within the lagoon and two on the open coast.

Sites Sampled

It was not possible to resurvey all the sites sampled in the original study, for reasons of time and because changes such as the development of some sites into official county or state parks had increased restrictions on scientific collecting. The coastal sites sampled in the restudy were the North Beach breakwater, Fort Pierce Inlet State Park (by special permit), and the Walton Rocks area, South Hutchinson Island, plus one site in the Indian River Lagoon, the Johnson House seagrass bed. One additional site was chosen for the 1998–1999 survey: the intertidal bridge pilings on the east side of the Route A1A causeway to the North Beach in Fort Pierce. This site was added because it was within the Lagoon, yet was close enough to the Fort Pierce Inlet, local marinas, and the commercial port in Fort Pierce to be a likely settlement spot for any newly arrived bryozoan species.

Collecting Methods

Some bryozoan species have colonies several centimeters or more in size and are recognizable in the field, but in many other species the colonies are microscopic and cryptic. Therefore collections were made by scraping hard surfaces and by gathering encrusted substrata: algae, hydroid stems, rocks, shells, or trash. As in the original study, sampling was not quantitative but was thorough. At each locality all microhabitats available—crevices of break-

waters, surfaces of rocks, shells, wood, algae, hydroids, octocorals, sabellariid worm tubes, etc.—were examined carefully for bryozoans. In addition, encrusted examples of each kind of substratum available were taken back to the laboratory and examined alive in seawater; attached bryozoans were identified under a dissecting microscope at 12–100×. Careful microscopic examination made it possible to identify the many tiny and/or uncalcified specimens that could not be identified or even detected in the field. The condition of the colonies and the presence of reproductive structures and/or embryos were also noted, as was the relative abundance of each species at a site. Voucher samples for the project are deposited at the Virginia Museum of Natural History.

Temperature and Salinity Data

Seawater temperatures and salinities were recorded at each census in this study. Temperature ranges are given in the results for each site. Salinity varied little. All readings were in the normal ocean range of the area (35–37‰). The salinity range in the Indian River Lagoon can be more variable than that recorded at any of the sites during the resurvey, but low salinities are connected with periods of heavy rainfall, and 1998–1999 was a drought year. No temperature or salinity data were collected in the original study, and no seawater temperature data were available for the area that covered the entire time period. Air temperatures for Fort Pierce were available (Figure 1) and are summarized in the Discussion section.

RESULTS

North Beach Breakwater, North Hutchinson Island, Fort Pierce Inlet

This site was located at the southern tip of North Hutchinson Island. Specimens were collected from the intertidal rocks on the north side of the north breakwater that protects Fort Pierce Inlet. Habitats sampled included the rocks of the breakwater; sabellariid tubes, hydroids, octocorals, and algae attached to the rocks; and driftwood and other debris wedged among the rocks. The bryozoan diversity at the breakwater is largely dependent on the presence of the hydroid *Thyroscyphus ramosus* Allman, 1877 and the soft coral *Carijoa riisei* (Duchassaing and Michelloti, 1860), whose colonies provide habitat for most of the epifaunal invertebrates at the site. The large

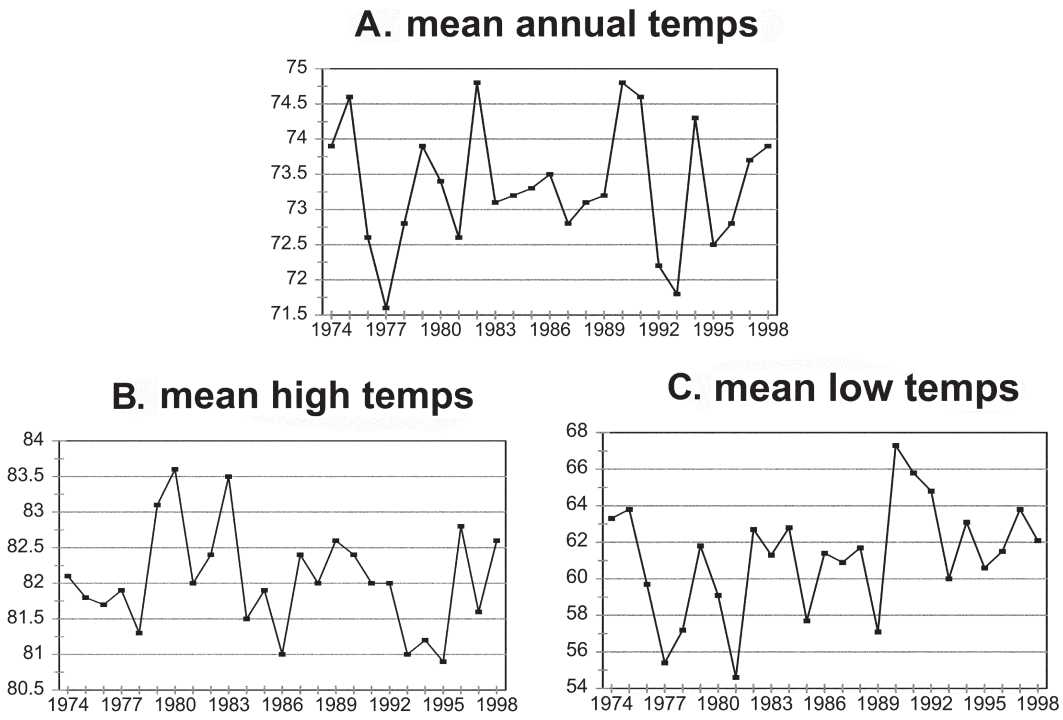

FIGURE 1. Mean annual (A), high (B), and low (C) air temperatures (in degrees Fahrenheit) for Fort Pierce, Florida, from 1974 to 1998. Note: lowest, 54°F = 12.2°C; highest, 83.6°F = 28.7°C.

mounds produced by the sabellariid worm *Phragmatopoma lapidosa* Kinberg, 1867 stay clean and unfouled when the worms are growing actively but break down as they age, the mounds dissolving or becoming riddled with holes and channels in which other organisms settle.

At the November census, water temperature was 23.3°C. New sabellariid tubes were covering the old eroded sabellariid mounds on many of the rocks. The hydroids *Thyroscyphus ramosus* and *Eudendrium carneum* Clarke, 1882 were in an active phase of growth. The cyclostome *Crisia elongata* Milne-Edwards, 1838 was the most common bryozoan found; masses of short young *Crisia* colonies were attached to hydroid roots and branches. Colonies of the encrusting cheilostome *Watersipora subtorquata* d'Orbigny, 1852 were also common, attached directly to the rocks near the low water mark.

At the February census the water temperature was 23.1°C. Hydroids had proliferated. *Thyroscyphus* and *Eudendrium* colonies were thriving, and *Tubularia* sp. and *Halocordyle disticha* (Goldfuss, 1820) were also present along with colonies of the octocoral *Carijoa riisei*. The worm reef was extensive and in healthy condition. *Watersipora* was abundant, with some small, recently recruited colonies present along with large mature colonies. *Crisia* was still a dominant, with large mature colonies producing gonozooids containing yellow embryos.

At the April census water temperature was 25.3°C. Old fouled colonies of *Watersipora* were still present, but the most abundant encrusting cheilostome was *Thalamoporella floridana* Osburn, 1940, which formed thin whitish crusts and bilaminate expansions around the stems of *Thyroscyphus*. *Crisia* colonies with gonozooids were still abundant.

At the July census the water temperature was 29.2°C. The worm reef mounds were crumbling in places but still showed areas of active growth. *Carijoa* was abundant, and there were large, well-grown colonies of *Thyroscyphus*, still active, with functional polyps and characteristic garlicky smell. The most abundant bryozoan at this census was the primitive cheilostome *Aetea sica* (Couch, 1844). This bryozoan has a runner-like growth form, producing uniserial rows of semierect zooids, and an ephemeral life history. Species of *Aetea* occasionally appear in an area in large numbers, encrusting almost every substratum. At other times they may be rare or absent at the same locality. At this census *Aetea* colonies were attached to sabellariid worm tubes, sponges, and *Codium* species of algae, as well as to hydroid stems. *Crisia* was still abundant, but colonies were short and there were very few gonozooids. The other species common at this census was the branching cteno-stome *Amathia distans* Busk, 1886, whose colonies form limp yellow-speckled clumps. They were attached to various substrata, including the senescent worm reef mounds.

Overall, the North Beach Breakwater site was remarkably stable in its bryozoan fauna over the 24-year interval. Thirty species were recorded at the site during this study, including 20 of the 31 species originally found there (Table 1). The species that were dominant in the original survey—*Amathia vidovici* (Heller, 1867), *Beania hirtissima* (Heller, 1967), *Beania klugei* Cook, 1968, *Celleporina hassalli* (Johnston, 1848), *Crisia elongata*, *Pasythea tulipifera* (Ellis and Solander, 1786), *Savignyella lafontii* (Audouin, 1826), *Synnotum aegyptiacum* (Audouin, 1826), *Thalamoporella floridana*, and *Watersipora subtorquata*—were still abundant and were present during at least three of the four censuses. In addition, two new species were common at this site. *Amathia alternata* Lamouroux, 1816 was present at other Indian River sites in the past and still occurs at those sites, but it had not previously been recorded at the North Beach Breakwater. *Caulibugula armata* Verrill, 1900 is new to the region since the original study was carried out.

Walton Rocks

This site is located about 13.7 km south of Fort Pierce Inlet on South Hutchinson Island, on the beach just south of the Hutchinson Island Nuclear Power Plant (which was not yet constructed at the time of the original survey). The habitat consists of exposed sandy beach, with intertidal beach rock ledges; their upper surfaces are covered year-round by a macroalgal turf and seasonally by mounds of sabellariid worm reef. Numerous loose slabs of beach rock are present in a sandy trough in the surf zone between the ledges and the low water line. The exposed location makes collecting at this site difficult or impossible under high surf or wind conditions, and the full extent of the ledges is revealed only during the lowest tides of the year, and then only under calm sea conditions. Encrusting bryozoan species and branching species such as *Scrupocellaria regularis* Osburn, 1940 occur on the undersides of both the beach rock ledges and loose beach rock slabs and stones. Other branching and encrusting species grow on the algae and hydroids attached to the ledges.

At the November census the water temperature was 23.3°C. Most common were the spiny mats of the cheilostome *Beania hirtissima*, which were found on the underside of almost every piece of beach rock. Also common were the beach rock-encrusting species *Exechonella antillea* (Osburn, 1927), *Schizoporella* "*unicornis*," and

TABLE 1. Bryozoans found (+) in 1998–1999 four-season resurvey in comparison with those found in original survey at the North Beach Breakwater, North Hutchinson Island, Fort Pierce, Florida. Dominant species are shown in **bold** type; a dash (–) indicates species not found during the resurvey.

Species	November 1998	February 1999	April 1999	July 1999
Species found in 1974–1975 survey				
Aetea sica	–	–	–	+
Aeverrillia armata	+	+	–	–
Amathia distans	–	–	–	–
Amathia vidovici	+	+	+	–
Anguinella palmata	–	–	–	–
Antropora leucocypha	–	–	+	–
Beania hirtissima	+	+	+	–
Beania klugei	–	+	+	+
Beania mirabilis	–	–	–	–
Bowerbankia imbricata	+	–	–	+
Bowerbankia maxima	+	+	–	–
Bugula minima	–	–	–	–
Bugula turrita	–	–	–	–
Celleporina hassalli	+	+	+	+
Crisia elongata	+	+	+	+
Cryptosula pallasiana	–	–	–	–
Exechonella antillea	–	–	–	–
Hippoporina verrilli	–	–	–	–
Jellyella tuberculata [a]	+	–	–	–
Nolella stipata	+	–	–	–
Pasythea tulipifera	+	+	+	+
Pourtalesella incrassata [a]	–	–	–	–
Savignyella lafontii	+	+	–	+
Scrupocellaria regularis	–	–	–	+
Synnotum aegyptiacum	+	+	+	+
Thalamoporella floridana	+	+	+	+
Valkeria atlantica	–	–	–	–
Vittaticella contei	+	+	–	–
Vittacella uberrima	–	–	–	+
Watersipora subtorquata [a]	+	+	+	+
Zoobotryon verticillatum	–	–	–	–
Additional species found, 1998–1999				
Amathia alternata	+	+	+	–
Caulibugula armata	+	+	+	+
Caulibugula pearsei	–	–	+	–
Biflustra arborescens [a]	–	–	–	+
Biflustra denticulata [a]	–	+	+	–
Bugula neritina	–	+	–	–
Bugula stolonifera	–	+	+	–
Parasmittina sp. 3	–	–	–	+
Rhynchozoon sp.	–	–	–	+
Schizoporella "*unicornis*"	–	+	–	–

[a] Species for which nomenclature has been revised since Winston (1982).

Pourtalesella incrassata (Canu and Bassler, 1928), actively growing peach or pink colonies with red embryos present in ovicells, along with the ctenostome *Nolella stipata* Gosse, 1855. *Nolella* zooids are straight mud-covered tubes resembling miniature polychaete tubes. They are connected by a thin stolon, but at this site zooids were so thickly aggregated that the stolons were invisible and the colony appeared as a fuzzy mat of tubes.

At the February census the water temperature was 23.0°C. The wind was strong because of a cold front, and the surf was high, making collection difficult. The macroalgal turf was thriving and mostly unfouled except

by epiphytic hydroids. There were few branching bryozoans. Loose rock in the trough was almost all buried under sand. The colonies of beach rock-encrusting bryozoans collected were abraded and bleached in color.

At the April census the water temperature was 23.7°C. The algal turf was growing luxuriantly. Many more beach rock stones, some freshly broken off the ledges, were uncovered. The undersides of most rocks were completely covered by a cryptic community that included zooanthids, didemnid ascidians, sponges, anemones, and branching and encrusting bryozoans. *Beania hirtissima* was again dominant, but other colonies of encrusting bryozoans, including *Schizoporella* "*unicornis*," *Exechonella antillea*, *Watersipora subtorquata*, and *Cryptosula pallasiana* (Moll, 1803), were brightly colored and healthy. *Nolella stipata* zooids were clean and translucent, less mud-coated than in February. Colonies were sexually reproductive, as well; many zooids brooded two or three yellow-ochre eggs near their distal ends.

At the July census water temperature was 30.9°C. Surf was moderate, sand had filled in around ledges again, and a considerable amount of detached beach rock ledge algae was washed up on the beach. The undersides of large beach rock slabs still had a healthy cryptic fauna consisting of zooanthids, ascidians, sponges, and bryozoans on their undersides, despite being buried in sand. Dominant bryozoans were *Exechonella antillea*, *Biflustra denticulata* (Busk, 1856), and *Beania hirtissima*, as well as *Nolella stipata* (which was still reproducing), plus two erect branching species, the ctenostome *Amathia vidovici* and the cyclostome *Crisia elongata*, both present as large, old, fouled colonies.

This site, Walton Rocks, had been the most diverse intertidal site in the original study, with 36 species recorded at that time. Twenty-five of the same species were found in 1998–1999 (Table 2). Of the dominant species in the original survey, all were still present in at least two of the four censuses, and all but one, *Parasmittina betamorphaea* Winston, 2005, was present at three of the four.

The biggest change at this site was a decline in abundance of *Cryptosula pallasiana* and its apparent replacement in beach rock undersurface habitats by *Exechonella antillea*, which in 1974 had been found only once, at the North Beach Breakwater, and which had not been collected at Walton Rocks.

Johnson House Seagrass Bed, Indian River Lagoon

This seagrass bed is located about 9.7 km north of Fort Pierce Inlet. It lies in a shallow cove just north of the Harbor Branch Canal, behind the Johnson residence on the campus of Harbor Branch Oceanographic Institution. The grass bed has been the site of several studies of seagrass and soft substratum communities (e.g., Mook, 1976; Kulczycki et al., 1981; Virnstein and Carbonara, 1985; Virnstein and Howard, 1987) and was one of the bryozoan sites studied monthly in 1974–1975. The turtle grass, *Thalassia testudinum* Banks and Soland. ex Koenig, is the most abundant seagrass at this site, but manatee grass, *Syringodium filiforme* Kuetz., is also common. Drift algae float among the grass blades.

At the November census water temperature was 24.4°C. Collections were made of all substrata: *Thalassia*, *Syringodium*, and drift algae. Most drift algae were fouled by a colonial ascidian, *Lissoclinum fragile* (Van Name, 1902). The stoloniferous ctenostome *Bowerbankia maxima* Winston, 1982, and the encrusting cheilostome *Conopeum tenuissimum* (Canu, 1908) were the dominant bryozoans.

At the February census, the water temperature was 15.2°C, with a strong north wind. Masses of drift algae had been cast up on shore. *Bowerbankia maxima*, *Conopeum tenuissimum*, and the branching cheilostome *Bugula neritina* (Linnaeus, 1758) were the dominant bryozoans on seagrass and drift algae, respectively.

At the April census the water temperature was 23.5°C. Drift red algae appeared bleached in color compared with their February condition; other algal species appeared to be thriving. There had been a new settlement of spirorbid polychaetes onto the seagrass since February, and *Conopeum* had decreased in abundance on *Thalassia*. However, there were larger numbers and larger sexually reproductive colonies of *Bugula neritina* on the *Syringodium*, along with small recent recruits.

At the July census the water temperature was 29.7°C. *Thalassia* and *Syringodium* blades were heavily fouled by filamentous algae and hydroids. Large colonies of *Bowerbankia maxima*, clean and healthy in appearance, with long free-trailing masses of stolons and zooids, occurred on the drift algae. *Conopeum tenuissimum* and *Schizoporella floridana* Osburn, 1914, with recently settled recruits and with embryos in mature colonies, were found on the *Thalassia*.

In the original study nine species of bryozoans were recorded from this site. Six of these were collected at least once in the re-study (Table 3). The dominant species, *Conopeum tenuissimum*, *Schizoporella floridana*, and *Bowerbankia maxima*, remained unchanged. Four additional species, *Aetea sica*, *Aeverrillia armata* (Verrill, 1873), *Hippoporina verrilli* Maturo and Schopf, 1968, and *Scrupocellaria* "*bertholletii*," not recorded here in

TABLE 2. Bryozoans found (+) in 1998–1999 four-season resurvey in comparison with those found in original survey at Walton Rocks, South Hutchinson Island, St. Lucie County, Florida. Dominant species are shown in **bold** type; a dash (–) indicates species not found at this location during the resurvey.

Species	November 1998	February 1999	April 1999	July 1999
Species found in 1974–1975 survey				
Aetea sica	–	–	–	+
Alcyonidium polypylum	+	+	+	–
Amathia alternata	–	–	+	+
Amathia distans	–	–	+	+
Anguinella palmata	–	–	–	–
Antropora leucocypha	–	+	–	–
Beania hirtissima	+	+	+	+
Beania klugei	–	+	+	+
Biflustra denticulata [a]	–	–	+	+
Bowerbankia gracilis	–	–	–	–
Bowerbankia imbricata	–	–	–	–
Bowerbankia maxima	–	+	–	–
Bugula neritina	–	+	–	–
Bugula stolonifera	+	+	+	–
Bugula turrita	–	–	–	–
Bugula uniserialis	–	–	–	–
Caulibugula pearsei	–	–	–	–
Celleporella carolinensis	–	–	–	+
Crisia elongata	+	+	+	+
Cryptosula pallasiana	+	+	+	
Electra bellula	–	–	–	–
Jellyella tuberculata [a]	+	+	–	+
Microporella umbracula	–	–	–	–
Nolella stipata	+	+	+	+
Parasmittina betamorphaea [a]	–	+	+	–
Pourtalesella incrassata [a]	+	+	+	
Savignyella lafontii	–	–	–	–
Schizoporella "unicornis"	+	+	+	+
Scrupocellaria regularis	+	–	+	+
Sundanella sibogae	+	–	–	–
Synnotum aegyptiacum	–	+	–	+
Thalamoporella floridana	–	–	–	+
Vittaticella contei	–	–	–	–
Vittacella uberrima	–	–	–	–
Watersipora subtorquata	–	+	+	+
Zoobotryon verticillatum	–	–	–	–
Additional species found, 1998–1999				
Aimulosia spp	+	–	+	+
Amathia vidovici	+	+	+	+
Celleporaria sp. 2	+	+	+	
Escharoides costifer	–	–	–	+
Exechonella antillea	–	+	+	+
Biflustra arborescens	–	+	–	+
Lichenopora sp.	–	–	–	+
Parasmittina sp. 2	–	–	–	+
Pasythea tulipifera	+	+	–	–
Scrupocellaria "*bertholletii*"	–	+	–	–

[a] Species for which nomenclature has been revised since Winston (1982).

TABLE 3. Bryozoans found (+) in 1998–1999 four-season resurvey in comparison with those found in original survey at the Johnson House Seagrass Bed, Harbor Branch Oceanographic Institution, Link Port, Fort Pierce, Florida. Dominant species are shown in **bold** type; a dash (–) indicates species not found at this location during the resurvey.

Species	November 1998	February 1999	April 1999	July 1999
Species found in 1974–1975 survey				
Amathia distans	–	–	–	–
Beania klugei	–	–	+	–
Bugula neritina	–	+	+	–
Bowerbankia gracilis	–	–	–	–
Bowerbankia maxima	+	+	+	+
Conopeum tenuissimum	+	+	+	+
Electra bellula	–	–	–	–
Nolella stipata	–	+	+	–
Schizoporella floridana	+	+	+	+
Additional species found in resurvey				
Aetea sica	–	–	+	+
Aeverrillia armata	+	–	–	–
Hippoporina verrilli	+	–	–	–
Scrupocellaria "bertholletii"	–	–	+	–

1974–1975, were also found at one or more censuses in 1998–1999. The three species not found during the re-study, *Amathia distans*, *Bowerbankia gracilis* Leidy, 1855, and *Electra bellula* (Hincks, 1881), were still present in the lagoon at other sites.

