

Effect of temperature and salinity on in-vitro population growth of copepode *Oithona* (Copepoda: Cyclopoidae). Temperature-salinity *Oithona* culture

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Abstract

The marine copepods culture has been considered a great resource for feeding in marine aquaculture. *Oithona* is the most abundant genus in the oceans and that is why they are the largest contributors of live prey in fish larvae. The present study aimed to identify the influence of temperature and salinity on the population growth of *Oithona* sp. under laboratory conditions. Copepods were cultivated (n: 15 ind/200 ml in three experimental temperatures: 21, 26, 31°C and in three salinities: 20, 30, and 40‰), which were monitored and adjusted daily. Copepods were fed based on a microalgae culture *Isochrysis* sp. at a continuous density of 600.000 cel/ml. for 15 days. At the end of the experiment, *Oithona* sp presented significant statistical differences ($p < 0.05$), in population growth, with a higher population density at 40‰ ($8.040 \pm 6.265.2$ ind/ 200ml), at a temperature of 31°C. Also, it was shown that the variable salinity alone did not influence the population growth of the copepod, while, if it was related to the water temperature, it presents statistically significant differences ($p < 0.05$), in all possible combinations. We concluded that *Oithona* sp. can be well adapted to higher temperature and salinity conditions in which the optimal salinity and temperature variables rate is 31°C and 40‰ to reach a higher development. These conditions improve the rate of reproduction, population growth, and its development in culture.

Introduction

Although rotifers and artemias occupy an important role in the aquaculture nutritional industry, they do not have the ideal size and nutritional quality characteristics that copepods represent, which have aroused great interest in its larviculture worldwide. For this reason, the success in the cultivation of around 60 species of this microcrustacean has been reported (Payne & Rippingale, 2000; Payne et al., 2001; Drillet et al., 2011; Raju et al., 2012; Anil et al., 2018; Anzeer et al., 2019), which in the oceans represents 70% of all animals of various stages and sizes (Ananth & Santhanam 2011; Raju et al., 2012). Much of their success is due to their characteristics, such as high reproduction rates, high population growth, short generation periods, acceptance of captive diets, and the ability to convert their food into lipid storage (Nanton & Castell, 1998, 1990; Knuckey et al., 2005; Dhont et al., 2013; Nogueira et al., 2019; Burbano et al., 2020). In addition, copepods have better rates of assimilation and digestion in the intestine of fish larvae compared to the group of artemia (Drillet, 2010).

On the other hand, it is essential to know aspects such as reproduction rates and life cycles, as well as the influence of temperature or salinity in the development and optimization of copepods culture. Knowledge about the production of tropical copepods is scarce. Therefore, their study is necessary to improve the development of marine larviculture in these latitudes and to standardize cultivation protocols as live food. Environmental parameters such as salinity and temperature influence aspects such as growth rate, spawning, ripening, osmoregulation, number of offspring, nutritional quality, fertility, or longevity, among others (Bergmans & Janssens, 1988; Miliou & Moraitou-Apostolopoulou, 1991; Nanton & Castell, 1999; Rhodes, 2003; Zaleha & Jamaludin, 2010, Ohs et al., 2010).

The order Cyclopoida belongs to one of the most abundant zooplankton groups in aquatic environments worldwide. They are part of the class Maxillopoda, subclass Copepoda and superorder Podoplea. They are distributed in all bodies of water in planktonic or benthic forms and are from commensals to parasites (Miracle, 2015). In Colombia, the Oithonidae family has been recorded in the genus *Oithona*, with nine species: *O. plumifera*, *O. ovalis*, *O. nana*, *O. atlantica*, *O. hebes*, and *Oithonidae. O. occulata*, *O. oswaldocruzi*, *O. robusta* and *O. setigera*. The species *O. plumifera* is distributed in most of the Colombian Caribbean Sea and the ballast waters (Owre and Foyo, 1976; Bernal, 2000; Rendón et al., 2003), while the other species are distributed in ballast waters in the ports of Magdalena and Bolivar (Rendón et al., 2003; Uribe and Calero, 2006; Medellín-Mora et al., 2010). This genus presents a broad food spectrum, considered an omnivorous group (Peralta and Lopez, 2015).

