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Effect of temperature increase on fertilization, embryonic development and larval survival of the sea urchin *Toxopneustes roseus* in the Mexican south Pacific

Leobarda Margarita Mejía-Gutiérrez[a], Francisco Benítez-Villalobos[b,*], Julia Patricia Díaz-martínez[a]

[a] División de Estudios de Posgrado, Universidad del Mar (UMAR), Campus Puerto Ángel, Distrito de San Pedro Pochutla, Puerto Ángel, Oaxaca, Mexico, C.P. 70902
[b] Instituto de Recursos, Universidad del Mar (UMAR), Campus Puerto Ángel, Distrito de San Pedro Pochutla, Puerto Ángel, Oaxaca, Mexico, C.P. 70902

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ABSTRACT

Toxopneustes roseus performs a key role in the eastern tropical Pacific as a strategic herbivore and bioturbation promoter. We evaluated the effect of temperature on the fertilization success, embryonic development and larval survival of *T. roseus* under laboratory conditions, to understand how the increase in ocean temperature could affect it in a global warming. The highest percentage of fertilization occurred in gametes that were exposed to 30 °C, and a significant negative effect of 32 °C was evidenced by the lowest percentage. There was also a deleterious effect in embryos exposed to 32 °C, resulting in an abnormal development at all the time points. The highest percentage of larval survival occurred at 30 °C, while the lowest percentage occurred at 32 °C. The results suggest that *T. roseus* probably lives near its upper thermal limit, and future ocean warming could threaten the permanence of the species in the eastern tropical Pacific, or at least lead to contraction or fragmentation of its range limits. Therefore if sea temperature rises globally, it could cause the disappearance of these populations that are living at the edge of their thermal tolerance, but for other populations located in more temperate latitudes, it could propitiate favorable conditions for fertilization and survival of embryos and larvae.

1. Introduction

The presence and success of the species in time and space depend on a complex number of environmental factors (Odum, 1959). Among these factors, temperature and pH are of greater importance within the marine ecosystem, because they directly influence every population of animals and plants, affecting their dynamics, ecological and physiological interactions, and their impacts on the ecosystem functioning (Fujisawa and Shigei, 1990; O'Connor et al., 2007).

Temperature is one of the most important environmental factors for optimal development in marine ectothermal organisms, which influences biological processes such as chemical reactions and physiological processes (Sanford, 2002). Given that future predictions exceed the optimum temperatures at which species are currently developing (IPCC, 2014), accelerated warming of the oceans could affect species, and take them beyond their tolerance threshold, by altering the costs of metabolic processes in the new conditions. It could also change the patterns of development and reproduction, thus reducing populations to cause the extinction of a significant number of species (Hoffmann and Sgró, 2011).

Like most marine invertebrates, echinoderms in general, and sea urchins, in particular, release their gametes into the water column where fertilization occurs. When the physico-chemical conditions of seawater change, their gametes are exposed to a variety of stressors, both natural and anthropogenic, which can affect the early stages of development, for example by limiting the ability to generate calcareous structures of the larvae (Kurihara and Shirayama, 2004; Melzner et al., 2009). Previous studies have shown positive effects of temperature increase on fertilization as well as negative effects on larval development (Byrne et al., 2010, 2011, 2013; Hardy et al., 2014; Hardy and Byrne, 2014, among others).

Toxopneustes roseus is a species of sea urchin with a wide distribution along the eastern Pacific coast, from the Gulf of California, Mexico, to northern Peru, including islands such as Isabel and Revillagigedo in Mexico, Isla del Coco in Costa Rica, the Galapagos in Ecuador, and Lobos de Afuera in Peru (Solís-Marín et al., 1997; James, 2000;

* Corresponding author.
E-mail address: fbv@angel.umar.mx (F. Benítez-Villalobos).

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Lawrence & Sonnenholzner, 2004; Alvarado et al., 2010). Along the Species distribution range, the different populations experience a thermal interval that goes from an annual average temperature of around 22 to nearly 30 °C. It is common to find this species on hard substrata, associated with coral structures, beds of non-geniculate coralline algae (rhodoliths) and rocky environments, from very shallow waters to depths of approximately 60 m. These organisms often form large aggregations of up to several tens of individuals, while feeding on rhodoliths and dead coral (James, 1998, 2000). The grazing of *T. roseus* can reduce the algal biomass and increase the production of carbonate sediments, which in turn constitutes one of the causes of bioturbation on these communities (James, 2000), affecting the general benthic community along the eastern tropical Pacific.