A1A Causeway

In addition to the three sites from the original study, one new site was also surveyed quarterly. The site is a shaded spot under the east end of the Route A1A causeway bridge to North Hutchinson Island. This site was chosen because of its position in the Indian River Lagoon, about 3 km north of the mouth of Fort Pierce Inlet, and close to Little Jim Island, where in 1989 a *Scrupocellaria* species previously unrecorded in the region had first been collected (Winston, 1995). Material was collected from bridge pilings, from drift algae, and from submerged wood.

At the November census water temperature was 23.3°C. The most abundant species were *Bugula neritina*, *Caulibugula armata*, *Bugula stolonifera* Ryland, 1960 (the latter two reproductive), and *Zoobotryon verticillatum* (Delle Chiaje, 1828). Medium-sized *Zoobotryon* colonies had some areas of new growth with actively feeding polypides.

At the February census the water temperature was 21.1°C with a cold north wind and turbid water conditions. *Bugula neritina* was again dominant, with large, bright wine red-colored, sexually reproductive colonies. Other abundant species were *Amathia vidovici* (colonies mostly mud coated, but with clean actively growing branch tips) and long stalks of *Caulibugula armata*. *Zoobotryon verticillatum* was present only as short, heavily fouled, and senescent clumps.

At the April census water temperature was 23.2°C, with almost no wind and extremely clear water. *Bugula neritina* was still dominant on bridge pilings, with more mature and senescent colonies than in February. *Zoobotryon verticillatum* was still present, as large colonies drifting among seagrasses and short clumps attached to pilings, all of them heavily fouled, but with some young actively growing branches. *Caulibugula armata* was still present, with large and unfouled colonies. *Amathia vidovici* was still abundant, but colonies were heavily fouled.

At the July census water temperature was 28.8°C, wind calm, with fairly clear water (visibility about 1 m). *Bugula neritina* and *Zoobotryon verticillatum* were absent. Dominant species were *Caulibugula armata* (old, fouled colonies, with many brown bodies in the lower parts of branches, but with zooids containing feeding polypides and ovicelled zooids containing creamy white embryos near branch tips), *Savignyella lafontii*, a delicate branching cheilostome, *Nolella stipata*, and *Amathia vidovici* (as small, heavily fouled colonies).

Twelve species were found at this site (Table 4), making it less diverse than the open coast sites but more diverse

TABLE 4. Bryozoans found (+) in the 1998–1999 four-season survey at the AIA Causeway Bridge, North Hutchinson Island, Fort Pierce, Florida. Dominant species are shown in **bold** type; a dash (–) indicates species not found at this location during the resurvey.

Species	November 1998	February 1999	April 1999	July 1999
Aetea sica	–	+	+	–
Amathia vidovici	+	+	+	+
Beania klugei	–	+	+	+
Bowerbankia gracilis	–	–	–	+
Bowerbankia maxima	+	+	+	–
Bugula neritina	+	+	+	–
Bugula stolonifera	+	–	–	–
Caulibugula armata	+	+	+	+
Nolella stipata	+	+	+	–
Savignyella lafontii	+	+	+	–
Scrupocellaria "bertholletii"	–	+	+	–
Zoobotryon verticillatum	+	–	–	–

than the Johnson House Seagrass Bed site (10 species) further up the lagoon from Fort Pierce Inlet. Species composition was stable; most species found there were collected in at least three of the four censuses. Overall dominants were *Amathia vidovici*, *Bugula neritina*, *Zoobotryon verticillatum*, and *Caulibugula armata*, a species that had not been collected in the area until about 1994.

DISCUSSION

In the 1974–1975 study, 55 species were recorded from all lagoon and shallow coastal sites. Forty-nine species were recorded at the three sites later resurveyed. During the 1998–1999 survey, 39 species were found at those three sites. Thus, 80% of the bryozoan species known originally from those sites were recollected after a 24-year interval, despite a smaller sampling effort (4 versus 12 collections). Seventy percent of the species found originally from all inshore sites combined were also found in the four-site resurvey, again with a much smaller sampling effort involved. There has been remarkable stability in species composition of the bryozoan fauna over the time period.

Sixteen species had additional localities (that is, they were present in the area originally, but occurred at a different site in the second study than that from which they had been recorded in the original survey), indicating that most species were not restricted to one site and could be expected at any or all sites provided the appropriate substratum and environmental conditions were present. Even though most of the species involved have nonfeeding, rapidly settling larvae, there is apparently enough dispersal and recruitment that disappearance from one site would not mean that a species would disappear from the region entirely. Only one species, *Schizoporella floridana*, was limited to one site, the Johnson Seagrass Bed, and to one substratum, *Thalassia testudinum*, and was not collected elsewhere in 1998–1999.

Species new for inshore intertidal sites, but known from offshore hard substrata or algae (Winston and Eisman, 1980; Winston and Håkansson, 1986), included *Aimulosia uvulifera* (Osburn, 1914), *Aimulosia pusilla* (Smitt, 1873), and *Escharoides costifer* (Osburn, 1914). Four species were newly recorded for the region during the study: two species of *Parasmittina*, a species of *Celleporaria*, and a *Lichenopora* species.

Although species composition remained very stable, species abundances changed considerably, not only from season to season but also between the two studies. The most notable changes involved the decline in abundance of the warm temperate species *Bugula stolonifera*, *Cryptosula pallasiana*, and *Hippoporina verrilli*, all of which have western Atlantic distributions extending northward to Long Island or Cape Cod. During the original study period abundant *Bugula stolonifera* colonies were found attached to the proximal portions of *Bugula neritina* colonies. In the re-study only a few colonies were found, and they were not in association with *Bugula neritina*. *Hippoporina verrilli* was a common species on Indian River Lagoon panels (Mook, 1976) and on panels and seagrasses in 1974–1975, and it was also found at two coastal sites

at that time. Reproduction and settlement were heaviest in the cooler months (October–January). In the re-study only a few small colonies were found at the Johnson Seagrass Bed. *Cryptosula pallasiana* is a cosmopolitan temperate fouling species. In 1974–1975 it occurred at four intertidal coastal sites. In the re-survey, however, it was found only at Walton Rocks where it was much less abundant under beach rock stones than originally. Instead, in the underrock habitat the dominant encrusting bryozoans in 1998–1999 included *Exechonella antillea*, a Caribbean species which, in the original study, had been collected only one time, at the North Beach Breakwater site. That original record itself may have indicated a range expansion for the species because a distributional survey by Maturo (1968) reported the species only from Miami south.

The other new species in the study are similarly warm-water species. *Caulibugula armata* was described by Verrill from Bermuda, and it is known from the Tortugas, Puerto Rico, and Brazil, according to Osburn (1940). *Aimulosia pusilla* was described from the Tortugas by Smitt (1873) and *Aimulosia uvulifera* and *Escharoides costifer* from the same locality by Osburn (1914). The typical *Scrupocellaria bertholletii* is a circumtropical species, often associated with coral reefs (Winston, 1986), but Indian River and other western Atlantic specimens show some morphological differences to those from other localities, indicating that *Scrupocellaria bertholletii* is a species complex rather than a single widespread species. It was first recorded in the Indian River lagoon in 1989 and continues to occur at both coastal and lagoon sites. The genera *Celleporaria* and *Parasmittina* contain numerous species that are extremely successful in both tropical fouling and cryptic coral reef communities (Winston, 1986). The addition of species in this group is not surprising.

The increase in warm-water species has continued since the re-study was completed. *Nellia tenella* (Lamarck, 1816), another circumtropical fouling and reef-associated species, was first found in the Indian River area in 1999, in intertidal collections in Fort Pierce Inlet. It has been found every year since then, although its abundance has varied. *Hippopodina irregularis*, a species described form Guanica Harbor, Puerto Rico, by Osburn (1940), was first found on *Syringodium* seagrass in Fort Pierce inlet in the summer of 2001. *Schizoporella pungens* (Canu and Bassler, 1928), the massive dark purple, Caribbean–Gulf of Mexico *Schizoporella*, whose colonies are characteristically found on submerged mangrove roots and in harbor fouling communities, had been on noted on drift plastic items washed ashore in the area for several years, always with an associated fauna of small corals and *Millepora* species that suggested the debris had been colonized further south, perhaps in the Straits of Florida or the Florida Keys. *Schizoporella pungens* colonies first recruited to panels in Indian River Lagoon (Faber Cove), as well as to numerous benthic substrata in Fort Pierce Inlet between July 2002 and July 2003. *Celleporaria sherryae* Winston, 2005, another Caribbean fouling and shallow reef-associated species, has also appeared at some coastal sites (2001) and within the Fort Pierce Inlet (2003).

Reasons for the increase in warm-water species are harder to identify. One explanation might be global warming. As noted by many recent studies, the decade of the 1990s was the warmest on record (Levitus et al., 2000). The effects of warming seawater temperatures on marine organisms, including bryozoans (Kelmo et al., 2004), have been noted worldwide. In addition to direct effects on growth and survival of benthic organisms, changes in water temperature also affect food supply (Menge et al., 2008; Richardson, 2008), as well as producing indirect effects via changes in ocean chemistry and circulation (Harley et al., 2006).

For these collecting sites no records of seawater temperature exist for the entire time period of the two studies (1974–1999). However, as these sites are all intertidal, it seemed reasonable to make use of the published air temperature data that were available for Fort Pierce as a substitute. Although mean annual temperatures and mean annual high temperatures (based on monthly averages) showed no discernible statistically significant pattern (Figure 1A,B), there is a suggestion in the data that mean annual low temperatures (Figure 1C) have increased over the time period. If warm-water species are more susceptible to cold-water shock than high temperatures, as has been shown in studies of Florida fish kills after freezes in the region (Gilmore et al., 1978), warmer winter temperatures might be a factor permitting the invasion and survival of populations of the more tropical species, as has been shown to be the case for some introduced marine invertebrates in other studies (Stachowicz et al., 2002).

However, other factors are involved. The Indian River Lagoon is part of the Intracoastal Waterway, a passage for boat traffic moving up and down the Atlantic coast, as well as in and out of the Gulf of Mexico and the Caribbean. Fort Pierce has a small commercial port with shipping traffic from the Bahamas (especially Freeport, where containers from China and other distant sources are transferred for transshipment into the USA), the Gulf of Mexico, and the Caribbean, as well as U.S. ports along the east coast. Species could be introduced through ballast water exchange by larger ships, as well as by hull fouling of small and large vessels.

Although the stability of the bryozoan fauna over this time period gives a positive picture of the health and stability of the lagoon epifauna overall, there is no way to predict the long-term impact of these factors. The dependence of many bryozoans on living substrata such as seagrasses, hydroids, and octocorals also makes it clear that disturbances affecting substratum organisms would have a major impact on the bryozoans and would probably be more destructive to their local diversity than the environmental fluctuations noted so far.

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The Turtles' Tale: Flagships and Instruments for Marine Research, Education, and Conservation

John G. Frazier

ABSTRACT. Marine turtles are classic flagship species. Their remarkable natural history—large body size, dependence on both terrestrial and oceanic environments, delayed maturity requiring decades to reach adulthood, regular migrations that crisscross ocean basins, massive reproductive output, mammal-like physiology, and other features—make them attractive to researchers and the general public alike. This attraction is further enhanced by the fact that these reptiles are widely recognized as endangered species. They are "biomagnets" for people around the world, from various sectors of society; incredible amounts of time, energy, and resources go into diverse types of investigation, public education, conservation, and international policy directed specifically at these "lowly reptiles." Oceanographers, ecologists, geneticists, marine biologists, and specialists from other related disciplines frequently begin basic research projects on marine turtles. These activities quickly evolve into large multifaceted programs including conservation activities, community-based approaches, and public education together with other forms of development and social projects, and even policy initiatives for promoting regional and global cooperation in the conservation of these shared resources and the habitats on which they depend. Besides enhancing better understanding of the biology and ecology of these animals and nurturing more active and diverse conservation and education initiatives, work on marine turtles also promotes much-needed initiatives in interdisciplinary and international cooperation, which are fundamental challenges to marine work in general. This paper provides a summary of the flagship species concept and gives examples of how work focused on marine turtles has promoted diverse initiatives in marine research, education, and conservation at multiple scholarly, social, and political levels; it argues that this approach serves as a critical integrating force to nurture a wider comprehension and appreciation of the scientific endeavor and its role in society.

FLAGSHIP SPECIES AND THE INCREASE AND DIFFUSION OF KNOWLEDGE

Scientists, educators, and conservationists who specialize on marine organisms and marine environments may all be convinced of the fundamental importance of such things as larval nectophores, pedunculate siphonophores, disappearing zooxanthellae, discharged nematocysts, mitochondrial cytochrome oxidase 1, maximum parsimony, and other indicators of "good science," but what of the rest of society? Marine biodiversity is unique yet poorly understood or appreciated by the general public or decision makers;

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and a central question with which we all must contend is "How can we promote it?"

Many marine organisms have complex, intriguing life histories, and marine turtles, comprising just seven living species, are classic examples. These air-breathing reptiles are typified by highly complex life cycles: they live with fish but nest on land, relying on terrestrial, coastal, benthic, and pelagic environments during different parts of their life cycle; they can occur in extremely dense concentrations both on land and in the sea; they are "highly migratory," crossing ocean basins; they take a decade or more to reach sexual maturity and can live for half a century or more; and they have highly specialized morphological and dietary adaptations, including mammal-like physiology. A single female often lays more than 100 eggs in a nest and can lay several nests in a season. Their large body size (up to 1 ton), striking coloration, and primeval appearance all add to the attractiveness of these marine reptiles. The fact that marine turtles are globally recognized as endangered species adds a further level of importance. Hence, these reptiles are flagship species: ambassadors of the oceans. The attraction has led to not only enormous interest on the part of the general public but also disproportionate attention in academic circles (Frazier, 2003a, 2005a, 2005b): nearly as much research is conducted on just seven species of marine turtles as is carried out for the remaining 300-some species of chelonians.

In addition, marine turtles are widely valued as sources of meat, eggs, oil, skin, and shell, which have been utilized, crafted, and traded for millennia. A global trading network that supplied elite urbanites of the Mediterranean with raw materials from the shores of the Indian Ocean and beyond was well established before the time of Christ, and the most frequently mentioned commodity was tortoise shell (the external keratinous scutes of the hawksbill turtle, *Eretmochelys imbricata* Linn.). Intricately fashioned toilet articles, particularly ornamental combs, some of which were 85 cm wide, as well as a special style of French furniture luxuriously inlaid with tortoise shell and metal ("Boulle"), and religious accoutrements have all been made famous by the tortoise shell used in their creation. In addition to the tremendous diversity of objects crafted from turtle parts, these animals have been portrayed for millennia on a wide variety of media, from cave walls to carved rocks to delicate ceramics to the cylindrical seals of ancient Arabia (Frazier, 2003b, 2004a, 2005c). Hence, they have had very important cultural, social, and spiritual values in many societies. During contemporary times marine turtles have been celebrated in many and diverse forms, ranging from symbols of sacred nature and "pristine" environments to evidence of the evils committed by modern society on the environment (Campbell, 2003). All this conveys upon these animals a wide variety of values, from cultural and historic to economic and spiritual.

ACTIVITIES FOCUSED ON MARINE TURTLE RESEARCH AND CONSERVATION

The national marine turtle program in Brazil, which began as a dedicated study of reproductive biology and natural history, has evolved into one of the best known long-term programs in South America and the world in general, and the attraction of the turtle flagship over the years has resulted in the incorporation of massive efforts in public education and community development, including alternate livelihoods for community residents, training, and facilitated interactions between different sectors of government and society, not to mention national counsel for regional and international policy actions (Marcovaldi et al., 2005). Similarly, multiyear programs in Uruguay (Laporta and Miller, 2005), northwestern Mexico (Delgado and Nichols, 2005), the Caribbean (Eckert and Hemphill, 2005), and Nova Scotia (Martin and James, 2005) conduct research on diverse topics such as feeding ecology, reproductive biology, genetics, migration, and fisheries interactions. All this research, as well as the associated educational and conservation activities, has been greatly facilitated—if indeed not made possible—by the attractiveness of marine turtles and the ease with which researchers have been able to make use of these flagship species to promote interest in collaborating with different research activities. It is not uncommon for fishermen to go out of their way not only to inform researchers about sightings and captures of marine turtles but also to take on extra work, requiring time, effort, and materials to deliver information and specimens to researchers. Frequently this means allowing, or even inviting, researchers to come onboard and make free use of the fishermen's vessels and materials. Swordfish fishermen in Nova Scotia provide their vessels as research platforms for the complicated process of capturing, boarding, measuring, instrumenting, and releasing turtles of half a ton in body weight or more; researchers are very much aware that the success of their work depends on the altruistic behavior of fishermen (Martin and James, 2005). Uruguayan fishermen, many of whom live at a subsistence level, not only invite researchers to make use of their boats but are active collaborators in the research, attending meetings and participating in presentations (Laporta and Miller, 2005). A dramatic example of

the level of dedication to, and investment in, marine turtle projects is *Theeram Pakriti Samrakshana Samiti* (Coastal Ecosystem Protection Committee) in Kolavipalam Village, Kerala, India. A group of artisanal fishermen decided to protect nesting turtles and their eggs, formed the committee, built a modest beach station, and now run nightly beach patrols, maintain an interpretation center with live turtles, and give public education presentations: all these activities have been self-organized and self-motivated, thanks to the attractive power of the turtles (Shanker and Kutty, 2005). This sort of material and moral support is difficult to evaluate adequately in simple financial terms, but it has been absolutely essential in supporting various aspects of basic research, education, and conservation activities. Indeed, many of these activities would not only be far outside the operational budgets of the organizations involved but simply impossible to achieve without the full collaboration of the fishing communities.

Adventure tourism, often referred to as "eco-tourism," has been widely promoted around the world with marine turtles as the central attractants; indeed, there is even an international travel guidebook that is dedicated specifically to marine turtle tourism (Devaux and De Wetter, 2000). In addition to paying their travel costs, it is not uncommon for tourists to actually pay for the privilege of working as volunteers in turtle research projects, some of which have been operating for decades (Campbell and Smith, 2005). In this way the flagship attraction directly supports research through both funding and the availability of trained volunteer assistants.

An incredible diversity of outreach and public education has been developed with marine turtles as the centerpiece, a phenomenon common around the world and far too diverse to summarize easily (Frazier, 2005d). There are national and regional training programs specific to marine turtle biology and conservation, and some of these have been active for more than a decade, during which time they have seeded well-trained and enthusiastic researchers, educators, and conservationists throughout vast areas, such as India (Shanker and Kutty, 2005), the Caribbean (Eckert and Hemphill, 2005), and Latin America (Buitrago et al., 2008; Marcovaldi et al., 2005). In some cases, the activities and festivals organized by conservationists have been appropriated by local people, who have completely taken over what were initially devised to "sensitize" and "motivate" them to collaborate with marine turtle projects. One of the clearest examples of the rapidly increasing and powerful attraction of marine turtles is the Annual Symposium on Sea Turtle Biology and Conservation, an event that is attended by about a thousand people, with representation from scores of countries and hundreds of presentations (Frazier, 2003a). By using the turtles as attractants "to get people in the door," these activities, events, and projects clearly transcend the turtles and provide a wide basis of information on a diversity of marine organisms and environments, thereby promoting greater interest, research, and appreciation for these topics.

There is ample evidence that the flagship attraction can be instrumental for developing popular and political support to affect local policy decisions, such as the creation of special protected areas and tourism management programs (Tisdell and Wilson, 2005). Moreover, international maritime and fisheries policies have been directly affected by international, regional, and national efforts to conserve marine turtles, particularly through such efforts as mitigation of fisheries bycatch (Bache, 2005). In fact, an extraordinary amount of attention has been paid to marine turtles in the field of international environmental law (Frazier, 2002). At present there are two bilateral agreements, an incipient trilateral agreement, a program under a United Nations Environmental Programme (UNEP) Regional Seas convention in the southeast Pacific, a memorandum of understanding for the Atlantic coast of Africa, and another memorandum of understanding for the Indian Ocean (both under the United Nations Convention for Migratory Species), and a "stand-alone" treaty for the Western Hemisphere, all focused specifically on the conservation of marine turtles. Every one of these instruments includes measures of habitat protection, and the term "habitat" is even included in the title of one accord. Hence, through activities to protect marine turtle habitats over vast areas, these instruments have direct relevance to a wide range of marine organisms and environments, again clearly transcending marine turtles.

In addition to these seven agreements specific to marine turtles, there are many other international agreements that are relevant to marine turtle research, conservation, and education: these include such major global treaties as the UN Convention on the Law of the Sea (UNCLOS), Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Convention on Biological Diversity (CBD), and the Convention on the Conservation of Migratory Species of Wild Animals (CMS) (Wold, 2002). Moreover, intense concern for the conservation of marine turtles has been instrumental in shaping policy and management directions in fisheries and other maritime issues (Bache, 2002). For example, the Inter-American Tropical Tuna Commission (IATTC), which was constituted nearly 60 years ago to develop regional management of tuna stocks in the Eastern Tropical Pacific, has

been dealing specifically with accidental capture of marine turtles since 2003 and has adopted at least eight resolutions to promote mitigation measures on turtle bycatch. Even the United Nations Food and Agriculture Organization (FAO), originally created to enhance the production of food, has become intimately involved in marine turtle conservation. In March 2004 the "Expert Consultation on Interactions between Sea Turtles and Fisheries within an Ecosystem Context" was held in Rome (FAO, 2004), followed by a technical consultation at which guidelines for mitigating turtle bycatch in fisheries were proposed (FAO, 2005). These technical considerations and recommendations were then taken up by the political body, FAO's Committee on Fisheries, where the proposal was adopted at a global level (COFI, 2005). The result is a set of recommendations for all States that are members of FAO (virtually every country that exists). Some of the specific actions that States are supposed to carry out include stock identification and assessment, tagging and genetic studies, testing mitigation techniques, "pay urgent attention . . . to collection of statistics," collect and share information, and harmonize conservation and management initiatives.