For a long time, the genus *Oithona* has stood out from other zooplankton for multiple reasons such as prolonged reproductive periods, low metabolic rates, life cycle adapted to its food availability, survival capacity, and production of eggs in limited food conditions, for its abundance as well as their high biomass (Paffenhöfer 1993, Cepeda et al., 2012; Peralta and Lopez, 2015). Although the genus has been used as live food in aquaculture (Santhanam & Perumal 2013, Vasudevan, 2013) and despite its ecological importance, there are few studies in Colombia related to the biology, physiology, or ecology of this genus and its applications as live food for larvae. To date, no published information is available as to the optimum temperature and salinity conditions for the culture of this species. Therefore, this study aimed to evaluate the optimal conditions and the influence of temperature and salinity on the copepod *Oithona* sp. grown under laboratory conditions. This information is important to maximize the intensive culture potential of this species, considering their high levels of omega 3 fatty acids (DHA, and EPA) which are important to meet the nutritional requirements in diets for marine larviculture, which faces great challenges for their development in the tropics.

Material and Methods

Study and sampling area

The copepods were collected with a 63 µm zooplankton net from natural populations from the Ciénaga Grande de Santa Marta (Fig. 1) and transferred to the Aquaculture Laboratory of the University of Magdalena. In the laboratory, they were sieved with a 63 µm zooplankton net and identified with a binocular stereoscopic (STEMI 305 model; Carl Zeiss microscope GmbH). After the selection, these were classified with a 200 µm filter to obtain only adults and then placed in beakers (200ml), for 1 hour, before the beginning of the experiment.

Experimental design

Once isolated, *Oithona* sp. were cultivated in three different treatments, consisting of two treatments factors (temperature x salinity): Three temperatures of 21, 26, and 31°C, and three salinities of 20, 30, and 40‰ with three replicates per treatment (Table 1). In each container (200ml), 15 adult copepods were used, and the presence of an ovate female was guaranteed.

Microcrustaceans were cultivated with filtered seawater sterilized with U.V., at a constant temperature, and salinity was monitored daily with a multiparameter (YSI mark) and adjusted according to the experimental design. The whole experiment was maintained with natural photoperiod (14L:10D), and without aeration. The microcrustaceans were fed with *Isochrysis* sp. (600.000 cel/ml), every three days. During the fifteen days of the culture, every three days (3, 6, 9, 12, 15 days), 10 ml of water sample were extracted and fixed in 5% formalin. The samples were analyzed with a conventional light microscope (Carl Zeiss) and a photographic record was made with a digital camera (AxionCam ERc5S). Copepods were counted to estimate the number of individuals present in each container, without discriminating the stages of development (nauplio, copepodit, and copepod).

Statistical analysis

The data were expressed as mean \pm standard deviation (SD) (Table 2). For the interpretation of the results, an analysis of a one-way ANOVA test was applied, to check the assumptions of normality with the Shapiro test and the Bartlett test to test the homoscedasticity. For this case, the assumptions were not met and a non-parametric test of Kruskal Wallis was performed to define the variable that influenced the growth and abundance of copepod. The analyzes were performed with the statistical software R.

Results

Results show the effect of salinity and temperature on copepod culture, development, and reproduction with three experimental treatments (Tables 2, 3, and 4).

Higher salinity and temperature (40‰; 31°C) recorded the highest *Oithona* sp. population growth (8.040 ± 6.265 individuals) (Table 3), instead of treatments 1 and 2 which showed less population growth (300 ± 273 individuals and 2.887 ± 2.493 , respectively) (Table 2). The ANOVA showed that the salinity does not influence the population growth of the copepod, while its interaction with the temperature does show a significant difference between them (Table 4). A Tukey test showed significant differences between the population growth of copepods grown at 21°C and 26°C (Table 5) (Fig. 2). In addition, a Kruskal-Wallis test (chi-squared) showed significant differences ($p < 0.01$) in population growth of treatments T1 and T3 between temperature and salinity (Table 5) (Fig. 3).

Discussion

The present study revealed the influence of temperature and salinity on the population growth of *Oithona* sp cultivated under controlled conditions. The highest growth was achieved at a salinity of 40‰ and a water temperature of 31°C. The variable salinity did not show statistical differences during the copepods' culture, but when it was confronted with the temperature, there was a statistical difference in the results observed about the total population and its development. The results of the combination of these water parameters indicate that they could improve copepod egg production and survival, thus influencing its population dynamics under culture conditions.

The high capacity of this genus to adapt to fluctuating environments of temperature and salinity, as observed in this study (31°C and 40‰), may be due to the origin of the strain, because it comes from the Ciénaga Grande de Santa Marta. This is a eutrophic estuarine system that has the affluence of river systems, saltwater flows from the Caribbean Sea, and precipitation and evapotranspiration processes (Espinosa et al., 2021), in which abrupt changes in temperature, salinity, and other environmental parameters, such as dissolved oxygen, occur permanently (Espinosa et al., 2021; Carrasquilla-Henao et al., 2022). For this reason, a good part of the species that inhabit this ecosystem have adaptive mechanisms to face different environments, like the *Oithona* genus.

The species of the genus *Oithona* have euryhaline and eurythermic capacities (Torres-Sorando et al., 2003; Hansen et al., 2004; Turner, 2004), low energy expenditure for breathing, low metabolic rate (Lampitt & Gamble 1982; Paffenhöfer, 1998; Castellani et al., 2005), and good prolonged reproduction rates (Paffenhöfer 1993; Zamora-Terol & Saiz, 2013). These capacities are evidenced by *Oithona* sp., which, according to our results, serve as an ideal candidate for mass culture for use in the larval rearing of finfish and shellfish, for its adaptability, easy manipulation, and great power of population regeneration.

To develop the culture of this species we suggest considering a mix of salinity x temperature of 40‰ x 31°C, since in 15 days of culture the population growth showed values of around 8.040 ind/200ml. These values are higher than those observed by Aldea (2022), where the largest total population was reported at a salinity of 25‰ with $3.634,75 \pm 869.44$ in *Oithona nana*. Chilmawati et al., (2019), reported in *O. similis*, a population density of $165,40 \pm 2.41$ individuals at a salinity of 18‰, between days 16 to 20 of culture. Finally, Vasudevan et al., (2013), reported a maximum growth of *O. rigida*, at a salinity of 35‰ and a total of individuals among nauplios, copepodites, and adults of 6.722/l. Mujica, et al., (1995), recorded in another order of copepods, (*Tigriopus* sp., Copepod: Harpacticoida), the highest growth with the combination of 21°C of temperature and 26‰ of salinity, ranges different from those stated here.

Also, Wilson et al., (2021), in the tropical copepod, *Acartia calanoid*, noted that salinity had significance on the final mean population and the intrinsic rate of increase of the population with values of 1.265 ± 209 at a salinity of 15‰ and followed by no significant difference to 20‰ with a population growth of 1.005 ± 129 . Punnarak et al., (2017), studied the optimal conditions for the culture of harpacticoid copepods at the laboratory scale, revealing the best survival rate at 30°C with a higher mean (\pm SE) ($46.67 \pm 5.85\%$), followed by those cultivated at 25°C and 35°C. The authors also evaluated the influence of salinity showing better results at 27‰ and 30‰ with an average survival rate of $44.72 \pm 6.35\%$ and $42.78 \pm 4.94\%$ respectively. Similarly, Chintada et al., (2023) determined the effect of salinity on production parameters to standardize the optimal salinity for *A. bilobata* culture. The results of the study showed that 25‰ to 30‰ is the optimal salinity range to achieve the maximum production of *A. bilobata*, with a total population number significantly greater than 1.539 ± 75 and an intrinsic population growth rate (0.50 ± 0.00) at 30‰. It also had the best average daily egg production at 25‰ and 30‰ (32 ± 1 and 34 ± 2 female eggs – 1 day – 1, respectively). Torres et al., (2021), identified the influence of temperature and salinity during the spawning of copepod *A. tonsaa* to improve the survival of cold-stored

eggs. The authors reported a better hatching rate of eggs stored for 2 and 4 weeks at low temperatures and high salinity, corresponding to 18°C and 30‰ with a hatching percentage of 58% and 73% respectively. However, fresh eggs had a higher hatching success ($\geq 80\%$). The authors concluded that eggs are affected by temperature and salinity and that if fresh eggs are used directly as live feed in aquaculture the temperature is not very critical, but they recommend spawning these copepods at a higher salinity. Other research corroborates this recommendation (Chen et al., 2006; Milione and Zeng, 2008; Ohs et al., 2010). They determined that for copepods, salinity influences population growth because it gets involved in productivity processes such as egg production, hatching success and survival as occurred in this study.