At an even greater level of political importance was a dispute brought before the World Trade Organization (WTO), which challenged the right of a Party to the WTO to enact unilateral measures that ban certain imports in an effort to protect marine turtles from capture and mortality in certain fisheries operations. After several years of contentious debate and the production and exchange of thousands of pages of documentation, an WTO Appellate Body decision released on 22 October 2001 concluded that because marine turtles are endangered species, countries can take exceptions to the all-powerful free-trade rules of the WTO and—following certain procedures—enact unilateral measures to protect turtles, including trade embargos (Bache and Frazier, 2006; Frazier and Bache, 2002).

COMPLEXITIES OF FLAGSHIP PERCEPTIONS

It is important to point out, however, that the inappropriate use of a flagship can lead to totally misguided policies and activities, counterproductive to both environmental and social needs. For example, easy access to highly attractive hatchling marine turtles led to an explosion of "sea turtle conservation hatcheries" along the coast of Sri Lanka, generously funded by unknowing tourists, despite the fact that these establishments were illegal and had negative impacts on hatchling recruitment (Tisdell and Wilson, 2005). Conservation programs that focus reflexively on an urgent need to do everything possible to protect marine turtles but ignore local sociopolitical complexities can create tremendous conflict, for different sectors of society often have divergent, even conflicting, views on how to respond to the flagship and what it primarily symbolizes (Shanker and Kutty, 2005; Frazier, 2008). Although conservationists view marine turtles as indisputable symbols of the need for people to cherish and protect the environment, other sectors of society—for example, certain ethnic groups—see the same turtles in very different ways, such as symbols of cultural identity and reclamation. This divergence in perceptions is true both on Pacific islands (Kinan and Dalzell, 2005) and on a Greek island in the Mediterranean, where contradictions in perceived value of the marine turtle flagship have resulted in violence, death threats, and other forms of intense conflict between different sectors of society (Theodossopoulos, 2005).

SHARED RESOURCES—THE ROOT PROBLEM

Because of their life history characteristics (particularly the long lifespan, dependence on a variety of diverse environments, and dispersal and migration across oceanic basins), marine turtles provide a classic case of shared resources, or "common property." Simple, but basic, questions such as "Who owns turtles?" or "Who has rights to turtles?" clearly show that many parts of many societies have direct impacts, rights, and responsibilities relating to these animals (Frazier, 2004b). This contention is easily illustrated by the fact that more than 2 million reproductive turtles were taken from the breeding grounds in Pacific Mexico between 1964 and 1980 (Frazier et al., 2007). Yet, animals from this population migrate widely throughout the eastern tropical Pacific, living at different times within the jurisdiction of different sovereign States or on the high seas (Morreale et al., 2007). Who had the right to slaughter so many reproductive animals that are part of the fauna of a vast region (an action that had enormous implications on the status of a shared population)? The same question can be asked of people who pollute the oceans with oil spills, plastics, or other wastes: What right do they have to contaminate a common resource? Similarly, when endangered species of marine wildlife, such as dolphins, whales, seabirds, and marine turtles, are caught and killed in fishing activities, the question arises: "What right does the fishing industry have to be killing (even if it is accidentally) wildlife species that are valued by the citizens of many nations?"

Dealing with shared resources is the root issue for nearly all questions regarding biological conservation—

particularly in marine environments. Hence, by highlighting the importance of this central problem, work on marine turtles brings even greater attention to this critical issue, and because these reptiles are regarded globally as endangered species, their importance is further enhanced. Investigations on marine turtles that help promote ways to resolve intractable issues of common property have implications that go far beyond chelonian biology and natural history: they bear on the way modern societies interact with the oceans.

CONCLUSIONS: PROMOTING MARINE RESEARCH, EDUCATION, AND CONSERVATION THROUGH FLAGSHIP SPECIES

The attention given to marine turtles spans the entire sociopolitical spectrum, from marginalized, politically insignificant fishing communities to the most politically powerful organizations on the planet. From one extreme of the political continuum to the other, these animals have been given extraordinary importance. These local, national, regional, and global policy decisions have enormous importance in the ways that individuals, governments, and organizations at various levels assign priorities and allocate resources. Even if the intent is only to comply superficially with obligations that are not enforced, the end result is resources and personnel allotted to some aspect of marine turtle research, education, and conservation.

Although the scientific enterprise and its practitioners strive to develop and maintain an objective, unbiased view of the world, there is no escaping the fact that both the enterprise and the practitioners are immersed within complex social and political systems. The result, despite the firmest of desires, is that there is close interplay and interaction between scientific activities and attitudes that dominate in the surrounding society (Rozzi, 1999). In fact, an anthropological study of the scientific establishment shows not that scientists and their practices are unique among humanity, but rather that they are immersed in a world of power struggles, politics, and myths—little different from the world of the lay public that is so often demeaned by the scientific community (Nader, 1996).

There is no inherent reason that information produced by scientific research will be read, understood, appreciated, followed, used, or even recognized in the halls of power; if practitioners of the scientific endeavor want their information to impact society outside the ivory towers of academia, it is essential that we learn how to "package" the information in digestible, understandable, interesting, and convincing ways (Frazier, 2005e). Flagship species greatly facilitate this exercise for they have values that are attractive to the general society. Used efficiently and appropriately, such species are powerful tools for promoting research, education, and conservation of countless marine issues.

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Latitudinal Gradients in Recruitment and Community Dynamics in Marine Epifaunal Communities: Implications for Invasion Success

Amy L. Freestone, Richard W. Osman, and Robert B. Whitlatch

ABSTRACT. Although the latitudinal diversity gradient, where species diversity peaks at low latitudes, is well documented, much less is known about how species life history strategies differ among regions and the implications of these differences for community development trajectories and particularly for invasion dynamics. As a first step in trying to understand these factors, we contrast spatial and temporal variation in recruitment rates and resultant community development of epifaunal assemblages in regions along a latitudinal gradient from the temperate zone to the tropics. We exposed settlement panels in four regions: Long Island Sound (Connecticut), Chesapeake Bay and Virginia's Eastern Shore (Maryland and Virginia), Indian River Lagoon (Florida), and a portion of the Mesoamerican reef in Belize. Panels were deployed for either one to two weeks, to evaluate recruitment patterns, or one year, to monitor community development. We found that both recruitment and community development rates were inversely correlated with diversity, with the highest rates seen in temperate latitudes and the lowest in tropical Belize. Seasonal variability in recruitment also varied latitudinally, with strong summer pulses of recruitment in northern latitudes shifting to low and year-round recruitment at low latitudes. However, species turnover through time in communities becoming established was highest in Belize. We conclude with predictions regarding the implications these patterns may have on invasion dynamics at different latitudes.

INTRODUCTION

Latitudinal patterns in diversity have remained an important theme in ecology for more than a century, yet we still continue to debate the relative contributions of processes that may cause these patterns (Currie et al., 2004; Mittelbach et al., 2007). There are many environmental variables that change with latitude, and it is easy to correlate species distribution patterns with these factors. Unfortunately, it is as easy to find exceptions to these correlations. In addition, with increased transport of nonnative species (Ruiz et al., 2000), species distributions continue to be altered. Although latitudinal gradients in native species diversity are well documented, studies on terrestrial and freshwater systems suggest latitudinal gradients in invasion success occur as well (Sax, 2001). However, little work to date has examined this question in marine systems. Therefore, we have been documenting latitudinal differences in both the recruitment and the community development of

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marine epifaunal invertebrates as a first step in understanding latitudinal differences in species invasions.

The mode by which species successfully invade new habitats is a pressing ecological research issue (Rejmanek and Richardson, 1996; Williamson and Fitter, 1996; Moyle and Light, 1996). Current theory predicting the attributes of successful invaders has largely been developed in terrestrial environments and usually stresses the importance of life history traits associated with rapid reproduction and wide dispersal ability (Rejmanek and Richardson, 1996). In the far more open marine environment, ocean currents can disperse larvae and adults of many species for great distances over relatively short time periods (Jokiel, 1984; Scheltema, 1986). Additionally, man inadvertently transports countless individuals and species between discrete biogeographic provinces (Ruiz et al., 1997; Carlton, 1999). Given the generally good dispersal abilities of marine species, those attributes of new species that allow them to coexist with, or even displace, native species will be as important as dispersal ability to a species invasion potential.

The sessile invertebrate or epifaunal community is an excellent system in which to examine rigorously both the life history attributes that characterize successful invaders as well as those attributes of native communities that govern their susceptibility to invasion. Epifaunal communities occur in all coastal habitats and can be found in all biogeographic regions. These communities contain species with a variety of life histories, yet their principal species are usually permanently attached as adults and are easy to manipulate. Although the species within these communities differ among regions, they function in similar ways. Most have planktonic larvae as the main means of dispersal, feed from the water column, compete for limited available space, and are preyed on by a variety of mobile vertebrate and invertebrate predators. Because epifaunal species are sessile and relatively small in size, natural communities can develop on small discrete substrates, with larval dispersal and recruitment linking communities within a site or habitat as well as within a region. These attributes make them ideal systems that can be experimentally manipulated in the field to test directly hypothetical relationships while maintaining natural levels of abundance, species composition, and diversity.

Among epifaunal communities, a major difference is the number of available species that have some reasonable probability of recruiting to a particular site within a region. Osman and Dean (1987) found that these regional pools of species varied by almost an order of magnitude and that both the mean number of species found on individual substrates and the correlated richness at each site varied greatly among the study sites within each region, with overlap among sites in different regions. These patterns potentially result from (1) the low probability of recruits of many species in the regional species pool actually reaching a particular site during the course of investigation and (2) the high probability of local, within-site dispersal of species already present at a particular site. Alternatively, high predation and local extinction rates at some sites may prevent certain species in the regional species pool from colonizing these sites (Osman and Whitlatch, 1996, 1998). As a first step in trying to understand factors contributing to the local and regional differences in diversity and how these are likely to influence species invasions, we have been contrasting temporal variation in recruitment rates and resultant community development in regions along a latitudinal gradient from tropical to temperate regions.

METHODS

We deployed experimental panels in four biogeographic regions along the eastern seaboard of the United States and in the Caribbean Sea. These regions were Long Island Sound in Connecticut (LIS; 41°N), Maryland and Virginia's Chesapeake Bay and Eastern Shore region (CB; 37°N), the Indian River Lagoon in Florida (IRL; 27°N), and the vicinity of Carrie Bow Cay in Belize (BEL; 16°N). Polyvinyl chloride (PVC) panels, 100 cm^2, were abraded to facilitate settlement of invertebrates and were suspended on racks underneath docks. The panels were held horizontal with the experimental surface facing the seafloor.

Recruitment

To estimate recruitment in all regions, panels were sampled either weekly (LIS) or biweekly (CB, IRL, BEL). At the beginning of each sampling period, four clean panels were exposed at each of the field sites. After the one- or two-week exposure period the panels were collected and new panels were deployed. In the laboratory, all panels were examined under a dissecting microscope, and all attached invertebrates were identified to the lowest possible taxonomic unit (usually species) and counted.

Sample schedules varied by region as necessitated by recruitment patterns and destructive storm activity. Weekly sampling at the LIS Avery Point (AP) site began in 1991 and has continued unabated to the present. In the years 1991–1996 sampling was suspended during the win-

ter months when almost no settlement occurs. From 1997 to the present, sampling was conducted continuously with biweekly sampling during the winter. The remaining LIS sites (Groton Long Point [GLP] and Mystic River [MR]) were added in 2001 and have been sampled on the same schedule as the AP site. Sampling in CB and IRL was begun in 2004 with two sites in each region. The CB sites were at the Smithsonian Environmental Research Center (SERC) in the upper Bay and at the Virginia Institute of Marine Science (VIMS) in the lower Bay. The IRL sites were the Smithsonian Marine Station (SMS) and the Ft. Pierce Inlet (Inlet). Sampling at the VIMS site was discontinued in 2007 after hurricane damage to the dock, and sampling at both IRL sites was suspended from September 2004 until March 2005 because of the loss of docks as the result of two hurricanes. Sampling in BEL began in December 2004 and continued through February 2006.

Data Analysis

Recruitment differences among sites within and across regions were compared by matching means for each sampling time and using paired *t* tests to analyze for significant differences. Wilcoxon signed-rank tests were also conducted for each pairing to eliminate the possible effects of large seasonal differences biasing the results. Because of the species differences among regions, analyses were done for total recruitment of all species, pooled invasive species, and pooled native species. Species identified as cryptogenic were included with the native species. The number of sampling periods varied greatly among the regions, and we conducted the analysis of each pair of stations using the maximum number of sampling periods in common based on the year and week of sampling. Data were corrected for exposure time to account for the one- and two-week sampling periods used in different regions.

Community Development

To measure difference in community development, experimental panels (same as above) were deployed for at least one year and nondestructively sampled for invertebrate richness. Four panels were deployed at each site (three per region) between July and August 2006 to a depth of 0.6 m below LLT and at least 0.5 m above the bottom. Panels in LIS, CB, and IRL were sampled iteratively 1, 3, and 12 months after deployment. Panels in BEL were sampled 3, 6, and 12 months after deployment. Panels were sampled with a dissecting microscope, and attached invertebrates were identified to the lowest possible taxonomic unit. Taxonomic richness on each panel was recorded.

RESULTS

Recruitment

Three types of recruitment patterns are evident. Within sites there are temporal patterns, among sites within regions there are fairly consistent relationships, and together these produce broader patterns among the regions.

Within-Site Temporal Patterns

Within each site there are temporal patterns in recruitment that result from seasonal cycles in reproduction and year-to-year variation in recruitment that can result from a variety of causes. Seasonal variability in recruitment is most evident in the two northern regions, LIS and CB, which experience large variations in temperature. In both regions recruitment is largely absent during the coldest winter months. The three sites in LIS are consistent in exhibiting peak recruitment in the late summer (Figure 1). Recruitment at the GLP site begins earlier and remains consistently higher than at the other sites throughout the whole season. This site is in shallower water and consequently experiences lower winter temperatures and higher summer temperatures (Osman and Whitlatch, 2007) and warms more quickly in the spring. At the CB sites, the majority of recruitment occurs in the spring and early summer, with a second, much smaller peak period in the autumn (Figure 1). Most dominant species in this region such as barnacles, bivalves, and polychaetes are planktotrophic with feeding larvae dependent on the spring and autumn plankton blooms. Recruitment in the remaining two regions, although temporally variable, exhibits no consistent seasonal cycle. Recruitment occurs year round at both IRL sites, with the inlet having somewhat higher recruitment in the summer (Figure 1). Recruitment at the SMS dock is dominated by several species of barnacles and has much more sporadic peaks. Finally, BEL recruitment was extremely low and demonstrated no obvious patterns.

Spatial Variability among Sites within Regions

Within the three regions with multiple sites we have observed fairly consistent differences among the sites. Based on the paired *t* tests of weekly differences in total recruitment over the period 2001 through 2007, the three sites in LIS were significantly different, with GLP > AP > MR

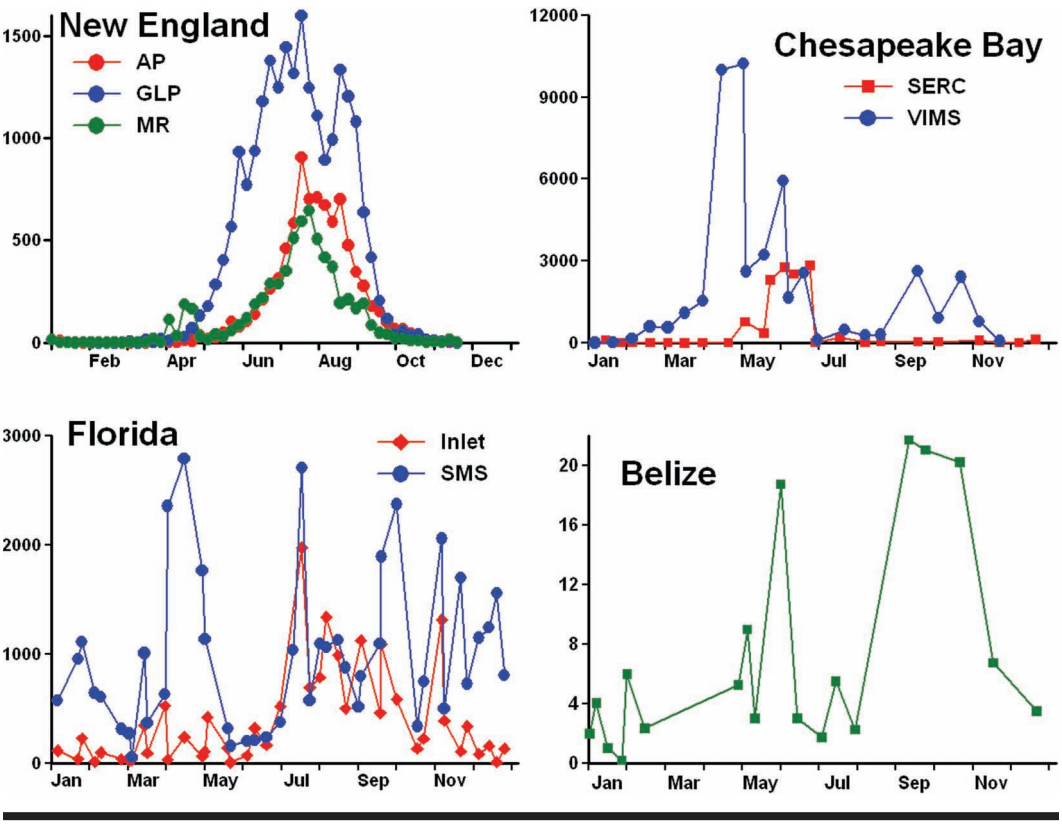


FIGURE 1. Comparison of temporal variation in mean recruitment in the four regions. Individual sites within regions are shown. Means were based on 1–6 years of data depending on region and the periods over which recruitment was measured (see Methods). Sampling sites are as follows: New England: AP = Avery Point, GLP = Groton Long Point, MR = Mystic River; Chesapeake Bay: SERC = Smithsonian Environmental Research Center, VIMS = Virginia Institute of Marine Science; Florida: Inlet = Ft. Pierce Inlet, SMS = Smithsonian Marine Station; Belize: Carrie Bow Cay.

(Table 1). Native species recruitment at the three sites showed the same pattern while the recruitment of invasive species was not significantly different among the sites (Table 2).

A similar analysis of the two CB sites for 2004 through 2006 found that total recruitment at the VIMS site was significantly greater than at the SERC site (Table 1). Recruitment at both sites was dominated by native barnacles, and invasive species recruitment was very low. Nevertheless both native and invasive species exhibited the same pattern as total recruitment (Table 2). Although experiencing similar variability in temperature, these two sites differ greatly in their salinity regimes. The SERC site is in the upper, low-salinity region of CB whereas the VIMS site is in the lower CB with higher salinities. In general fewer species recruit at the SERC site, and barnacle recruitment is much less.

Similarly, the two IRL sites differed significantly (2004–2006) in total recruitment, with SMS greater than Inlet (Table 1). Native and invasive species exhibited the same pattern (Table 2). Although there was little difference between the sites in temperature and salinity, they did differ in dominant species, which resulted in strong differences in total recruitment. Barnacle recruitment (six different species) was consistently much higher at the SMS site and this contributed greatly to the overall site differences. Most species of bryozoans as well as spirorbid worms had higher recruitment at the Inlet site. Figure 2 illustrates these differences.

The nonparametric paired analyses of the data from all three regions were almost identical to those above. The only difference was that in LIS invasive species recruitment was significantly greater at GLP than at either AP or MR.

Regional Patterns

The regional differences in temporal and spatial patterns in recruitment can be seen in Figure 1. In LIS the strong

TABLE 1. Results of paired analysis of mean recruitment between each pair of sites. Recruitment data were paired by sampling time. Mean values are for 2-week sampling periods and vary based on the number of sampling dates in common between any two pairs of sites (*df* = degrees of freedom;). Significant probabilities (Prob) are in **bold**.

			Total Annual Recruitment				
Site 1 [a]	**Site 2** [a]	***df***	**Mean 1**	**Mean 2**	***t*-ratio**	**Prob > |*t*|**	**One-sided**
Avery Point	Mystic River	229	474.5	299.2	6.39	**< 0.0001**	**< 0.0001**
	Groton LP	252	446.6	1000.4	8.60	**< 0.0001**	**< 0.0001**
	SERC	21	208.4	550.8	1.44	0.16	0.08
	VIMS	21	251.3	2206.2	2.99	**0.007**	**0.004**
	SMS	38	638.2	1010.0	2.22	**0.03**	**0.02**
	Inlet	36	607.5	607.5	1.37	0.17	0.09
Mystic River	Groton LP	234	296.7	996.8	9.63	**< 0.0001**	**< 0.0001**
	SERC	23	152.3	506.2	1.65	0.11	0.06
	VIMS	23	184.1	2097.8	3.22	**0.004**	**0.002**
	SMS	39	425.4	1068.1	4.15	**0.0002**	**0.0001**
	Inlet	38	451.2	459.9	0.09	0.93	0.46
Groton LP	SERC	21	698.5	550.8	0.52	0.61	0.31
	VIMS	21	761.3	2275.1	2.11	**0.05**	**0.02**
	SMS	39	1288.4	1050.5	0.91	0.36	0.18
	Inlet	37	1192.5	471.3	3.60	**0.0009**	**0.0005**
SERC	VIMS	17	381.6	1519.7	2.11	**0.05**	**0.03**
	SMS	10	341.6	685.3	1.18	0.26	0.13
	Inlet	10	341.6	242.1	0.42	0.68	0.34
VIMS	SMS	15	2694.9	903.9	2.38	**0.03**	**0.02**
	Inlet	15	2694.9	169.6	3.01	**0.009**	**0.004**
SMS	Inlet	71	1045.5	399.6	7.20	**< 0.0001**	**< 0.0001**

[a] Groton LP = Groton Long Point (GLP); SERC = Smithsonian Environmental Research Center; VIMS = Virginia Institute of Marine Science; SMS = Smithsonian Marine Station.

seasonality produces a relatively normal distribution in recruitment centered on the summer months of peak temperatures. Peak periods are relatively broad, with 1,000 to 2,000 recruits per panel per week. This overall pattern reflects the concentration of recruitment by most species in the summer period. Recruitment in CB is also seasonal but generally dominated by a few species, with sharp peaks in recruitment of 3,000 to 10,000 individuals per panel. The pattern in IRL is more diffuse with recruitment occurring throughout the year and several sharp peaks of 2,000 to 3,000 recruits per panel (barnacles) over a background of continuous recruitment. Individual species do have peaks in recruitment but they do not occur at the same time as in the northern regions. Thus, some species recruit in the winter and others in the summer, and this difference is reflected in the continuous total recruitment throughout the year. Finally, recruitment at the BEL site was extremely low, despite the much greater species diversity in the region.

Given these patterns, we examined whether total annual recruitment was influenced by the regional differences in variability and peak abundances. Figure 3 shows the total mean annual recruitment for each of the sites; no general pattern is discernible from these data. Except for BEL, within-region differences in total annual recruitment are as great as, if not greater than, differences among regions. Figure 3 also shows the dominance of barnacle recruitment in both the low-diversity CB and high-diversity IRL regions, whereas bryozoans and ascidians dominate recruitment in LIS. Interregional differences in total recruitment, regardless of strong differences in temporal patterns, exhibited no pattern that could be associated with diversity or latitude.

Results from the paired analyses did show some regional differences (Tables 1, 2; see Figure 3). For total recruitment the VIMS site in CB had significantly greater recruitment than all other sites. The GLP in LIS and SMS in IRL were significantly greater than the Inlet IRL, SERC CB, and AP LIS

TABLE 2. Results of paired analysis of mean invasive and native recruitment between each pair of sites. Recruitment data were paired by sampling time. Mean values are for 2-week sampling periods and vary based on the number of sampling dates in common between any two pairs of sites. Significant probabilities (Prob) are in **bold**.

			Invasive					Native				
Site 1 [a]	Site 2 [a]	*df*	Mean 1	Mean 2	*t*-ratio	Prob > |*t*|	One-sided	Mean 1	Mean 2	*t*-ratio	Prob > |*t*|	One-sided
Avery Point	Mystic River	229	144.9	144.9	0.00	0.99	0.50	329.6	154.3	6.69	**< 0.0001**	**< 0.0001**
	Groton LP	252	133.7	145.9	0.67	0.50	0.25	312.9	854.5	8.91	**< 0.0001**	**< 0.0001**
	SERC	21	66.6	1.3	2.18	**0.04**	**0.02**	141.8	549.5	1.81	0.08	**0.04**
	VIMS	21	95.8	18.8	1.94	0.07	**0.03**	155.6	2187.4	3.14	**0.005**	**0.003**
	SMS	38	218.9	345.5	1.23	0.22	0.11	409.0	666.6	1.84	0.07	**0.04**
	Inlet	36	203.2	191.0	0.39	0.70	0.35	381.3	270.6	1.46	0.15	0.08
Mystic River	Groton LP	234	143.3	149.3	0.31	0.75	0.38	153.4	847.5	9.87	**< 0.0001**	**< 0.0001**
	SERC	23	35.8	1.2	2.27	**0.03**	**0.02**	116.4	505.0	1.86	0.08	**0.04**
	VIMS	23	28.9	24.0	0.60	0.55	0.28	155.1	2073.8	3.24	**0.003**	**0.002**
	SMS	39	199.9	355.9	1.85	0.07	**0.04**	225.4	712.3	4.37	**0.0001**	**<0.0001**
	Inlet	38	200.4	186.1	0.21	0.83	0.42	250.8	273.8	0.37	0.71	0.36
Groton LP	SERC	21	163.7	1.3	2.31	**0.03**	**0.02**	534.8	549.5	0.05	0.96	0.48
	VIMS	21	220.7	18.8	2.47	**0.02**	**0.01**	540.7	2256.3	2.47	**0.02**	**0.01**
	SMS	39	218.9	126.6	2.01	**0.05**	**0.03**	1069.5	705.0	1.50	0.14	0.07
	Inlet	37	203.2	−12.2	0.30	0.77	0.38	989.3	280.3	3.55	**0.001**	**0.0005**
SERC	VIMS	17	1.2	25.6	2.11	**0.05**	**0.02**	380.3	1494.2	2.05	0.06	**0.03**
	SMS	10	0.4	173.1	3.11	**0.01**	**0.006**	341.2	512.2	0.63	0.54	0.27
	Inlet	10	0.4	48.7	1.95	0.08	**0.04**	341.2	193.4	0.61	0.56	0.28
VIMS	SMS	15	11.0	239.4	3.23	**0.006**	**0.003**	2683.9	664.5	2.78	**0.01**	**0.007**
	Inlet	15	11.0	26.6	2.38	**0.03**	**0.02**	2683.9	143.0	3.02	**0.009**	**0.004**
SMS	Inlet	71	374.8	138.7	5.11	**< 0.0001**	**< 0.0001**	670.7	260.9	5.96	**< 0.0001**	**< 0.0001**

[a] R. = River; Inlet = Ft. Pierce Inlet.

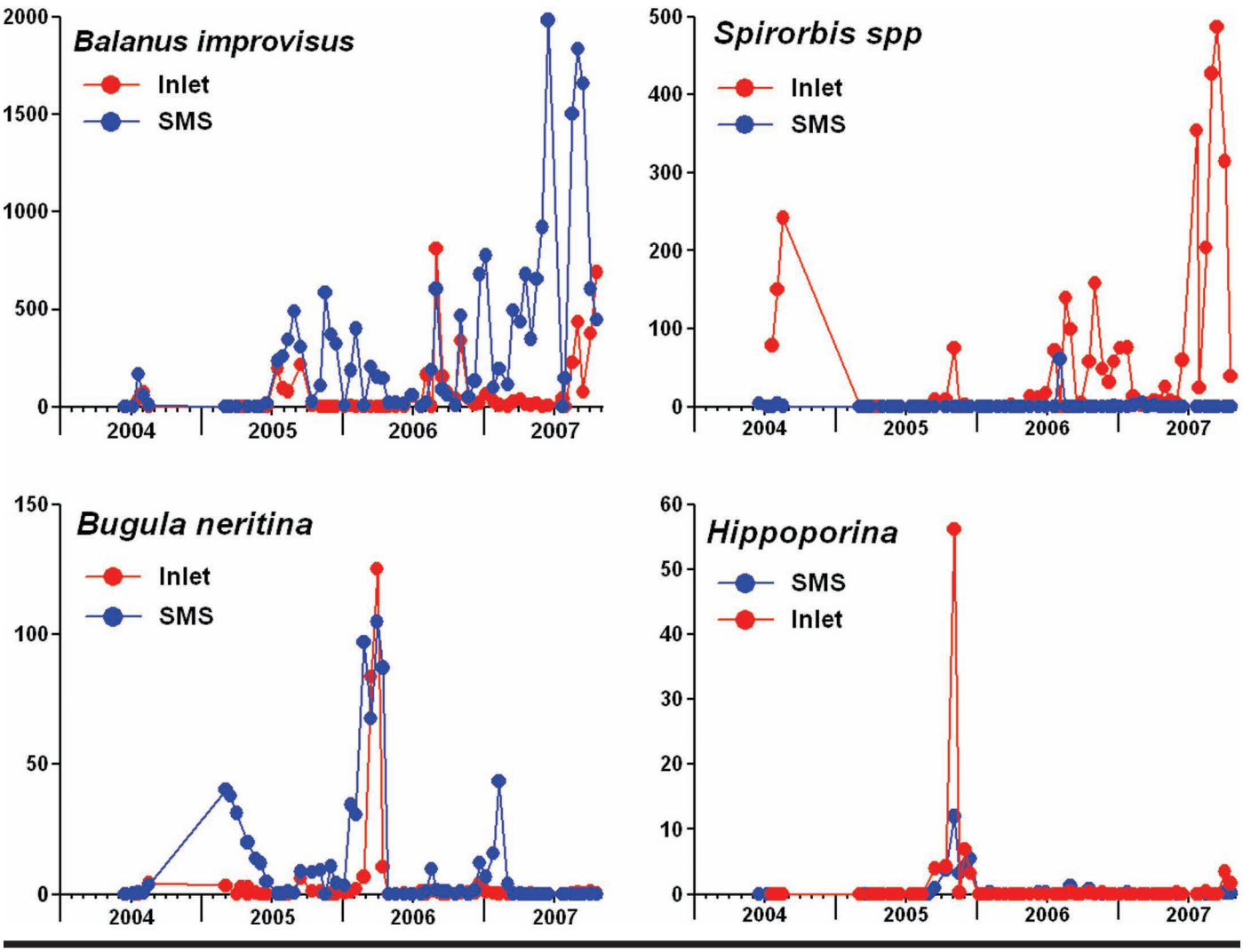


FIGURE 2. Comparison of recruitment at the two sites in Indian River Lagoon, Florida, for the barnacle *Balanus improvisus*, spirorbid polychaetes (*Spirorbis* spp.), arborescent bryozoan *Bugula neritina*, and encrusting bryozoan *Hipporina* sp.

sites, and all were greater than the MR LIS site. Recruitment of native species exhibited similar interregional patterns. However, for invasive species, recruitment was significantly higher in IRL than CB, with the LIS sites intermediate.

Community Development

Spatial and Temporal Variability

The speed of community development varied dramatically with latitude. The primary limiting resource, space, was quickly occupied in the temperate and subtropical regions by three months, compared to the tropical communities, which took close to a year to attain comparable spatial coverage (Figure 4). In the northernmost region, LIS, growth rates were particularly high, and at one site (AP) panels were completely covered after only one month, which is a striking comparison to comparably aged communities in BEL (Figure 5). These productive communities in AP were primarily composed of *Diplosoma listerianum*, an invasive colonial tunicate, and *Mogula manhattensis*, a solitary tunicate. These animals quickly became too heavy to remain attached to the panel and sloughed off, providing another flush of open space to recruiting species. This punctuated seasonal pulse of productivity of *Diplosoma* and *Mogula* did not occur at the other two sites in LIS, but growth rates remained high throughout the region.

Overall, communities in LIS experienced less temporal turnover in species composition than more southerly sites,

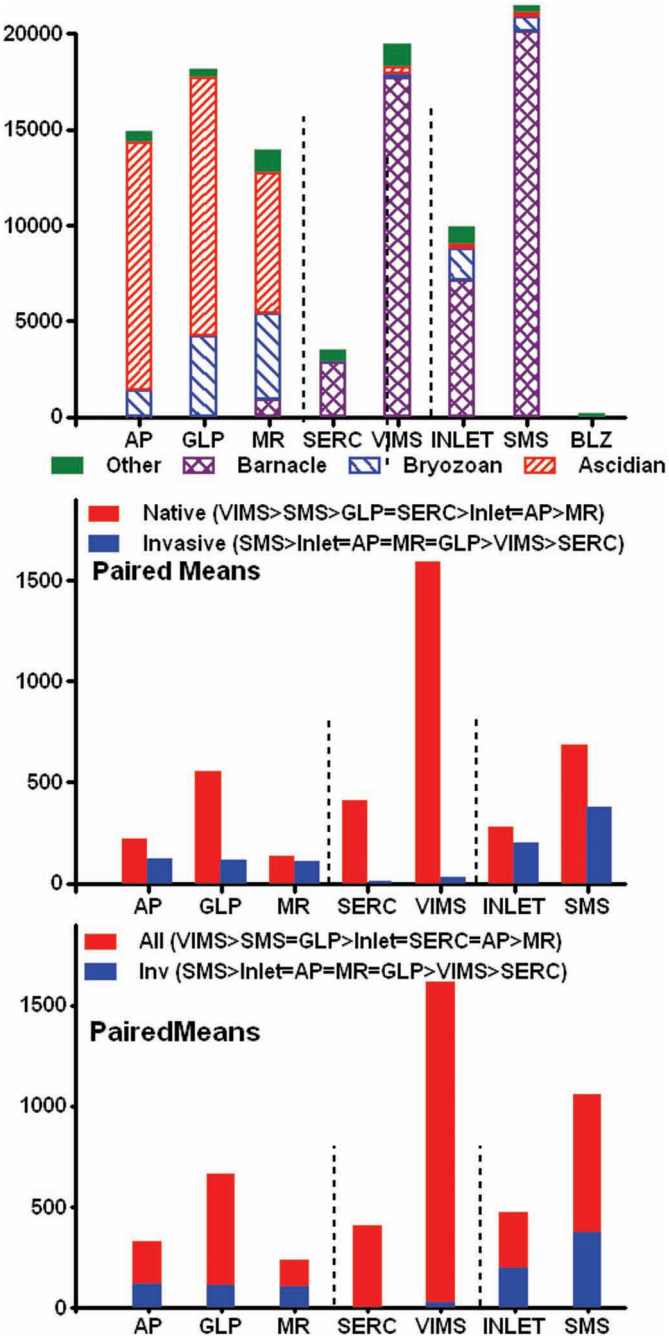


FIGURE 3. Comparison of recruitment among regions. Top: Total annual recruitment at each of the sites within regions. The contributions in each region of major taxonomic groups are represented by colored shading and/or scoring within the histogram bars. Middle: Mean recruitment of invasive (blue) and native (red) species by sites within region; significant differences are based on paired analyses (see Table 2). Bottom: Total mean recruitment by site showing the contribution of invasive (Inv) and native species. Dashed lines separate regions in all graphs.

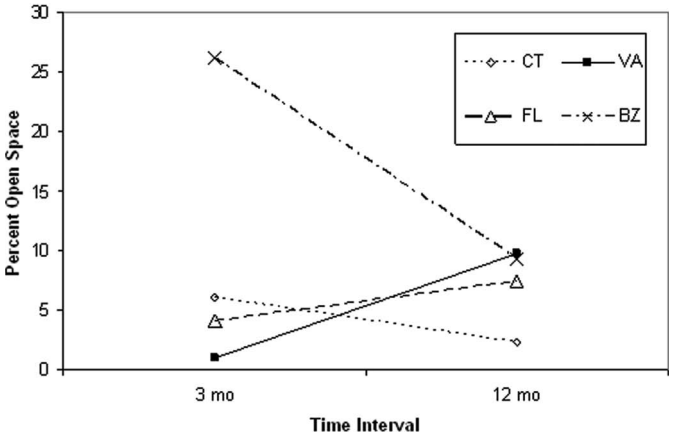


FIGURE 4. Total percent cover of open space on panels in the four regions after 3 and 12 months (CT = Connecticut; VA = Virginia; FL = Florida; BZ = Belize). In Belize, space occupied by algae was included as covered space, so percent cover of invertebrates was even lower than shown.

particularly BEL (Figure 6). Communities in LIS were consistently dominated by bryozoans, particularly *Bugula turrita*, and both solitary and colonial tunicates (Figure 7; personal observations). This observation is in contrast to the higher rates of species turnover that characterized communities in tropical BEL (Figure 6; Freestone, unpublished data).

Epifaunal communities in CB had low temporal and spatial variability in species composition compared to other regions. Barnacle recruitment occurred soon after deployment, in July 2006. After the first month, all panels had 99% to 100% cover (see Figure 4) and were almost completely covered with barnacles, with few other coexisting species. After three months, community structure still closely resembled the one-month communities; however, barnacles began to die and other species, such as *Mogula*, various hydroids, and sabellid polychaete worms recruited (see Figure 7). After one year, the primary layer of barnacles was less visible, having been covered with a thick layer of sediment tubes, mostly from amphipods and worms. Anemones were also common throughout. Panels deployed at the three sites were also very similar. Porifera were least common in CB when compared with other regions throughout the experiment.

Overall, communities in IRL retained almost complete phyla representation through time (Figure 7). All focal phyla were found on all panels in IRL after three months, and only Porifera had a very modest decline by one year. Species in these communities coexisted at very small spatial scales, with the result that IRL had the most diverse invertebrate assemblages at the panel scale after

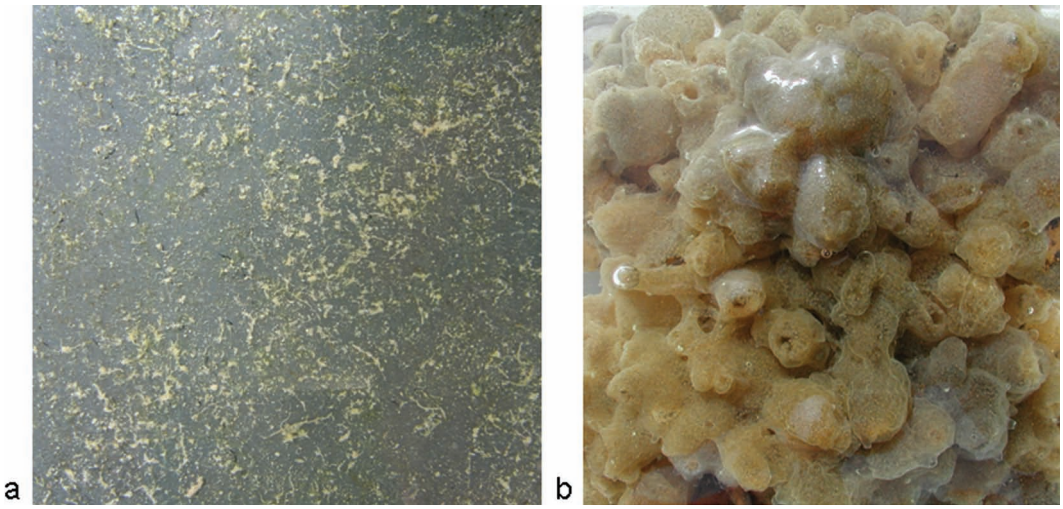


FIGURE 5. Growth rates were higher at northern latitudes, a pattern that is clearly visible in this comparison between (a) a 3-week-old community from Carrie Bow Cay, Belize and (b) a 4-week-old community from Avery Point, Long Island Sound (100-cm^2 panels are shown).

one year (Freestone, personal observation). More species turnover was apparent over the course of the year at IRL than in LIS or CB, but not as much as at BEL.

Communities in BEL were characterized by significant temporal and spatial variability in species composition. Communities developed much more slowly than did more northern communities (see Figure 4), and community composition clearly changed with time. These communities also varied at very small spatial scales, as panels that were deployed within a meter of each other harbored very distinct community assemblages with differing amounts of open space. In contrast to more northern communities, BEL communities were more consistently dominated by polychaetes, Cnidaria (sea anemones, hydroids, coral), and Porifera. After one year, Porifera clearly dominated the panels (Freestone, personal observation). Compared to the bushy and common bryozoan colonies that occur in LIS, bryozoans in BEL were generally very small, delicate, and rare.

Similar to the recruitment study, the largest difference in taxonomic composition of developing communities across all regions was the presence of barnacles. Barnacles were common in temperate and subtropical zones but were completely absent in BEL at 3 months. After 12 months, their dominance was still seen in CB and IRL, but barnacles were less common in LIS. However, only one barnacle on one panel was found in BEL. Although barnacles in temperate and subtropical zones are both intertidal and subtidal, barnacles are almost exclusively intertidal in BEL.

DISCUSSION

Based on our preliminary examination of these ongoing studies, it is clear that there are both strong intraregional and interregional patterns in both the recruitment and the development of epifaunal communities. Seasonality in recruitment clearly varies with latitude. Strong summer peaks coupled with the almost complete absence of any recruitment in the winter were found for most species in the temperate regions (LIS and CB). The strong dominance of barnacles in CB resulted in a bimodal pattern generally associated with the spring–fall plankton blooms upon which barnacle larvae feed. In IRL there was also a strong temporal variability in recruitment but neither a consistent seasonal pattern nor any similarity among species. Finally, in BEL recruitment was too low to discern any pattern. There were also fairly distinct patterns among sites within regions. In the temperate regions, sites showed consistent differences in numbers of recruits but little difference in the species recruiting at any one time or in the relative abundances of these species. In the subtropical IRL, there were greater differences between the two sites in the composition of the fauna recruiting at any one time. Based on the low recruitment and greater community variability among sites in BEL, it would appear that site differences in recruitment in the tropics are likely to be even greater.