The ranges used in this experiment are within those established for temperature in the studies performed by Velásquez, et al., (2001), where *Apocyclops distans* (Copepoda, Cyclopoidae) showed the highest average number of copepods in darkness at room temperature (26°C to 33.8°C). Osorio (1998) found that in *Pseudodiaptomus euryhalinus Johnson* (Crustacean: Copepoda Calanoidea), population growth rates are influenced by temperature than by salinity (0.98 to 30°C and 0.68 to 23°C). In this sense, through this study, we confirmed that *Oithona* sp., has thermoregulatory capacity, supporting high thermal increases with good reproduction rates under controlled conditions, and therefore are excellent candidates for mass production under laboratory conditions. Therefore, as stated by Ban (1994) and Santer & Bosch (1994), temperature and food will affect the development and life cycle of copepods, specifically cyclopoids, calanoids, and chaetognaths, as they are considered poikilotherm organisms, which will be subject to the temperature of the environment where they are.

Likewise, several authors report that temperature and salinity have a direct influence on parameters such as egg production, hatching rate, and survival of copepods nauplii (Peterson, 2001; Chen et al., 2006; Holste and Peck, 2006; Milione and Zeng 2008). As, for example, Wilson et al., (2021), reveal that, in *A. tropical*, salinity at 15‰ influenced their final population. Punnarak et al., (2017), demonstrated a high survival rate of harpacticoid copepods when cultured at 30°C, and at 27‰ to 30‰ in Likewise. Chintada et al., (2023), revealed the optimal range of production and the best production of *A. bilobata* eggs at 25‰ to 30‰. However, in this study, the salinity variable itself did not show statistical differences during cultivation, although it was observed that when it was confronted with temperature, there was a difference in the observed results in relation to the total population and its development. This result would be favorable because it presents a higher reproduction rate, a situation that maximizes the constant production of live food in the larviculture of different species.

The ability to maximize copepod production is one of the main objectives of aquaculture. Most copepods exhibit tolerance to salinity changes, which may or may not be related to their osmoregulation capacity (Gaudy et al., 2000; Peck et al., 2015; Dutz & Christensen, 2018). Other studies have also reported the influence of these two parameters on the production of the species, such as spawning, survival, the rate and percentage of hatching of eggs, and population growth (Chen et al., 2006; Milione and Zeng, 2008, Ohs et al., 2010; Torres et al., 2021), which directly occur in their production processes.

In summary, the high capacity of this genus to adapt to fluctuating environments of temperature and salinity, as observed in this study, may be due to the origin of the strain, that comes from the Ciénaga Grande de Santa Marta a eutrophic estuarine system that has the influx of a river system, the salt water that flows from the Caribbean Sea; in addition to precipitation and evapotranspiration processes (Espinosa et al., 2021) where abrupt changes in temperature, salinity and other environmental parameters, such as dissolved oxygen, occur permanently (Espinosa et al., 2021; Carrasquilla-Henao et al., 2022). For this reason, a good part of the species that inhabit this ecosystem has adaptive mechanisms to face different environments. In addition, the species of the genus *Oithona*, have euryhaline and eurythermic capacities (Torres-Sorando et al., 2003; Hansen et al., 2004; Turner, 2004), low energy expenditure for breathing, and low metabolic rate (Lampitt & Gamble 1982; Paffenhöfer, 1998; Castellani et al., 2005), In addition to good reproduction rates that are also prolonged (Paffenhöfer 1993; Zamora-Terol & Saiz, 2013). These capacities are evidenced by the species *Oithona* sp., which, according to our result, give it an important value to be selected as a species cultivated as living food for the aquaculture of larvae.

Conclusions

The results of this study showed that there is an intrinsic relationship for the cultivation of *Oithona* sp, between temperature and salinity, and an optimal range of culture at 31°C and 40‰ is suggested. The control of these two physical parameters improves the productivity of the culture to achieve the use of this resource as potential live food for aquaculture. However, these culture methods will need to be further studied to ensure a better development of the production of the species on an industrial scale. Based on these results, there is also a need to address new research related to survival, culture yield, egg production, and hatching, among others.