The interregional variation in recruitment for native species was influenced by the variation in barnacle dominance, with the CB and IRL sites showing significantly

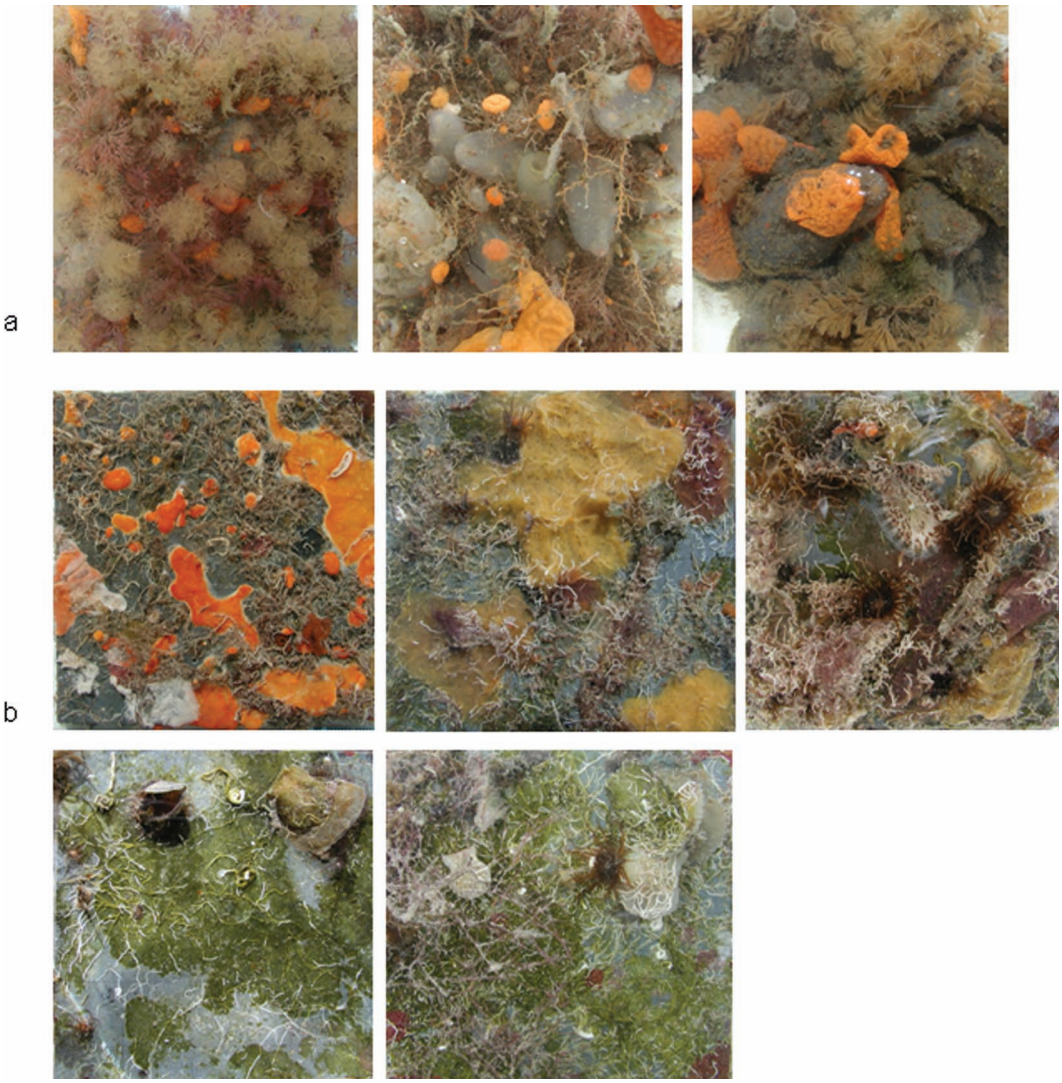
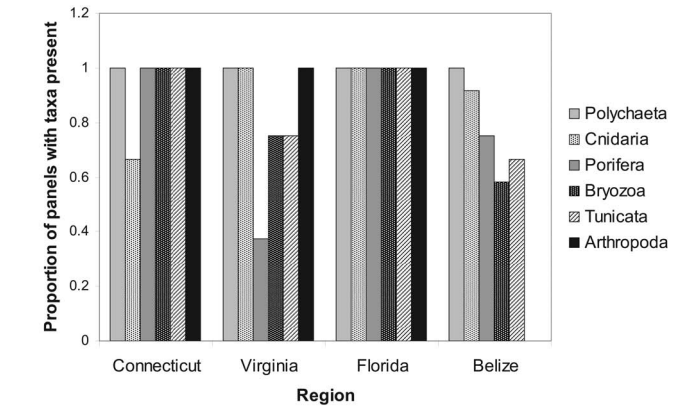


FIGURE 6. Time-series comparison of community development in (a) Long Island Sound after 1, 3, and 12 months and (b) Belize after 3, 6, 9, 12, and 20 months (100-cm^2 panels are shown). Greater species turnover occurred in Belize than in Long Island Sound.

higher recruitment than the LIS sites. However, the recruitment of invasive species varied in a similar manner to patterns described by Sax (2001). The tropical BEL site had little overall recruitment and no recruitment of invasive species, the subtropical IRL sites had the highest recruitment of invasive species, and the higher latitude temperate sites had lower recruitment of invasive species. The combined patterns of native and invasive species resulted in invasive species representing a much higher proportion of overall recruitment in IRL than in all other regions. In the low-diversity estuarine CB, invasive species contributed a very small proportion of total recruitment (see Figure 3).

Finally, even with the strong differences in recruitment reflected in the paired analyses, similarities in total annual recruitment were found between some sites in the three northern regions. This result suggests that the cumulative recruitment in more seasonal regions with strong peaks and in regions with little or no seasonality in recruitment can be similar.

In BEL, the epifaunal communities were quite spatially variable in community composition even at the smallest scale of panels at each site. This pattern is consistent with the spatial heterogeneity hypothesis for the latitudinal diversity gradient, which states that abiotic variability in tropical systems allows more species to coexist (see Davidowitz and



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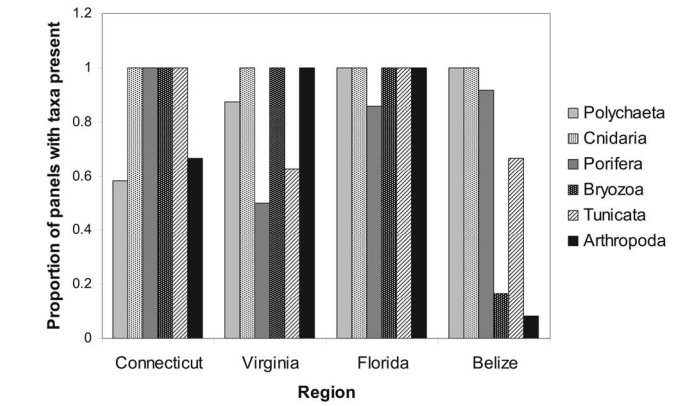

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FIGURE 7. Proportion of panels that had listed taxa present at (a) 3 months and (b) 12 months by region.

Rosenzweig, 1998). Interestingly, in contrast to the abiotic variation that characterizes other systems, such as terrestrial plant–soil relationships, the settlement panels were identical in size and material, so substrate composition was not a source of variability. While it is possible that differences in subtle small-scale variation in currents or eddies could drive community variability, a more parsimonious explanation is that propagule supply is very low and sporadic. Community developmental trajectories may therefore be more a result of random recruitment from a limited larval pool rather than spatial variability in abiotic conditions.

Another possible explanation that has strong theoretical underpinnings is that biotic interactions, such as predation, are also strong and spatially variable in the tropics (Schemske, 2002). Although this hypothesis has not been empirically tested in a comprehensive experiment, the idea that biotic interactions are stronger in the tropics has received much theoretical attention (Mittelbach et al., 2007). Visual observations of the communities in BEL support this hypothesis. For example, we commonly observed grazing or saw indirect evidence of grazing (i.e., abrasions) on the panels from indiscriminate consumers, including gastropods, crabs, and fish. While predation undoubtedly occurs in temperate environments (Osman and Whitlatch, 1995, 1998, 2004), overall interaction strengths may be weaker and more spatially predictable in northern latitudes. Sporadic and low larval recruitment, spatially variable predation, and low growth rates in areas of low productivity are all potential contributors to the spatial and temporal variability of tropical epifaunal community development.

Our main goal has been to document and contrast recruitment and community development patterns among regions along a latitudinal gradient to ascertain potential differences in the ability of nonnative species to invade these systems. Except for our sites in BEL, all the regions we have been studying have nonnative species present, and such species are often dominant within these epifaunal communities. In LIS we have found that early recruitment of invasive ascidians in years with warm winters (Stachowicz et al., 2002a) and their dominance at harbor sites without native predators that prey on their recruits (Osman and Whitlatch, 1995, 1998, 2004) have contributed to their successful invasion. The strong and consistent timing of recruitment of native species certainly can create an opening for invaders that can recruit outside this window. In CB recruitment is even more constrained temporally with much higher numbers of recruits, again creating potential temporal windows for invasion. However, as our community development data have shown, the communities in both these temperate systems develop rapidly and thus quickly limit resources for new species. Studies in LIS (Stachowicz et al., 2002b) have shown that as community diversity within these systems increases, the communities become more resistant to invasions, mostly by increasing the likelihood of limiting open space.

In IRL, space was also rapidly occupied and the amount of open space remained low after three months. In addition, the diversity within this system is higher, and some species are recruiting at any time of the year. Based on the results of the LIS study (Stachowicz et al., 2002a) these factors should increase the resistance of this system to invaders. Although we have found several invasive species at our study sites, none of these species appears to be particularly abundant or dominant. Finally, in BEL we have observed much more diverse and spatially variable communities. Recruitment and the rates of community development are low, and this situation certainly allows spatial resource to be available for much longer periods of time, which should create a greater window for species invasion. However, the extremely high diversity of both epifaunal

species and predators may inhibit invasion success. It is also likely that, given the spatial variability in communities, invaders will face completely different communities at each site as well as temporal variability in communities within sites. Although it is much too early in our studies to link latitudinal variation in recruitment or community development to invasion success, our preliminary results do suggest that there is a correlation between decreasing invasion success and increasing diversity, increasing community variability, and the reduction in recruitment windows in less seasonal environments, with species varying greatly in the timing of recruitment.

Acknowledgments

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Ex Situ Culture of Caribbean and Pacific Coral Larvae Comparing Various Flow-Through Chambers

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ABSTRACT. Coral reefs are some of the oldest and most diverse ecosystems on our planet, yet throughout their range coral reefs are declining precipitously, mainly as the consequence of human activities. In situ conservation practices, such as habitat preservation, are an important way to protect coral reefs. However, reefs now face global threats in addition to local impacts. It is therefore critical that ex situ conservation activities are incorporated into conservation practices for coral reefs. Many coral species reproduce sexually during a limited yearly breeding season. If the resulting larvae are cultured, their husbandry can be very time consuming: time that is often taken away from larval research. Three different types of flow-through larval rearing systems were designed and tested during breeding seasons of the elkhorn coral *Acropora palmata*, the mushroom coral *Fungia scutaria*, and the cauliflower coral *Pocillopora meandrina*. The flow-through systems were tested against static bowl rearing, and no difference was observed in the survival of the larvae in two of the species: $P = 0.12$ for *A. palmata* and $P = 0.99$ for *F. scutaria*. These results suggested that these chambers may result in significant savings of limited research time during a coral spawning event. However, *P. meandrina* larval survival was better in bowls than in the flow-through chamber ($P = 0.03$). Rearing the maximum number of larvae possible with minimal maintenance will enhance opportunities for larval research, settlement, and growth. This is especially important for species that are now threatened, for which time and information are critical during the breeding season.

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INTRODUCTION

Coral reefs are some of the oldest and most diverse ecosystems on our planet. They are essential nurseries and feeding grounds for fish and invertebrates, act as natural storm barriers for coastlines, and are a potential source for novel pharmaceuticals (Colin, 1998). Throughout their range, coral reefs are declining precipitously, mainly because of human activities. These negative influences induce stress and can increase diseases in corals. Even in the most

remote marine bioreserves, such as the northwestern Hawaiian Islands (Maragos et al., 2004), human activities are damaging fragile coral ecosystems (Bellwood et al., 2004). Additionally, other environmental pressures, such as El Niño-Southern Oscillation events, result in bleaching and coral mortality (Glynn and D'Croz, 1990; Glynn, 1996). As greenhouse gases increase, atmospheric and sea-surface temperatures and ocean acidification are also expected to increase (Kleypas et al., 1999; Hoegh-Guldberg et al., 2007). When these effects are coupled with human-induced stresses, reefs will remain in crisis, their existence worldwide increasingly threatened (Hoegh-Guldberg, 1999; Hughes et al., 2003).

Scientists speculate that unless committed efforts are made to remedy this situation functional coral ecosystems may disappear in less than 50 years (World Wildlife Report, 2004; Hoegh-Guldberg et al., 2007). Although all the oceans in the world have corals, reef-building corals in the Caribbean are showing the greatest signs of disease-related mortality, and these corals may have far less than 50 years left to survive (Hoegh-Guldberg et al., 2007). The massive elkhorn coral, *Acropora palmata*, has historically been the most ecologically important reef-building coral in the Caribbean, but its populations have declined 90% to 99% since the mid-1980s, primarily because of disease (Aronson and Precht, 2001). Because of this decline and its critical role for Caribbean reefs, *A. palmata* has been one of the first two corals listed as "threatened" under the Endangered Species Act (*Acropora* Biological Review Team, 2005). As stony corals continue to die, they are being replaced with sponges, gorgonians, and algae (Hughes, 1994; McClanahan and Muthiga, 1998), altering the composition of Caribbean ecosystems.

In situ conservation practices, such as establishment of marine protected areas, are an important way to protect coral reefs. However, reefs now face global rather than just local threats. Therefore it is critical that ex situ conservation techniques are incorporated into conservation actions for coral reefs. Ex situ conservation techniques, defined as protecting organisms outside their native habitat, such as rearing sexually produced larvae in seminatural enclosures for future restoration purposes, hold strong promise for improvements in preserving species and genetic diversity within ecosystems. This stage is particularly needed to help diversify some of the declining endangered populations in Florida where many of the stands of *A. palmata* are genetically identical (Baums et al., 2005).

To address the ex situ conservation needs for coral reefs, SECORE (www.secore.org) was initiated by the Rotterdam Zoo in 2001 with the primary goals of studying sexual coral reproduction, specifically developing ex situ breeding techniques, disseminating techniques among aquarium and research communities through workshops and publications, developing a cooperative international network of public aquariums and research institutions, and establishing breeding programs to help sustain ex situ and field populations. In 2006 and 2007 SECORE members representing several national and international institutions held workshops in Puerto Rico with goals to successfully rear elkhorn coral from spawn produced during the annual mass spawning at Rincón and Bajo Gallardo sites. Gametes were collected and fertilized, producing close to a million larvae, of which hundreds of thousands were raised in the field laboratory and more than 400,000 were brought into captivity, resulting in approximately 2,300 juvenile larval recruits now living in public aquaria around the world (Petersen et al., 2007). These larvae were the first juveniles of this species ever reared in captivity, constituting a major step that will help with the conservation of their genome and restoration of this species in the wild.

Although ex situ conservation practices have yet to be applied to coral populations in conjunction with restoration, extensive work has been conducted in the zoological community on maintaining gene diversity in populations with ex situ techniques (Ballou, 1992; Harnal et al., 2002; Pukazhenthi et al., 2006). In particular, the black-footed ferret was rescued from the brink of extinction, with only 18 individuals remaining in the population, using ex situ conservation practices in parallel with restoration practices (Howard et al., 2003). Enhancing reproductive success of endangered coral through ex situ practices may be key to their future restoration and preservation (Richmond and Hunter, 1990). There are a number of ex situ techniques that have enhanced larval survival and settlement. Heyward et al. (2002) used a seminatural enhancement procedure for maintaining acroporid corals in open floating pools in the ocean. Water was pumped into the pools throughout the larval growth period, and then the contents were pumped into an enclosed area on the sea bottom with conditioned ceramic tiles. Heyward et al. started with $\sim 10.5 \times 10^6$ larvae/pool and after 144 h post-fertilization had $\sim 7.5 \times 10^5$ larvae/pool ($\sim$0.7% survival), resulting in $\sim$1,500 settled recruits in the best treatments versus 0 on the control tiles. Although this settlement rate was relatively low, it was far greater than the natural settlement rate and indicated a robust enhancement of recruits for this area.

Most current coral larval husbandry practices are low-cost efforts, such as bowls or aquaria filled with fil-

tered seawater, and these methods are very successful at rearing larvae (Babcock and Heyward, 1986; Schwartz et al., 1999; Petersen et al., 2007). The problem is that these time-consuming and labor-intensive husbandry practices compete with the limited time available for research during a coral breeding season, especially if the coral species is limited to a single annual breeding, as is *Acropora palmata*. For coral in need of replenishment, rearing the maximum number of larvae possible with the least time invested in husbandry would enhance opportunities for larval growth and settlement (Richmond and Hunter, 1990; Petersen and Tollrian, 2001; Borneman, 2006). The goal of this paper was to design and test simple flow-through systems in the field that would minimize husbandry and yet successfully rear large numbers of coral larvae without compromising survival.

Three species of coral larvae were tested in three different types of rearing chambers. These larvae were selected because they represented a good cross section of coral larval types with different buoyancies, swimming behaviors, and rates of development that might benefit from these chambers. *Acropora palmata* are large lipid-filled floating larvae (Figure 1a) that develop slow swimming ability in the water column after 48 h. *Fungia scutaria* are small negatively buoyant larvae containing modest lipid stores. These larvae develop rapid swimming behavior in the water column within 12 to 24 h (Figure 1b). *Pocillopora meandrina* are negatively buoyant larvae with modest lipid stores (Figure 1c); these larvae develop slow swimming behavior along the bottom after 24 h. In designing and constructing these low-tech chambers, we made an effort to use materials for their components that are affordable and available in most hardware stores throughout the world.

MATERIALS AND METHODS

Larval Collection and Rearing

Acropora palmata eggs and sperm were collected during the annual spawn from Tres Palmas Reserve (Rincon, Puerto Rico) and the offshore submerged bank Bajo Gallardo (Boqueron, Puerto Rico) in August 2007. Egg/sperm bundles were collected in the water over the spawning coral with 1 L plastic Nalgene bottles attached to fine mesh nets. The egg/sperm bundles were brought to shore in the plastic bottles, separated by gentle agitation, and then combined with the eggs and sperm from at least three to four individuals to yield a sperm concentration of approximately 10^6 cells/mL (final concentration in water). The eggs and sperm were gently agitated for 2 h, cleaned with 1 μm-filtered seawater, assessed for fertilization rates, and released into rearing chambers for subsequent development.

Fungia scutaria eggs and sperm were collected from captive animals held in flowing seawater tanks from June through October 2006 at Coconut Island, Hawaii. Animals were prepared for spawning following the methods of Krupp (1983). Briefly, as a female spawned, these eggs were gently moved into a plastic bowl and fertilized with ~150 mL sperm (10^6 cells/mL, final concentration in water) from four or five males. The embryos, resulting from several male and female gametes, were kept in a single plastic bowl (8 L) and left overnight to develop. In the morning the developing larvae were cleaned with four changes of 0.5 μm-filtered seawater and then released into their rearing chambers for subsequent development.

Egg/sperm bundles were collected from *Pocillopora meandrina* fragments in April and May 2008 from Coconut Island, Hawaii. The eggs and sperm were separated

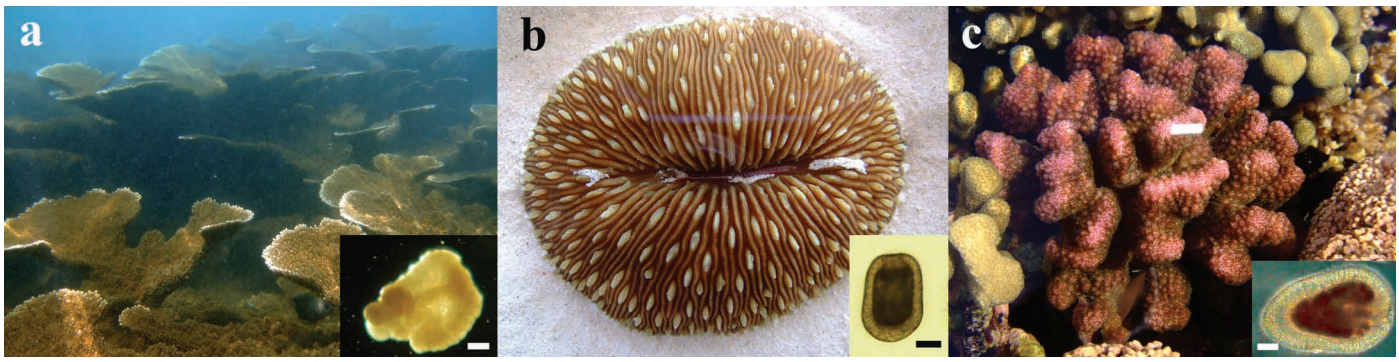

FIGURE 1. Three species of coral were reared in this study. a, *Acropora palmata*, elkhorn coral; inset: larvae at 24 h postfertilization. b, *Fungia scutaria*, mushroom coral; inset: larvae at 96 h postfertilization. c, *Pocillopora meandrina*, cauliflower coral; inset: larvae at 96 h postfertilization. All scales = 50 μm.

by gentle agitation, and then combined with the eggs and sperm from at least three or four individuals to yield a sperm concentration of approximately 10^6 cells/mL. The eggs and sperm were gently agitated for 0.5 h, cleaned with 0.5 μm-filtered seawater, and left overnight to develop. The next morning the developing larvae were cleaned with 0.5 μm-filtered seawater and released into their rearing chambers for subsequent development.

Digital images of the larvae from all three species were captured with an Olympus BX41 microscope with an attached digital camera Sony DFWV300, and the major and minor axes were measured with NIH Image software.

Construction of Rearing Tanks and Measurement of Densities

Larval corals were reared in three different designs of flow-through chambers (Figures 2–4), as well as static bowls that required daily cleaning and water changes. The names of these chambers were chosen to describe the major water movement they provided to the larvae. All developmental times reported throughout the paper are in hours postfertilization.

Up-Flowing Tanks

These tanks were made from 20 L heavy-walled plastic pans (U.S. Plastics Corp., Lima, Ohio) modified by covering the handles in a buoyant foam and removing four panels from the bottom and replacing them with nylon screening (240 μm mesh). A central cross-shaped area was left intact to create an inlet for upward-directed water flow; then additional shear flow was added with four additional adjustable water inlets around the edges approximately 16 cm above the bottom, yielding a final volume in the chambers of ~23 L (see Figure 2). All flow was regulated by valves to optimize the slow tumbling movement of the larvae in the chamber. The floating chambers were immersed in large 2,400 L pools to stabilize their temperature (28°–31°C) and mimic natural temperature cycles throughout a 24 h period. To maintain water quality close to that which the larvae would experience in open water, the chambers were attached to a filtered (1 μm) flow-through system with seawater pumped from the reserve, so that water was completely exchanged in the chambers several times each day. Flow rates through the chambers were maintained at approximately 2 L/min, and the bath of fresh seawater surrounding the chambers was turned over about one to two times per hour. Salinity, temperature, and pH closely mimicked natural conditions without additional effort.

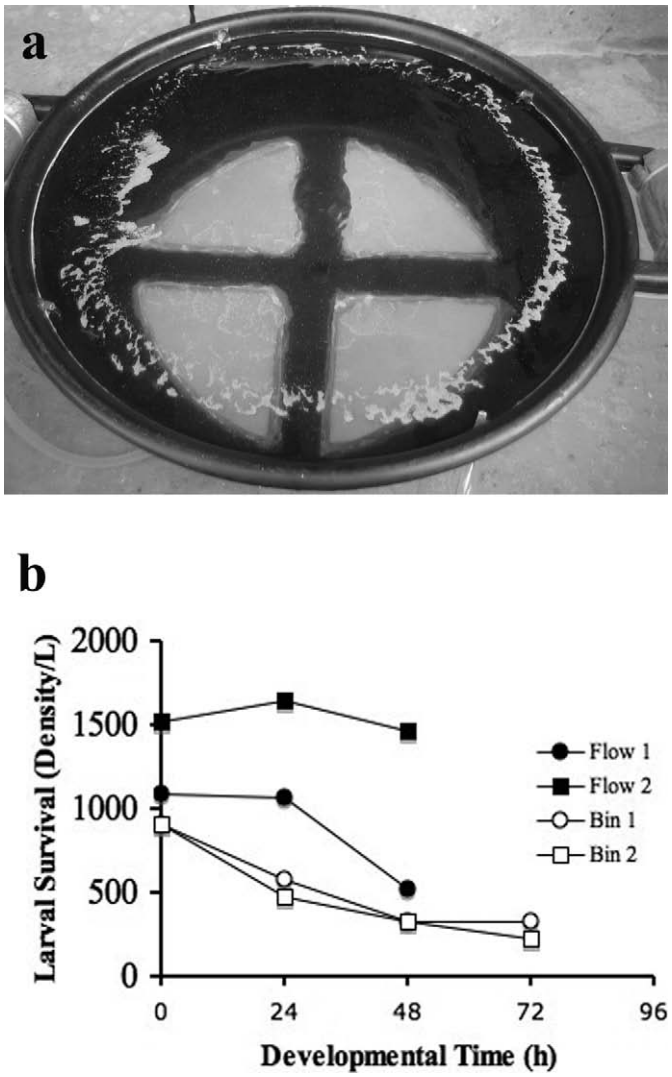

FIGURE 2. Larval rearing of *Acropora palmata* larvae. a, Up-flowing tank used to rear the *A. palmata* larvae. b, There was no significant difference in survival between the up-flowing tanks and static bins (ANCOVA, $P = 0.12$). Points show mean counts for each trial. Larvae in the upward-flow tanks were developing rapidly (~28°C) while those in the static bins were maintained at a slightly lower temperature (~25°C). Larvae in the flow chambers were removed from the experiment one day earlier than those in the bins to ship them to an aquarium for settlement and rearing.

Approximately 1,000 to 1,500 larvae/L were fertilized in 50 mL conical plastic tubes, then placed into either the up-flowing tanks ($n = 2$) or static bins. Counts were taken immediately, and then daily for all groups. Bin density began at ~900 larvae/L, and the two flow chambers contained either 1,100 or 1,500 larvae/L. The fertilization rate for *A. palmata* spawn used for these tests was ~90%.

The static treatments used for comparison with the up-flowing tanks were plastic rectangular bins (length [L] × width [W] = 51 cm × 36 cm) with water depth of 12 cm, yielding a volume of 22 L. These tanks were maintained in an air-conditioned room; the water was maintained at 25° to 26°C and changed twice daily. To keep the floating *A. palmata* larvae from clumping and forming an anoxic layer, the water and floating larvae were stirred every hour with a bubble-wand (2-mm-diameter rigid air line attached to a small air pump) throughout the rearing period. The previous year, a stocking density of approximately 1,000 *A. palmata* larvae/L was used successfully in each bin and we used this same level for these tests. Larvae from the same spawn and bulk fertilization as were used in the chambers were placed in the static bins (n = 2) 2 h after fertilization.

To determine larval survival, the chamber and static containers were stirred to suspend the larvae evenly in the water column, and five 15 mL samples were taken and the number of larvae counted each day. The number of larvae/mL was multiplied by 1,000 to determine the density per liter (density/L). The larvae in both systems were only allowed to develop for two to three days, and then they were packaged for shipment. Approximately 4,000 larvae were placed into a 2 L Nalgene bottle with filtered seawater (FSW); the bottles were filled to the top with FSW and capped leaving no bubbles, taped for security, placed horizontally in a cooler (8–12 bottles were placed in a single cooler), and sent by express mail to aquaria throughout the USA.

Spiral-Flowing Tanks

Small conical fiberglass tanks (~75 L) were fitted with a central standpipe covered with 40 μm nylon mesh to rear *Fungia scutaria* larvae (see Figure 3). To maintain the water quality close to that which the larvae would experience under natural conditions, the conical tanks were attached to a filtered (0.5 μm) flow-through system with seawater

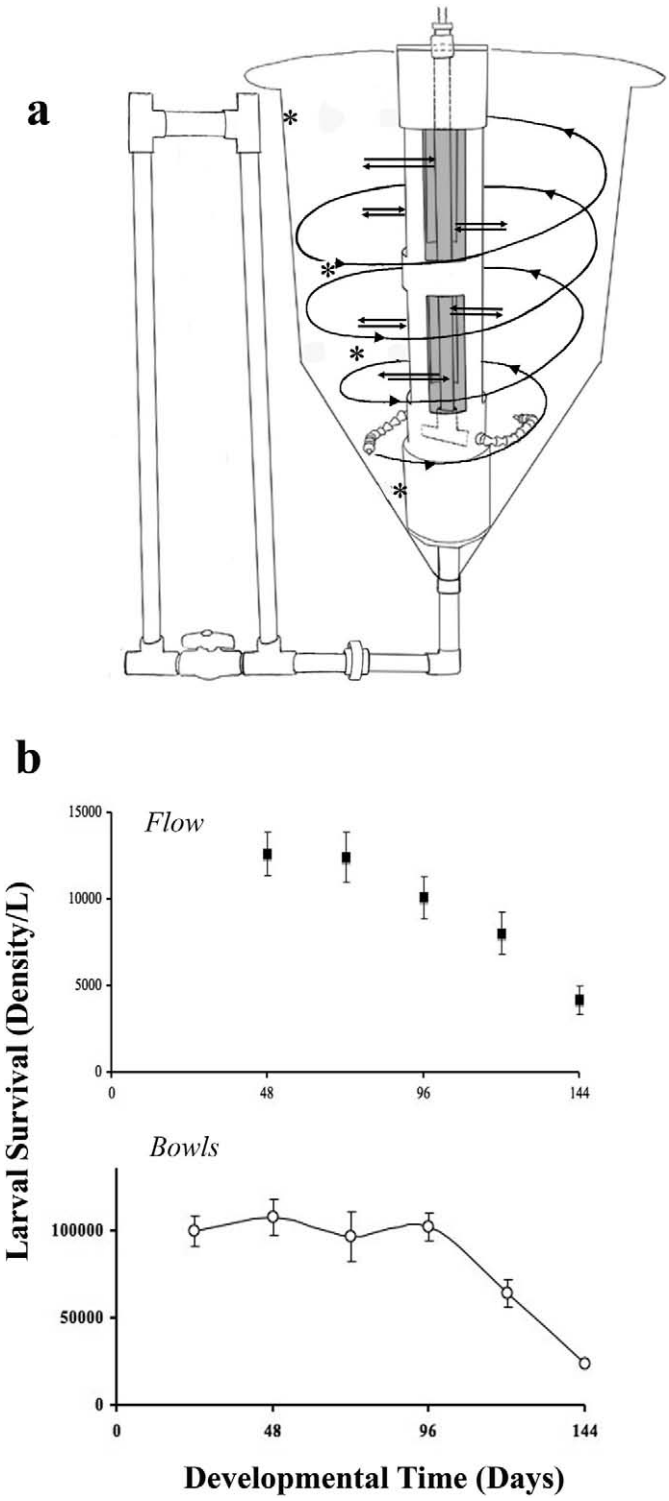

FIGURE 3. Larval rearing of *Fungia scutaria*. a, Drawing of assembled spiral-flowing tank and its flow, mixing, and position of dye experiments. Curved lines with arrowheads indicate direction of water flow spiraling upward from one inlet of a Loc-Line (both inlets had flow, but for simplicity flow from only one is drawn). Double arrows indicate water freely flows into and back out of the mesh areas on the standpipe. Asterisks (*) indicate locations in the water column where dye was injected for dye tests. b, Survival rate of *F. scutaria* larvae maintained in the spiral-flowing tank (upper graph, "Flow") and the static bowls (lower graph, "Bowls") between 24 and 144 h postfertilization. Each point shows mean and standard error. There was no difference in survival of larvae from flow chambers and static bowls (ANCOVA, P = 0.99).

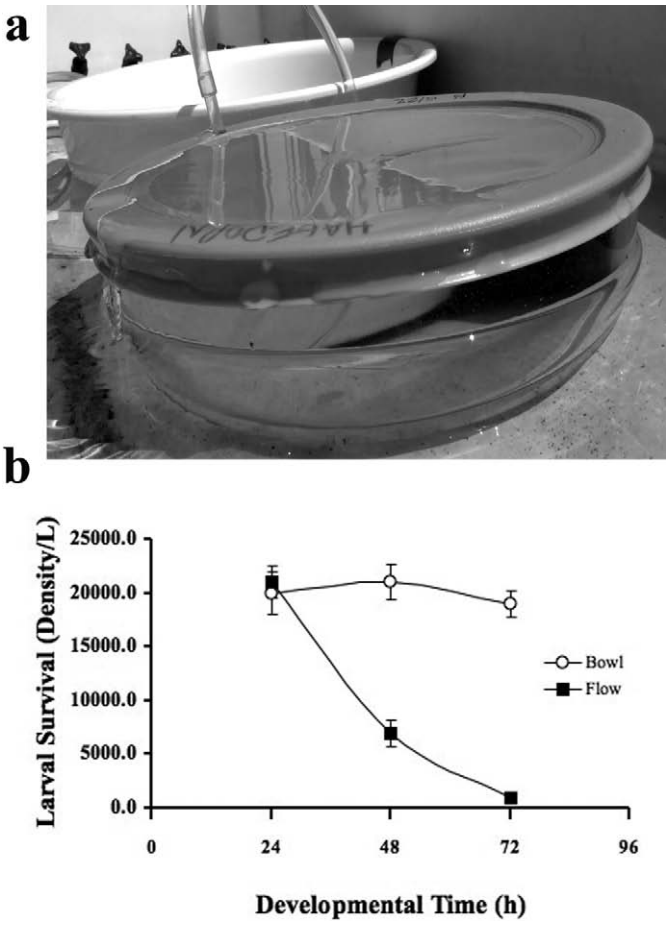

FIGURE 4. Larval rearing of *Pocillopora meandrina*. a, Down-flowing tank used to rear *P. meandrina*. b, Curves for larval survival in the static ("Bowl") and down-flowing ("Flow") tanks did not have the same slope (ANCOVA, $P = 0.03$); bowl rearing for this species produced substantially better survival than the down-flowing tank. Points show mean counts for each trial and standard error of the mean.

pumped from the reef. Flowing filtered seawater entered the top of the central tube and moved through nozzles at the tank base to produce gentle circular movement throughout the water column, and the wastewater exited the tank through the mesh-covered outflow. The flow rate was 150 to 300 mL/min, producing a complete turnover in the tanks approximately every 4 to 8 h. To test whether the conical tank could support the growth and development of *F. scutaria*, approximately 10,000 larvae/L were stocked in the conical tanks ($n = 4$) tanks. To reduce mortality of the early fragile stages (0–24 h postfertilization) from excessive motion in the flow-through chambers, the spiral-flow tanks were tested with 24 h postfertilization larvae. Therefore fertilization rate was not an issue, because all the larvae used for these tests were intact and developing. A comparison of the static bowl method (with daily cleaning) and the flow-through method was then performed to determine survival rate over time (up to 144 h postfertilization).

The static treatments used for comparison to the conical tanks were 3 L plastic bowls that had been the standard for successfully rearing *F. scutaria* larvae for many years (Schwarz et al., 1999). At 24 h postfertilization, *F. scutaria* larvae from 10 bowls were combined and evenly distributed into 2 larger bowls. Counts (see below) were taken for each bowl, and a standard rearing density was redistributed into 8 separate smaller bowls at a larval density of 100,000 larvae/L filtered seawater.

Fungia scutaria larvae were counted each day by gently stirring to homogeneously suspend them in the water column. Ten 1 mL samples were taken midwater from each conical flow-through tank and placed into a Sedgewick-Rafter counting chamber; the larvae were counted with a dissecting microscope and their numbers averaged. In addition, 10 samples (20 μL each) were taken midwater from each bowl, the number of larvae counted under a dissecting microscope, and their numbers averaged. These smaller 20 μL samples were assessed from the *F. scutaria* because of their high densities in the chambers.

Down-Flowing Tank

In April 2008 we attempted to rear *Pocillopora meandrina* larvae in the spiral-flowing tanks, but because of the negatively buoyant nature of the larvae, this method resulted in 100% mortality. Therefore, we developed a down-flowing tank for rearing *P. meandrina* larvae in May 2008 (see Figure 4). The tank was constructed of a 1.65 L glass bowl with a plastic lid. The center of the lid was removed and replaced with 40 μm mesh, leaving a 2 cm ring around the outside in which a hole was made to insert a 2 mm plastic rigid air line attached with air-line tubing to a manifold for controlling water flow. To maintain the water quality close to that which the larvae would experience in the open water, the down-flowing tanks were attached to a filtered (1 μm) flow-through system with seawater pumped from the reef. Flow to the tanks was maintained at 120 mL/min.

The larvae were fertilized in 50 mL tubes, rinsed with sterile filtered seawater, and placed into two bowls at 28°C to develop overnight at a density of approximately 80,000/L. After 24 h postfertilization, larvae were counted and then cleaned using a 40 μm mesh and 0.5 μm-filtered

seawater. One group was placed into the downward-flow chamber at a flow rate of 120 mL/min with a 40 μm mesh top to allow the water to flow out. The other bowl remained static. *P. meandrina* larvae were cleaned (static bowl only) and counted daily from the flow tanks and static bowl (maintained as described for *F. scutaria* above) for comparison.

Statistics

To determine the differences between survival in flow chamber versus static treatment, the data from all experiments was normalized and the *y*-values linearly transformed; analysis of covariance (ANCOVA) was then performed to determine whether the slopes were significantly different, using GraphPad Prism 5 software for the Macintosh GraphPad Software (San Diego, CA).

RESULTS

Rearing chambers were designed for three different coral species exhibiting different larval swimming behaviors, buoyancy, and sizes.

Up-Flowing Tank

Acropora palmata was the largest of the larvae studied (~700 × 500 μm depending on the developmental stage) and had the slowest rate of development (see Figure 1a). *A. palmata* larvae float at or near the surface of the water approximately 48 to 60 h postfertilization (depending on the temperature) until they began swimming. Even once they had begun swimming, they swam at or near the surface and were considered positively buoyant for most of their larval development before metamorphosis and settlement (~144 h). Clearly, all the larvae must become negatively buoyant before settlement; therefore, these categories only apply to the early larval periods (up to ~120 h, depending on the species).

During the first 24 h of development, the larvae developed asymmetrical, small protrusions of cells that could have easily be damaged in the up-flowing tank, but the chamber produced normally developed *A. palmata* larvae (as compared to the bins) because it simulated the gentle tumbling that the larvae would experience in the natural water (see Figure 2). Larval survival in the up-flowing tanks was similar to that in the static bins ($P = 0.12$) (Figure 2a). However, the up-flowing tank did not produce viable larvae for *F. scutaria* and *P. meandrina*. This tank produced 100% mortality in *F. scutaria* larvae within 24 h, even if the larvae were slightly more developed when placed in the chambers.

Spiral-Flowing Tank

Dye injection studies were performed on the spiral-flowing tank, using food coloring to examine the mixing properties of the vessel with an inlet flow of 150 mL/min. Figure 3 illustrates the mixing pattern in the flow-through vessel. Flow into and out of the system was equal, but the open area of the slits in the 10 cm central polyvinyl chloride (PVC) tube dictated the velocity through the screens. The nozzles were angled slightly downward to promote turbulence at the bottom to keep the larvae well mixed. The 180° positions of the nozzles provided rotation within the water column and encouraged mixing. Dye studies with separate injections were made at positions noted by asterisks (*) in Figure 3. At a flow of 150 mL/min, full vertical mixing occurred within minutes.

Developing *F. scutaria* larvae were fairly small (~200 × 100 μm) and fragile during the first 12 h of development and were just negatively buoyant during their early embryonic period (0–12 h postfertilization) (Figure 1b). However, once they began swimming, they were evenly distributed in the water column, and we considered this species to be neutrally buoyant. There was no difference in the survival between the spiral-flowing tank and bowls ($P = 0.99$). Both rearing systems produced similar survival rates in which the densities remained relatively steady through 96 h postfertilization and then dropped off at 120 to 144 h postfertilization (see Figure 3). This decrease in densities may reflect the complete absorption of stored fats (M. Hagedorn, unpublished data), as these larvae did not have zooxanthellae. *P. meandrina* larvae were tested in the spiral-flow tank, but 100% mortality was observed after 48 h postfertilization.

Down-Flowing Tank

Pocillopora meandrina larvae were the smallest of the larvae tested (~120 × 40 μm); they began slow swimming at 24 h but were negatively buoyant for the remainder of their larval development, remaining on or near the bottom (Figure 1c). Similar to *F. scutaria*, *P. meandrina* larvae were relatively susceptible to damage within the first 24 h, so they were reared in 3 L bowls for the first 24 h. The down-flowing tank was used for rearing *P. meandrina*; however, the static bowls appeared superior to the down-flowing tank for this species ($P = 0.03$) (see Figure 4).

DISCUSSION

For many years, large numbers of coral larvae have been reared successfully using simple husbandry methods such as static bowls and tanks. We have demonstrated that species of buoyant and neutrally buoyant coral larvae have similar survival in either static or flow-through chambers (see Figures 2, 3). These devices have proven to be very useful in improving culture conditions to reduce husbandry labor because neither embryos nor fresh water needed to be constantly transferred.

Modified examples of the up-flowing tank have already been used successfully by coral restoration biologists in the field (Margaret Miller, NOAA Southwest Fisheries Center, personal communication). *Montastraea faveolata* and *Diploria strigosa* were reared successfully in the up-flowing tanks and shipped to Columbus Zoo and Aquarium for settlement with 3-month survival as high as 65% and 45% for each species, respectively. Thus, the up-flowing tank has proven to be both practical, in that it can be adapted to the researcher's needs, and valuable, because it reduced husbandry time and facilitated restoration science under field conditions.

In weighing the benefits of each rearing system, one of the biggest factors to consider is time. For species that have only a single breeding season consisting of a few days, time available for conservation and restoration research is precious, and any time savings is a benefit. Moreover, the time remaining for some species that are threatened has become critical, and restoration practices need to be improved. *Acropora palmata* (elkhorn coral) and *Acropora cervicornis* (staghorn coral) were the first corals to be listed as threatened species under the U.S. Endangered Species Act. These major reef-building species once formed dense thickets and stands in the Caribbean. Today, these two species are currently at 1% to 20% of their historical levels throughout their range (Bruckner, 2003). Here we describe only one aspect of an ex situ conservation process, namely improved rearing associated with yielding better time management.

However, both the static and flow-through methods described here have their strengths and weaknesses. The static method was inexpensive to set up in terms of equipment and space. For example, 60 bowls can be maintained in two double-tiered flowing water tables taking up only about 2.5 m^2; however, this method was very expensive in terms of labor needed for cleaning ($\sim$5,000 h $year^{-1}$). The flow-through system was more costly to set up because it required a filtered flow-through water system and specially constructed rearing chambers. The amount of salary needed to pay one person for a season cleaning larvae, however, far exceeds the cost of the filtered seawater system and rearing chambers. The flow-through chambers required more space than the bowls, but each flow-through vessel could maintain almost three times the density (in $\sim$0.25 m^2) than was ordinarily maintained in a static bowl and with little maintenance time required.

One of the major issues facing biologists in rearing coral larvae is how to keep them cool (28°–30°C) under field conditions. During daylight hours, static bins left outside without any cooling mechanism can easily reach 31° to 33°C, which is lethal for most species. The rearing data in Figure 3 reflect some of these issues. These data were not exactly comparable, because they did not have the same developmental temperatures. Had the static bins been maintained at 28° to 30°C (as were the flow chambers), possibly their survival would have been far worse, because their water quality would decay so rapidly. Because *A. palmata* is an endangered species, our goal was to produce the most larvae for captive maintenance in public zoos and aquaria (Petersen et al., 2007), this required having static "backup" bins maintained at a slightly cooler temperature to provide the larvae sufficient development time in transit to reach their respective sites before settlement. However, without an air-conditioned room to cool the bins, this would not have been possible, making this impractical under some field conditions.

Within the first 24 h of development, many coral larvae are susceptible to fragmentation by mechanical disruption. However, the water movement within the up-flowing tank and potential contact with the walls did not cause substantial fragmentation of *A. palmata* during early development, even when the *A. palmata* larvae were placed in the chambers within the first few hours after fertilization. In contrast, *P. meandrina* was far more delicate, did not develop strong swimming behaviors, and could not withstand the water movements in the flow chambers. *F. scutaria* larvae are negatively/neutrally buoyant larvae that develop strong swimming behaviors within the first 12 to 24 h, and the spiral-flow system shown in Figure 3 functioned well for them, because the water flow is upward and any disintegrating unfertilized oocytes and larvae passed through the mesh, allowing for the maintenance of excellent water quality in the rearing chambers. However, no one type of rearing chamber can be applied universally across species. Instead, the type of water flow within the chamber must be matched with the buoyancy and early swimming behavior of the larvae. Regardless, these readily built and easily maintained flow-through chambers may be a substantial aid to coral conservation and restoration.