Declarations

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Example statements:

Competing Interests

Authors declare we have no financial interests.

Author Contributions

Katrina Medina-Lambraño: Methodology, Investigation, Validation and Formal analysis

Adriana Rodríguez Forero: Supervision, Writing- Reviewing and Editing

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Tables

Table 1

Experimental design for the evaluation of population growth of *Oithona* sp, "in vitro".

Treatments	Independent variable			Dependent variable Population growth (Egg production)
	Temperature (°C)	Salinity (‰)		
T1	21°C	20	30	40
T2	26°C	20	30	40
T3	31°C	20	30	40

Table 2

Mean \pm standard deviation (SD) of population growth in the different culture temperatures of the copepod *Oithona* sp.

Treatments	Temperature (°C)	Mean	SD
1	21	300	273
2	26	2.887	2.493
3	31	5.820	4.805

Table 3
 Mean ± standard deviation (SD) of population growth with different salinity and temperature treatments of *Oithona* sp.

Treatments	Temperature (°C)	Salinity (‰)	Mean	SD
1	21	20	260	194,9
		30	320	268,3
		40	320	383,4
2	26	20	3.420	2.999,5
		30	4.120	2.512,4
		40	1.120	531,04
3	31	20	6.100	4.933,05
		30	3.320	1.772,6
		40	8.040	6.265,2

Table 4

ANOVA test results on the total population growth of *Oithona* sp. with different temperature and salinity treatments.

Population growth (individuals /200ml)	G.I	S.C	Mean square	Value F	Value P
Temperature °C	2	228828444	114414222	16.34	0.0000129 ***
Day	4	106269778	26567444	3.79	0.0123 *
Salinity ‰	2	3960444	1980222	0.28	0.7555
Temperature: Salinity	4	76968889	19242222	2.75	0.0452 *
Residuals	32	224042222	7001319		

Table 5
Comparison of population growth of the copepod *Oithona* sp. between treatments. Tukey test and HSD intervals.

Comparison	Value P
T1 - T2	0.0304*
T1 - T3	0.0000***
T2 - T3	0.0128*

Where: T1: 21°C; T2: 26°C; T3: 31°C; P-Value: Probability; ***: Significance.

Figures



Figure 1

Map of Ciénaga Grande de Santa Marta (CGSM) and Isla del Rosario sampled place. (Retrieved from Google earth).