Acknowledgments

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Worldwide Diving Discoveries of Living Fossil Animals from the Depths of Anchialine and Marine Caves

Thomas M. Iliffe and Louis S. Kornicker

ABSTRACT. Inland (anchialine) and offshore submarine caves in limestone and volcanic bedrock are extreme environments inhabited by endemic, cave-adapted (typically eye- and pigment-reduced) fauna. Specialized cave diving technology is essential for investigating this habitat. A number of new higher taxa are represented herein, including closely related species inhabiting caves on opposite sides of the Earth, thus suggesting an ancient common ancestry. Because many of these species are known from only a single cave, pollution or destruction of caves will result in their extinction.

INTRODUCTION

Definition of Anchialine and Marine Caves

Anchialine caves are partially or totally submerged caves situated within a few kilometers inland from the coast in volcanic or karstic limestone terrain. Tidal marine waters in these caves have a long residence time, of months to years. Such caves are locally termed "cenotes" in the Yucatan Peninsula of Mexico, "blue holes" in the Bahamas and Belize, and "grietas" in the Galapagos Islands. The caves typically possess a highly stratified water column, with surface layers of freshwater or brackish water, separated by a thermo–chemocline from underlying fully marine waters low in dissolved oxygen (Iliffe, 2000). Animals that are restricted to the anchialine habitat and show pronounced morphological, physiological, biochemical, and behavioral adaptations are termed stygofauna or stygobites. In some areas such as Yucatan, freshwater and marine stygobites inhabit their respective water masses within the same caves.

In contrast to anchialine caves, marine caves are located either directly on the coastline (e.g., tidal springs) or are wholly submerged beneath the seafloor (e.g., offshore blue holes) and contain marine waters that freely exchange with the sea on each tidal cycle. The stygophilic fauna of marine caves can also be found in suitable and similar habitats outside of caves (e.g., under rocks or in crevices within the reef) and lack specialized adaptations for subterranean life.

Moderate to strong tidal currents are present in many marine caves. As a result, encrusting and low-growing, filter-feeding animals such as sponges, hydroids, anemones, tube worms, and even some corals may completely cover all

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hard surfaces. Other organisms are swept into caves by tidal currents but can only survive there for short periods of time and are termed accidentals. Some species of fishes, lobster, and mysidaceans seek shelter within marine caves but must venture out into open waters to feed and are classified as stygoxenes.

Some extensive marine caves extend far or deep enough so that a more or less gradual transition to long water residence times takes place and conversion to a true anchialine habitat occurs. Similarly, a number of inland anchialine systems have submerged entrances in the sea, with significant water exchange occurring in the entrance sections but with a transition to anchialine characteristics and fauna taking place as distance from the sea increases and the magnitude and impact of tidally exchanging water decline.

Biological Significance

Anchialine caves contain a rich and diverse, endemic stygobitic fauna (Sket, 1996; Iliffe, 2000, 2004) but, because of the specialized technological demands and potential dangers of cave diving, are relatively unstudied. These habitats serve as refuges to "living fossil" organisms, for instance, members of remiped crustaceans, and to animals closely related to deep-sea species, such as the galatheid crab *Munidopsis polymorpha*. Such stygobites typically possess regressed features including loss of eyes and body pigmentation. For reasons that remain unclear, the invertebrate fauna is dominated by crustaceans and includes the new class Remipedia, plus three new orders, nine new families, more than 75 new genera, and 300 new species. This extraordinary degree of novelty qualifies anchialine habitats as uniquely important. Because anchialine species commonly have a highly restricted distribution, often being found only in a single cave system on one island, pollution or destruction of the caves will result in their extinction.

Stygobitic anchialine fauna often have highly disjunct biogeographic distributions, inhabiting caves in isolated locations on opposite sides of the Atlantic and Pacific Oceans, as well as in the Mediterranean, and are considered Tethyan relicts. Various hypotheses have been proposed to explain the origin of anchialine fauna. In general, these theories invoke either vicariance (geological) or dispersal (biological) processes. Recently initiated molecular genetic comparisons of cave populations from distant locations may help provide data for determining the age and dispersal sequence of anchialine stygobites (Zakšek et al., 2007; Hunter et al., 2008).

Lifestyle Adaptations

The extreme environmental conditions in anchialine caves, such as the absence of light, hypoxia, and limited food reserves, present a unique set of challenges for the organisms that reside there. The lack of light precludes photosynthetic (primary) production of oxygen and food. Without light, organisms receive no visual information for orientation or communication and must function without diurnal timing mechanisms.

Adaptations to anchialine and marine caves can be morphological, behavioral, and physiological (Iliffe and Bishop, 2007). As a result of both food scarcity and hypoxia, there is a high selective advantage for economy of energy observed in many taxa, with possible adaptations including enhanced chemo–mechano-receptors for improved food finding capability, starvation resistance, and reduction in energy demand via reduced metabolism.

Methods

Diving Investigations

Because anchialine stygobites are commonly found only at significant depths or distances from the water surface, cave diving is an essential component of the collection and study of anchialine fauna (Iliffe and Bowen, 2001). Cave diving requires specialized training, equipment, and techniques because a direct assent to the surface is not possible and divers may be hundreds of meters from outside access. In case of equipment failure or loss of air supply, cave divers must have readily available backups. Special techniques for cave diving may include the use of side-mounted, instead of back-mounted, scuba tanks to allow divers to pass through low bedding plane passages. Closed circuit rebreathers, which recycle the diver's exhaled gases, reduce the amount of percolation, that is, of silt dislodged from cave ceiling or walls by the exhaust bubbles produced in conventional open circuit scuba, and lessen contamination of the cave waters, which are low in dissolved oxygen (Figure 1). Rebreathers allow for much longer dives and generally less decompression time. Deep dives, depths below 40 m, require the use of special breathing gas mixtures that replace part or all of the nitrogen with helium to reduce the effect of nitrogen narcosis. As many cave dives are for longer durations and/or to deeper depths, they frequently involve long decompression.

Sampling and Fixation

The exceptionally clear waters of anchialine caves facilitate visual observation and collection of stygobitic

FIGURE 1. A diver uses a Megalodon closed-circuit rebreather with full face mask to collect a small shrimp, *Typhlatya* sp., from a cave in Yucatan. Rebreathers recycle expired gas so that no bubbles are produced.

species. Collectors generally lead the dive to have undisturbed water in front of them. As they slowly sweep their dive lights back and forth in an arc, observing the water column illuminated by the light beam, animals as small as a few millimeters can be distinguished as white pinpoints, sharply contrasting with the black background of the cave. Specimens recognized in this manner can be collected either individually in clear glass vials or plastic tubes or in larger numbers using a type of suction device known as the "Sket bottle" (Chevaldonné et al., 2008). Plankton nets, of 93 μm mesh with a 30 cm mouth diameter and 1 m length, can be used to collect smaller animals, such as copepods, from the water column. When collecting animals from the surface layer of sediments, divers can gently fan up the sediments with a hand and then sweep the plankton net through the disturbed water. This agitation should be done with care so as not to obscure overall visibility, which could cause the dive team to lose sight of their guideline leading back to the surface. Larger amounts of sediment can be collected in sealable plastic bags for later sorting in the laboratory. Finally, minnow traps or similar funnel-shaped traps made from plastic bottles (Manning, 1986) can be baited with a small amount of fish, crab, or other attractant and left within the cave for 6 to 24 h. If the trap is carefully placed inside a sealed plastic bag when it is recovered, even small invertebrates can be collected.

If temperatures are kept close to cave temperature after collecting, specimens will remain alive for up to 24 h. Photographic documentation of color pattern and natural body position in live specimens is highly desirable. Smaller animals can be photographed using a phototube attachment on a dissecting microscope and larger specimens with the macro setting found on many digital cameras. If animals are too active to be photographed easily, they can be chilled in a small dish placed in a refrigerator or an ice bath until they stop moving. Digital video segments showing swimming and other behaviors can be made in the same manner. Specimens are sorted under a dissecting microscope using small pipettes to transfer them to individual dishes for each taxon. Depending upon the type of animal and its intended use, various fixatives can be used. Most animals are best preserved in 70% to 95% pharmaceutical grade ethanol, which allows them to be used for either morphological or molecular investigations. Specimens for confocal laser scanning microscopy can be fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) buffer (1:1 in seawater), while those intended for scanning or transmission electron microscopy are fixed in 2% glutaraldehyde in seawater.

GEOLOGICAL ORIGINS, AGE, AND DISTRIBUTION OF ANCHIALINE HABITATS

Anchialine caves occur in both volcanic bedrock and karstic limestone. Lava tube caves form during volcanic eruptions of basaltic lava. They typically occur close to the earth's surface and are thus relatively short lived (thousands to a few tens of thousands of years). Anchialine lava tubes may originate on land and extend out under the coastline and beneath the seafloor or can form from submarine eruptions. Anchialine lava tube caves are known from the Canary Islands, Galapagos Islands, Hawaii, and Western Samoa. The longest of these is the Jameos del Agua (Atlantida Tunnel) on Lanzarote in the Canary Islands, the submerged portion of which extends 1.6 km beyond the coastline, reaching a depth of 50 m (Iliffe et al., 2000).

The most extensive of known anchialine habitats are solutionally developed limestone caves that typically contain both freshwater and marine waters. Such caves are sometimes referred to as flank margin caves and were formed by mixing dissolution in a fresh groundwater lens (Mylroie and Carew, 1990). The largest anchialine cave is Sistema Ox Bel Ha located on the Caribbean coast of the Yucatan Peninsula in Mexico; it contains 180 km of

surveyed underwater passages interconnecting 130 cenote entrances. Extensive anchialine limestone caves are also known from the Bahamas, Bermuda, Belize, Dominican Republic, and Bonaire in the Caribbean, plus the Balearic Islands and Sardinia in the Mediterranean. Smaller anchialine caves are present on many islands in the Indo-South Pacific and in Western Australia.

Limestone caves last much longer than lava tubes and can be hundreds of thousands to many millions of years old. Commonly, massive stalactites and stalagmites occur underwater to depths in excess of 50 m in coastal limestone caves. Because speleothems form very slowly and only in air, these caves must have been dry and filled with air for long periods of time when glacial sea levels were as much as 130 m lower than today. The last low stand of Ice Age sea level occurred only 18,000 years ago.

Coastal tectonic faults that extend below sea level constitute another form of anchialine habitat. On Santa Cruz in the Galapagos Islands, vertical faults in coastal volcanic rock are locally called "grietas" (Iliffe, 1991). Wedged breakdown blocks have partially roofed over submerged portions of grietas so that they are in total darkness. Similar faults are present in Iceland. Fault caves also occur in uplifted reef limestone on the island of Niue in the Central Pacific, producing deep chasms containing anchialine pools. The Ras Muhammad Crack in the Sinai Peninsula consists of a water-filled crack in an elevated fossil reef formed by a 1968 earthquake (Por and Tsurnamal, 1973). Many of the offshore ocean blue holes of the Bahamas consist of submarine faults running parallel to the platform edge. Ocean blue holes typically exhibit exceptionally strong, reversing tidal currents created by an imbalance between tides on opposite sides of the islands.

ANCHIALINE CAVE ECOLOGY

Physical and Chemical Characteristics

The water column in most anchialine caves is highly stratified (Iliffe, 2000). The largest changes in chemical and physical parameters typically occur at the halocline where freshwater or brackish water is separated from underlying fully marine waters (Figure 2). It is not uncommon for caves to possess multiple haloclines. On larger islands and in continental regions such as Yucatan and Western Australia, freshwater occurs in the shape of a lens with thickness increasing in a direct relationship with distance inland from the coast. In Yucatan, the depth of the halocline and corresponding thickness of the freshwater lens increases from 10 m at 2 km distance inland to 20 m at 10 km inland.

Water temperature in Yucatan caves generally increases with depth, although in the Bahamas the inverse occurs and water below the halocline is generally cooler than surface water. Warmer waters below the halocline could be caused by geothermal heating at depth or evaporative cooling at the surface.

In the lightless interior of caves, there are no plants and hence no photosynthetic oxygen production; stable and stratified water masses also restrict vertical mixing and exchange of oxygen with surface waters. Thus, cave waters are typically hypoxic to anoxic. Where deeper, water-filled vertical shafts extend to the surface, such as in many cenotes and inland blue holes, input of leaves and other organic detritus has caused the total depletion of dissolved oxygen with resulting anoxia and hydrogen sulfide production. A cloud-like layer of hydrogen sulfide several meters thick occurs just below the halocline and may reduce underwater visibility to near zero, but water clarity improves considerably below the H_2S layer. In some caves, dissolved oxygen levels can recover to 1 mg/L or less and populations of stygobitic animals occur.

A pH minimum generally occurs at the halocline, possibly arising from microbial oxidation of organic matter suspended at the density interface and resulting CO_2 production. Increased acidity at the halocline may explain the dissolution of limestone and the resulting development of cave passages at this depth.

Trophic Relationships

Determination of stable carbon and nitrogen isotopes values from animals, sediments, and other sources of organic matter in Yucatan caves has been used to examine the trophic ecology of these systems (Pohlman et al., 1997, 2000). Four potential sources of organic matter were identified in Yucatan caves: the soil from the surrounding jungle, algae from the cenote pool, chemoautotrophic bacteria, and, to a lesser extent, organic matter originating from marine waters. Stable nitrogen isotope data determined that the food web comprised 2 to 2.5 trophic levels.

The paucity of food in anchialine caves drives organisms toward a generalist diet. Mysids and isopods tend toward omnivory, while ostracods and thermosbaenaceans occupy the roles of detritivores. The thermosbaenacean *Tulumella* and atyid shrimp *Typhlatya* have modified appendages that allow them to filter out even the tiniest particles. Remipedes, fishes, and some amphipods, operating either as top-level predators or as scavengers, feed on ostracods, thermosbaenaceans, copepods, isopods, amphipods, and shrimps.

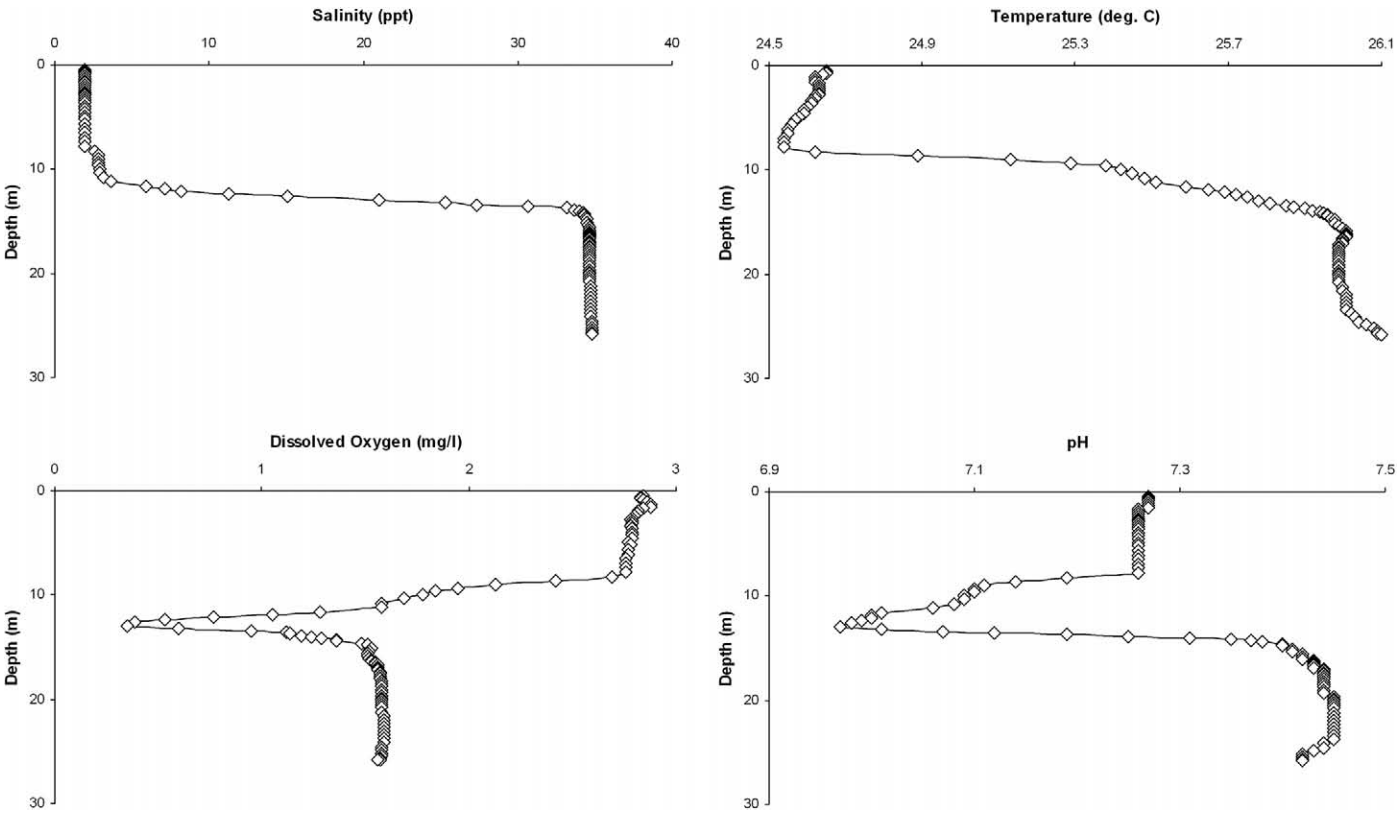

FIGURE 2. Depth profiles of salinity, temperature, dissolved oxygen, and pH from an anchialine cave, Cenote 27 Steps, Akumal, Mexico, 7 December 2003, recorded with a YSI 600 XLM multiparameter water quality monitor. Individual measurements (diamond symbols) were taken at 4 s intervals between the surface and 26 m water depth.

BIODIVERSITY

Fishes

Stygobitic anchialine fishes (Figure 3a) are represented in the families Bythidae (eight species in two genera from the Bahamas, Cuba, Yucatan, and Galapagos Islands), Eleotridae (one species from Northwestern Australia), Gobiidae (three species in two genera from the Philippines and Japan), and Synbranchidae (two species in one genus from Northwestern Australia and Yucatan) (Romero, 2001).

Non-Crustacean Invertebrates

Although most stygobitic anchialine invertebrates are crustaceans, a variety of non-crustacean invertebrate stygofaunal species have been described. Anchialine species include four sponges, one turbellarian, five gastropods, ten annelids, four chaetognaths, one tantulocarid, and three water mites. Although some of these species are questionable stygobites, several are clearly cave adapted. The polychaetes *Gesiella jameensis* from the Canary Islands and *Pelagomacellicephala iliffei* from the Caicos Islands and Bahamas (Figure 3b) conserve energy by slowly swimming in the cave water column, while the chaetognath *Paraspadella anops* from the Bahamas lacks eyes and pigment.

Crustaceans

Crustaceans are the most abundant and diverse group present in both freshwater and anchialine cave habitats. Among the anchialine Crustacea, the largest numbers of species are represented by amphipods, copepods, decapods, ostracods, isopods, mysids, and thermosbaenaceans, approximately in that order.

Remipedia

Remipedes are a class of Crustacea originally described from Bahamian caves by Yager (1981). Although their multi-segmented trunk and paired swimming appendages

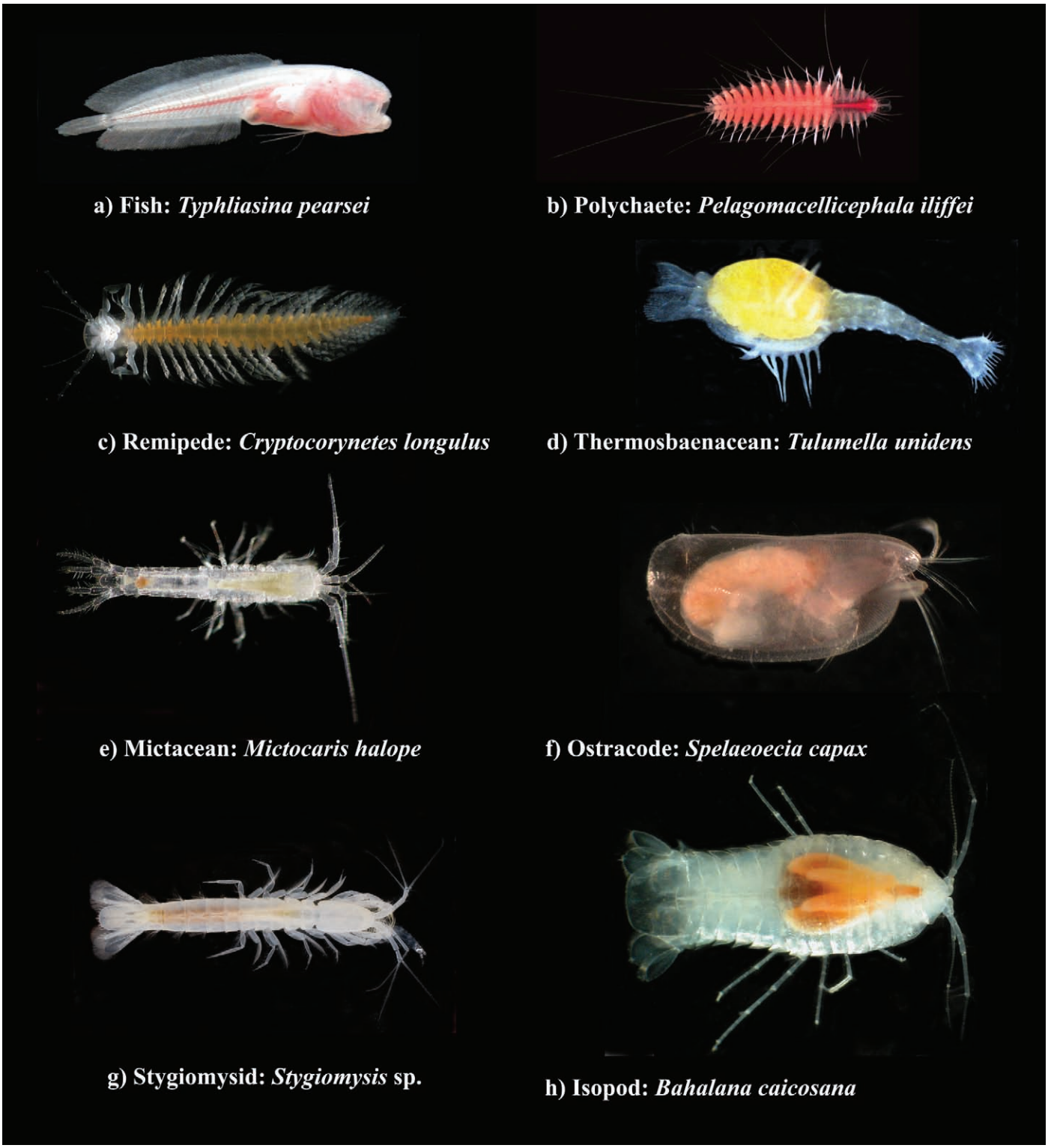

FIGURE 3. Characteristic anchialine cave animals include the (a) Yucatan cave fish *Typhliasina pearsei*; (b) polynoid polychaete worm *Pelagomacellicephala iliffei* from the Bahamas; (c) remipede *Cryptocorynetes longulus* from the Bahamas; (d) thermosbaenacean *Tulumella unidens* from Yucatan; (e) mictacean *Mictocaris halope* from Bermuda; (f) halocyprid ostracod *Spelaeoecia capax* from the Bahamas; (g) stygiomysid *Stygiomysis* sp. from Yucatan; and (h) cirolanid isopod *Bahalana caicosana* from the Caicos Islands.

appear primitive, their head and mouth parts are highly specialized (Figure 3c). Remipedes have paired hollow fangs for capturing prey and are among the top predators in anchialine habitats. They are up to 4.5 cm in length, usually colorless and blind, with elongate, centipede-like bodies. Twenty species of remipedes inhabit fully marine, oxygen-deficient waters in caves from the Bahamas, Caicos Islands, Cuba, Yucatan Peninsula, Dominican Republic, Canary Islands, and Western Australia (Koenemann et al., 2008b; Daenekas et al., 2009). The recent discovery of free-living, nonfeeding remipede larvae promises to yield information on the reproduction and development as well as the evolutionary affinities of this enigmatic group (Koenemann et al., 2007, 2009).

Thermosbaenacea

Thermosbaenaceans (Figure 3d) are small (5 mm or less), eyeless or eye-reduced, anchialine and freshwater peracarid crustaceans with a dorsal brood pouch in females (Wagner, 1994; Jaume, 2008). They include at least 34 species with a wide distribution in caves and thermal springs around the Mediterranean and Caribbean, as well as in Australia and Cambodia.

Mictacea

Mictaceans (Figure 3e) are small (3–3.5 mm), eyeless and depigmented, nonpredatory crustaceans. This peracarid order is represented by only a single species that inhabits anchialine caves in Bermuda (Bowman and Iliffe, 1985).

Bochusacea

Bochusaceans are very small (1.2–1.6 mm), semitransparent, and eyeless peracarid crustaceans that include two anchialine species from the Bahamas and Cayman Islands, plus two deep-sea species (Gutu and Iliffe, 1998; Jaume et al., 2006).

Copepoda

Platycopioid, misophrioid, cyclopoid, harpacticoid, and calanoid (especially epacteriscid and ridgewayiid) copepods inhabit anchialine caves in tropical regions around the globe. They are small (typically 1–2 mm long) and have a short, cylindrical body with head and thorax fused into a cephalothorax. Most are planktonic filter feeders, but some, such as the harpacticoids and cyclopoids, are benthic, while epacteriscids are predators on other copepods.

Ostracoda

Halocyprid ostracods (Figure 3f) include anchialine species with a distribution and co-occurrence similar to that of remipedes (Kornicker et al., 2007). *Danielopolina* is the most widely distributed stygobitic genus with species on opposite sides of both the Atlantic and Pacific, inhabiting caves in the Bahamas, Cuba, Yucatan, Jamaica, Canary Islands, Galapagos, Western Australia, and Christmas Island. More than 300 species of podocopid ostracods have been found in springs, caves, and anchialine habitats.

Mysidacea

Stygobitic mysids are found in freshwater and anchialine habitats in Africa, the Caribbean, Mediterranean, and India. Their distribution suggests that they were stranded in caves by lowering of the sea level in the Tethys and Mediterranean. Recent molecular phylogenies of the mysids suggest that a new order is justified for the stygiomysids (Figure 3g), which inhabit caves in the Caribbean and Italy (Meland and Willassen, 2007).

Isopoda

Stygobitic isopods (Figure 3h) range from several millimeters to several centimeters in length. Anthurid isopods occur in anchialine and freshwater caves in the Canary Islands, Caribbean and Indian Ocean islands, Mexico, and South America. Asellot isopods inhabit anchialine and freshwater caves in the Caribbean, Europe, Galapagos, India, Indonesia, Japan, Malaysia, North and Central America, and Polynesia. Cirolanid isopods have been found in freshwater and anchialine caves clustered in Mexico and the Caribbean (Iliffe and Botosaneanu, 2006), as well as in Europe and the Mediterranean.

Amphipoda

Amphipods occur in freshwater and marine cave habitats. Stygobitic representatives are present in the bogidiellid, crangonyctid, hadziid, and niphargid families of the amphipod suborder Gammaridea. They are very widely dispersed, with large numbers of species inhabiting caves in Central and Southern Europe, the Mediterranean, eastern and southern North America, and the Caribbean.

Decapoda

The anomuran galatheid crab *Munidopsis polymorpha* inhabits an anchialine lava tube in the Canary Islands

(Wilkens et al., 1990). Brachyuran crabs are widely distributed in caves of the tropics and subtropics. Anchialine stygobitic shrimp include representatives from the caridean families Agostocarididae, Alpheidae, Atyidae, Hippolytidae, Palaemonidae, and Procarididae; the stenopodid family Macromaxillocarididae; and the thalassinid family Laomediidae.

Other Crustacean Stygofauna

One tantulocarid, an exceptionally tiny ectoparasite on anchialine harpacticoid copepods, occurs in the Canary Islands (Boxshall and Huys, 1989). A species of stygobitic nebaliacean inhabits anchialine caves in the Bahamas and Caicos Islands (Bowman et al., 1985). Several species of cumaceans and tanaidaceans have been collected from anchialine caves in Bermuda and the Bahamas, but it is not clear whether they belong to the stygofauna.

BIOGEOGRAPHY

Upon examining the biogeography of anchialine fauna, some extraordinary patterns are evident. A number of anchialine genera, including the remipede *Lasionectes*, ostracod *Danielopolina*, thermosbaenacean *Halosbaena*, and misophrioid *Speleophria*, inhabit caves on opposite sides of the Earth and are believed to be relicts whose ancestors inhabited the Tethys Sea during the Mesozoic (Humphreys, 2000). Some anchialine taxa are represented in the Mediterranean, but others, notably remipedes and *Halosbaena*, are absent. The presence of anchialine taxa at all in the Mediterranean is remarkable considering that this basin was completely dry for long periods of time during the Miocene. The aytid shrimp *Typhlatya* shows an especially interesting distribution with 17 known species inhabiting freshwater and anchialine caves in the Mediterranean region, Bermuda, Ascension Island, Caribbean locations including Cuba and Yucatan, and the Galapagos Islands (Alvarez et al., 2005). The shrimp family Procaridae contains one genus with species in the mid-Atlantic and Caribbean, as well as Hawaii.

Based on numbers of stygobitic species, the Bahamian archipelago appears to have been a possible center of origin for anchialine fauna. Among the Remipedia, 15 of 20 described species inhabit caves in the Bahamas (Koenemann et al., 2008b; Daenekas et al., 2009), whereas among anchialine halocyprid ostracods, Bahamian species account for 4 of 11 in the genus *Danielopolina*, 6 of 11 in *Spelaeoecia*, and all 8 species of *Deeveya* (Kornicker et al., 2007).

The Bahamas archipelago consists of a series of broad, shallow-water, highly karstified, carbonate platforms rising abruptly from the deep sea. The islands and cays consist of Pleistocene limestone covered by a thin veneer of Holocene carbonate reefs and sediments. Underlying these younger limestones is a continuous section of Tertiary and Cretaceous limestones and dolomites exceeding 11 km in thickness. If the position of the tectonic plates before the development of the Atlantic Ocean is reconstructed, virtually all the Bahamas overlap the African continent and its continental shelf. This finding suggests that the Bahama platform developed over oceanic crust during the earliest phase of the creation of the Atlantic. The extended shallow-water history of the Bahamas, coupled with the cavernous nature of the limestone, may help to explain its rich and diverse anchialine fauna.

ORIGINS OF ANCHIALINE BIOTA

A number of theories have been proposed to explain the trans-oceanic distribution of many anchialine taxa. The *vicariance model* suggests that plate tectonics served as a mechanism for the dispersal of anchialine fauna (Rosen, 1976; Wiley, 1988). This model mainly describes the Tethyan track of ancient taxa that were rafted on the drifting continents to their present positions (Stock, 1993; Jaume et al., 2001). However, the existence of anchialine fauna on mid-ocean islands such as Bermuda, Ascension, and Hawaii that have never been part of or closer to a continent cannot be explained by this mechanism (Iliffe, 2000).

The *regression model* suggests that sea-level regressions, caused by tectonic uplift or eustatic glacial lowering of sea levels, stranded crevicular or interstitial marine littoral species that subsequently adapted to brackish or freshwater conditions (Stock, 1980). This model is supported by the observed correlation between the distribution patterns of numerous, marine-derived cave organisms and the position of shorelines during the Late Mesozoic or Tertiary seas. Nevertheless, the presence of anchialine fauna in caves that were completely dry and air filled (as evidenced by their now-submarine speleothems) less than 10,000 years ago indicates that these animals can migrate vertically with raising postglacial sea levels (Iliffe, 2000). Also, small islands such as Bermuda offer little chance for marine species to be stranded.

A *deep-sea origin* has been proposed for some anchialine species having close relatives that inhabit bathyal depths (Hart et al., 1985). Both caves and the deep sea are old, climatically stable, lightless, and nonrigorous environments. Anchialine habitats on islands and continental

margins could be connected via a continuum of crevicular corridors extending from shallow depths to the deep sea (Iliffe, 1990). However, evidence against a deep-sea origin of cave faunas includes the questionable ability of deep-sea species to cross the oceanic thermocline, the relatively recent nature of deep-sea species (resulting from the lack of oxygen in Atlantic bathyal waters during the late Oligocene), and phylogenetic analyses of morphological characters supporting independent colonization of deep-sea and anchialine habitats (Stock, 1986; Danielopol, 1990).

The *active migration model* involves the inland dispersal and colonization of subterranean habitats by expansionistic marine species with a high degree of salinity tolerance (Rouch and Danielopol, 1987). This process is independent of climatological and geological variations.

Passive oceanic dispersal of larval or postlarval stages of anchialine species by currents could explain the wide distribution of some anchialine shrimp species within the Indo-Pacific. Rafting on floating objects, such as wood, algae, kelp, and coconuts, or on mobile and migratory animals, for instance, sea turtles, fishes, and larger arthropods, could disperse anchialine species, even those without a free larval stage. However, oceanic dispersal is unlikely for many anchialine groups that produce few offspring or have narrow physiological tolerances.

ADAPTATION TO LIFE IN ANCHIALINE CAVES

Behavioral Adaptations

Behavioral adaptations are the most immediate adaptations for survival and colonization in cave systems. Cave organisms, in particular amblyopsid cave fishes, use a glide-and-rest technique to conserve energy in their search for food. Remipede locomotion is also designed for the economy of movement. Remipedes swim slowly, using less energy for the same distance than if they swam at higher speeds (Koenemann et al., 2008a). The power stroke produces drag by individual legs, but the recovery stroke is completed with the legs folded with other legs to reduce water resistance.

The stygobitic galatheid crab *Munidopsis polymorpha*, inhabiting an anchialine lava tube in the Canary Islands, has a number of specialized behaviors (Parzefall, 1992, 2000). These small crabs are most abundant in a dimly illuminated pool where they hide in rock crevices during the day but come out at night to feed on diatoms. Because of the large numbers of individuals in this pool, they spread in an almost regular pattern determined by the length of the second antennae.

Munidopsis crabs remain aggressive throughout the year. They detect intruders from water movements and attack with extended chelipeds. Male crabs are attracted by a molting hormone released by females. To prevent the females from fleeing, males rhythmically move their chelipeds as they approach, until the female responds by vibrating one of her chelipeds. The male then seems to turn the female over on her back to initiate insemination.

Morphological Adaptations

Regressive Features

The loss of features that in cave environments no longer have a function, such as eyes and pigmentation, is regarded as regressive evolution. There are two main theories explaining the driving force for regressive evolution. In an environment with a depauperate food supply, natural selection should favor reallocating energy from developing unused features, such as eyes and pigment, to growth and survival. A second explanation is that regressive evolution may be the result of nonselective processes such as neutral mutation and genetic drift. Features such as eyes and pigment that abruptly lose their biological function when animals enter caves are free to be turned off by now non-lethal mutations.

Unfortunately, the theory of energy economy by character reduction in stygobites is not well tested, especially with anchialine stygobites, yet the anchialine environment is dominated by blind, depigmented organisms.

Constructive Features

In the case of constructive features, priority is given to life history, metabolism, development, and starvation resistance, with sensory development such as mechano- and chemoreceptors being subordinate. For troglomorphy to occur, two factors must be present: (1) selective pressure in favor of the development and (2) genetic, physiological, or behavioral ability of the organism to respond to the selective pressure. A prerequisite for constructive traits is their genetic availability in epigean forms: if traits are not present in epigean ancestors, they will not be present in hypogean descendants.

There are several areas of the body where constructive features occur. In crustaceans, appendages may be elongated, in particular, the antennae, and in fish, the head may become enlarged or flattened. Corresponding to the morphological changes, there is an increased sensitivity to chemical and mechanical stimulants. As a result of compensatory enhancement of extraocular senses, the signal-processing structures in the brain are altered.

Physiological Adaptations

Adaptations to a Food-Poor Environment

Food in the stygobitic environment may be in general scarce or at best patchy; therefore, the stygofauna need to cope with temporal periodicity of food availability and potentially tolerate long periods of starvation. This adaptation occurs through lipid accumulation or energy economy. In comparison with pelagic crustaceans, anchialine crustaceans sacrifice protein mass for increased lipid stores (Iliffe and Bishop, 2007). Lipids provide neutral buoyancy without energy expenditure, while also serving as an energy reserve when food is limiting. Anchialine stygobites also tend to be smaller than their epigean counterparts. Their small size is a mechanism for energy economy.

Adaptation to Hypoxia and Anoxia

As mentioned previously, the anchialine environment, especially at or below the halocline, is commonly hypoxic or even anoxic. As a result, hypogean organisms tend to have substantially lower oxygen consumption rates than their epigean relatives (Bishop et al., 2004). Many organisms are capable of obtaining energy when faced with a reduction or absence of oxygen, but few are able to survive indefinitely without a return to oxygen. When the oxygen supply becomes inadequate, organisms switch to anaerobiosis to compensate for adenosine triphosphate (ATP) demand.

During periods of anaerobiosis, organisms conserve their energy stores by a loss in physiological functions such as motility, ingestion, and digestion, combined with a dramatic depression of their energy (ATP) demand. When oxygen is temporarily unavailable, many organisms switch to anaerobic glycolysis. Anaerobic glycolysis is, however, a fundamentally inefficient metabolic strategy and thus not an attractive solution for anchialine organisms.

By examining the activities of enzymes critical to metabolism and energy conversion, it is possible to determine the rate at which food is converted to cellular energy (Bishop et al., 2004). Citrate synthase (CS) is an indicator of an organism's maximum aerobic potential, or how fast an organism can aerobically convert glucose to energy. Malate dehydrogenase (MDH) functions in the presence as well as absence of oxygen, whereas lactate dehydrogenase (LDH) contributes to both aerobic and anaerobic metabolic pathways and serves as an indicator of glycolytic potential.

Anchialine organisms are anaerobically poised with both LDH:CS and MDH:LDH ratios tending to be greater than one. The higher the MDH:LDH ratio, the greater is the tolerance to hypoxia. Such high ratios indicate an evolutionary adaptation to the anaerobic anchialine environment.

CONSERVATION

Over the past 25 years, more than 400 new species of anchialine stygobites have been discovered and described. A high percentage of these species are known only from a single cave or cave system. Even within caves, species are characteristically found only at specific depths or locations as defined by a narrow range of environmental parameters. In many parts of the world, tourism development, limestone quarries, and groundwater pollution are either destroying or grossly polluting numerous caves, resulting in extinction of untold numbers of species.

Anchialine species qualify for inclusion on endangered species lists for reasons of their limited distribution and the declining environmental quality of their habitat. In Bermuda, 25 cave species are on the IUCN (International Union for Conservation of Nature) Red List of endangered species. Other cave species from the Yucatan Peninsula are on the official Mexican list of threatened and endangered species.

Maintaining groundwater quality is essential to the environmental health of the subterranean environment. For example, the small oceanic island of Bermuda is the third most densely populated country in the world and has the largest number of private cesspits per capita. Disposal of sewage and other wastewater into cesspits or by pumping down boreholes is contaminating the groundwater and cave water with nitrates, detergents, toxic metals, and pharmaceuticals; depleting the very limited amounts of dissolved oxygen in cave water; and generating toxic levels of hydrogen sulfide.

Some ocean caves such as the Blue Holes of the Bahamas have strong tidal currents sweeping through them for very considerable distances. In one such cave, plastic bottles and other trash have been observed littering the floor of the cave nearly a mile back into previously unexplored passages. Far too many caves and sinkholes are viewed as preferred locations for the dumping of garbage and other waste products.

Another serious environmental problem concerns the destruction of caves by limestone quarries or construction activities. Half a dozen or more Bermuda caves have been totally destroyed by two limestone quarries that produce crushed aggregate for construction purposes. Untold other

caves have been lost to enormous limestone quarries in the Yucatan Peninsula. Many caves have been filled in and built over by golf courses, hotels, and housing developments in Bermuda. Recently, a series of luxury town homes was built directly on top of the largest cave lake in Bermuda.

Sometimes even seemingly innocent activities can threaten caves and cave animals. Along the Caribbean coast of the Yucatan Peninsula, many open water cenote pools are inhabited by the freshwater fish *Astyanax fasciatus*. Some of these fish frequently follow divers into caves, moving in front of the dive team and voraciously darting in to consume any cave fish or crustaceans that are illuminated by the beam of a dive light. Considering the many thousands of cave divers who use these systems each year, it is not surprising that the caves most heavily visited by tourist divers are now essentially devoid of life.

Even the gas exhaled by divers may have adverse effects on cave animals. Because anchialine cave waters typically contain extremely low levels of dissolved oxygen, exhaust bubbles from open circuit scuba could have profound effects on the cave ecosystem. Several anchialine caves in Western Australia with unique fauna are currently off limits to open circuit divers and may only be visited by those using rebreathers (Humphreys et al., 1999).

Some anchialine caves in Bermuda, the Canary Islands, and Mallorca have been developed into commercial tourist attractions. Unfortunately, many of the tourists visiting these sites have viewed the deep clear water cave pools as natural wishing wells in which to throw a coin or two. Copper coins tend to rapidly deteriorate and dissolve in saltwater, producing high levels of toxic copper ions in the cave waters. In one such cave in the Canary Islands, the endemic crab *Munidopsis polymorpha* has shown a marked decline in abundance during the past decade or longer, probably in response to high levels of copper in the cave water.

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Decimating Mangrove Forests for Commercial Development in the Pelican Cays, Belize: Long-Term Ecological Loss for Short-Term Gain?

Ian G. Macintyre, Marguerite A. Toscano, Ilka C. Feller, and Maria A. Faust

ABSTRACT. The unique, biologically diverse ecosystems of Pelican Cays, Belize, are in serious danger from sediment suffocation related to the recent clear-cutting of mangroves for commercial development in what is currently designated Southwater Cay Marine Reserve. Field observations in the Pelican Cays in March 2007 revealed extensive clear-cutting of mangroves and covering of exposed peat surfaces with sediment dredged from the adjacent seafloor to create false sand cays. On Manatee Cay, introduction of dredge spoils taken from the nearby seabed resulted in fine sediment plumes spilling into the adjacent ponds, smothering the attached benthic communities on mangrove roots and burying *Thalassia* bottom communities. In addition, comparative studies of microalgal (phytoplankton) assemblages in a Manatee Cay pond before and after mangrove clearing indicate a dramatic loss in this group. This change, related to high turbidity observed in the water column, signals a serious impact to this aquatic ecosystem. In March 2007, clear-cutting, burning, and dredge and fill operations were taking place on Fisherman's Cay, with additional survey lines visible on Fisherman's, Manatee, and Cat Cays. We used a series of aerial photographic surveys from 2003 to 2007 to document the extensive loss of mangroves on both Manatee and Fisherman's Cays. To date, additional clearing of mangroves has occurred on Northeast Cay, Bird Cays, and Ridge Cay, resulting in a total of 15.3 ha or more than 29% of the mangrove community that have been destroyed in the Pelican Cays. Furthermore, several survey lines through still-forested areas on these islands indicated that additional clearing of mangroves was planned. The Pelican Cays ponds contain unique, biologically diverse ecosystems dominated by delicate sessile photosynthetic and filter-feeding populations; these rare communities will be lost as a result of sediment suffocation caused by the clearing and filling of these islands. However, the conversion of mangrove ecosystems for residential, tourism, and commercial uses is both widespread and accelerating in Belize and throughout the global tropics. This pressure is having an adverse effect on the health of coral reefs and the biomass and viability of commercial fisheries, which are essential for both tourism and local livelihoods.

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INTRODUCTION

The Pelican Cays group is an oceanic coral reef boundary environment (Macintyre et al., 2000a), containing a network of coral ridges and semi-enclosed or enclosed ponds (Figure 1) where shallow mangrove cays are immediately adjacent to channels approximately 20 to 30 m deep. The lagoon-like ponds, which