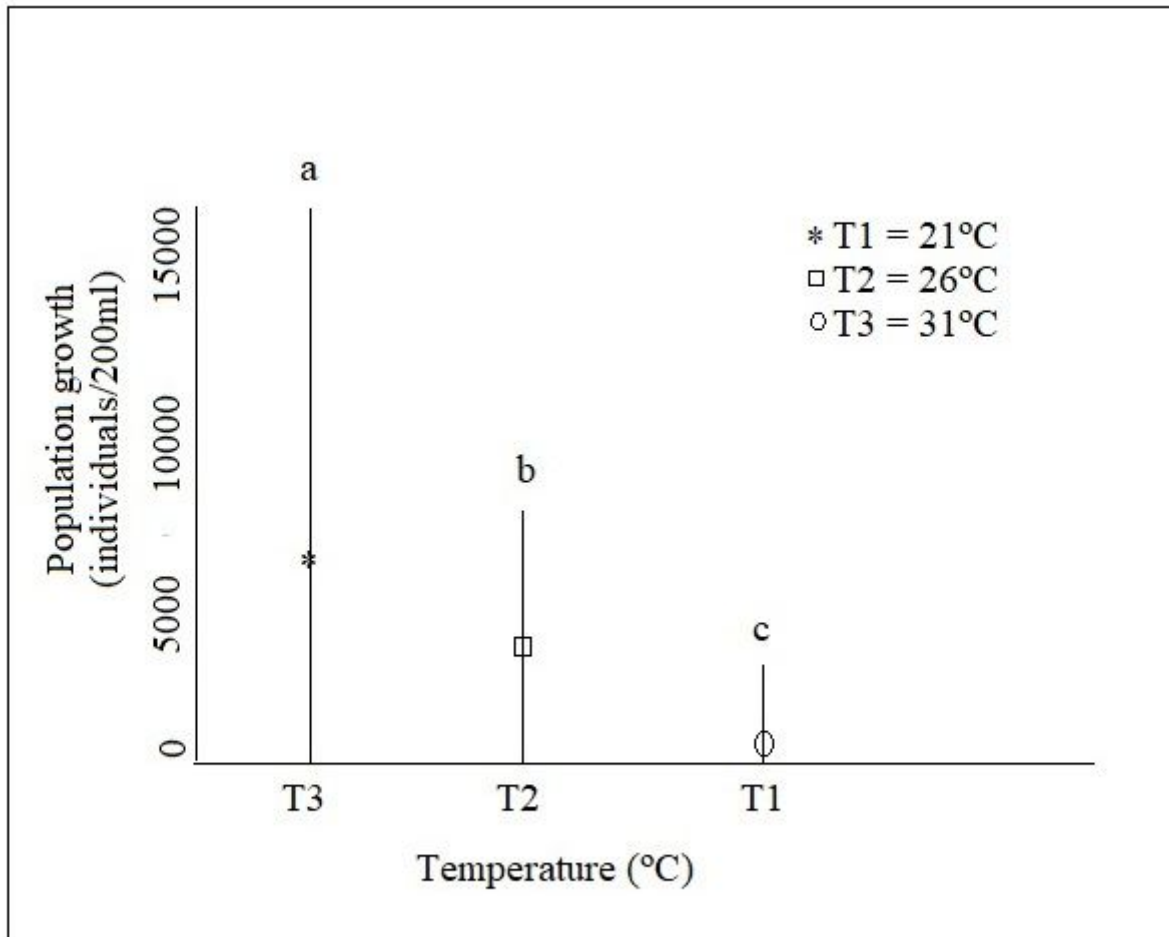


Figure 2

Comparison of means of treatment concerning the population growth of *Oithona* sp. during 15 days of culture. Tukey test (Mean \pm SD).

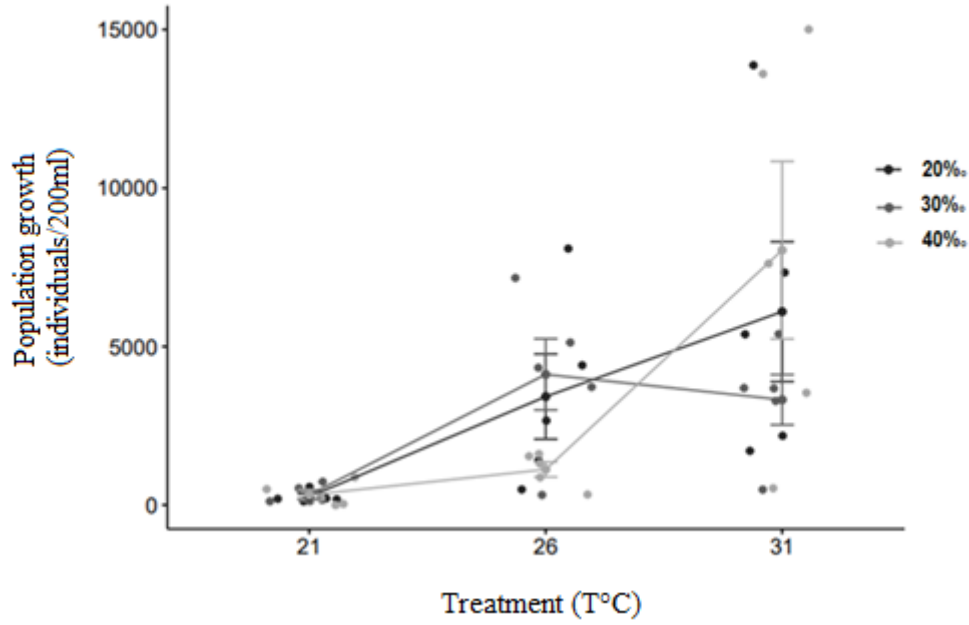


Figure 3

Population growth of *Oithonasp*, between temperature and salinity, during 15 days of culture. Kruskal-Wallis test (Mean \pm SD).