Recent evidence indicates that environmental factors, mainly sea surface temperature, exert an influence over reproductive cycles and seasons, and act as the main trigger for gamete maturation of several marine invertebrates, as it has been shown for scleractinian corals across the eastern tropical pacific (Santiago-Valentín et al., 2018). At the Mexican south Pacific scale, López-Pérez et al. (2016), established a historical (1870–2008) average temperature for the region of around 28 °C, and an increase of up to 1.8 °C above historical data during the ENSO event. An important influence of the temperature over the intensity of reproductive activity has been reported for three echinoderm species (Benítez-Villalobos and Martínez-García, 2012; Benítez-Villalobos et al., 2013, 2015) and two mollusks (Avila-Poveda, 2013; Alejo-Plata and Gómez-Márquez, 2015) in this area. This intensity in reproductive activity indicates that the increase in temperature during the warm period (May–October), reaching almost 30 °C, probably acts as one of the proximate causes that determine the seasonal reproduction observed in those marine invertebrates.

Several studies have evaluated the influence of temperature during embryonic and larval development, settlement, metamorphosis and larval survival of different marine invertebrates (O'Connor and Lawler, 2004; Dove and O'Connor, 2007; O'Connor et al., 2007; Saunders and Metaxas, 2009). The effects of this environmental factor on development in echinoderms from temperate zones have been studied in numerous investigations (Farmanfarmaian and Giese, 1963 and Andronikov, 1967, among others); however, there is little information on the effects of temperature in the early development of subtropical and tropical echinoderms (Chen and Chen, 1992; Sheppard et al., 2010; Hardy et al., 2014). In the eastern Pacific, Díaz-Pérez and Carpizo-Ituarte (2011) identified the limit of thermal tolerance, survival and delay of the metamorphosis of *S. purpuratus* in Baja California, obtaining a thermal tolerance limit of 27 °C in pre-competent and competent larvae. Temperatures above this value were lethal for both stages of development. Regarding the eastern tropical Pacific, not a single study has been carried out on the effect of rising temperatures over the early stages of development of sea urchins or other echinoderm species.

Considering the above, it is important to understand how a significant increase in ocean temperature could affect important organisms of the marine ecosystem, as well as the way in which they will respond to such a stress factor. The importance of this work is that for the first time the effect of temperature variation in the early developmental stages of a tropical eastern Pacific echinoderm is evaluated. The objective of this study was to evaluate the effect of temperature (28, 30 and 32 °C) on the success of fertilization, embryonic development and larval survival of the pink sea urchin *T. roseus* under laboratory conditions, considering the wide range of distribution of the species and the average temperature at which the different population inhabit (22–30 °C). There are no data about the reproductive cycle of *T. roseus*; however, there is information on several species of invertebrates (especially echinoderms) in the study area, which exhibit a spawning period between May and October, when the sea temperature fluctuates between 28 and 30 °C (Benítez-Villalobos and Martínez-García, 2012, Avila-Poveda, 2013; Benítez-Villalobos et al., 2013, 2015). According to the climatic projections of increases in temperature throughout Latin America by 2100 (IPCC, 2014), in a series of medium and high emission scenarios, the warming varies from +1.6 to +4 °C in Central America to + 1.7- +6.7 °C in South America (average confidence level). In a low emission scenario, an increase of the warming in the whole region is projected from +1 to +1.5 °C.

2. Material and methods

2.1. Study area

The study area is located on the western border of the Gulf of Tehuantepec (GT), Mexico, in the eastern tropical Pacific. This area is influenced by coastal currents, upwelling, climatic variations and epicontinental discharges due to the large number of marshes and coastal lagoons that exist on its coastline (Tapia et al., 2007). The region experiences a dry season that extends from November to April, and a rainy season (800–1500 mm) from May to October. The dry season is characterized by high-speed winds (30–50 m/s) from the North and an average sea surface temperature of 18–21 °C. As the winds intensify, they lead to the formation of ocean fronts in the southwest part of the GT and upwelling on the southeast side (Ortega-García et al., 2000), allowing to harbor high biological productivity, in comparison to the waters of temperate zones (Yánez-Arancibia, 1985). This high productivity is maintained throughout the year, influencing the biological processes of spawning, rearing and feeding of various species of marine communities (Ortega-García et al., 2000). On the contrary, during the rainy season there is an absence of winds and the water temperature varies between 25 and 30 °C (Monreal-Gómez and Salas de León 1998).

Toxopneustes roseus were collected from the bay of Estacahuite, Puerto Angel, Oaxaca, Mexico (15° 40′5.15 ″N, 96° 28′ 51.94″ W), which has a depth ranging from 0.5 to 12 m (Fig. 1). The bottom is sandy with rocky crests associated with well-developed coral communities, with the coral *Pocillopora damicornis* predominating (Leyte-Morales, 1999), and non-geniculate coralline algae (Reyes-Bonilla and Leyte-Morales, 1998).

2.2. Field work

The collection of the organisms (10 organisms for every experiment) was done manually by scuba diving at approximately 3 m depth. The organisms were individually placed in hermetically-closed plastic bags with filtered seawater to prevent the gametes from mixing in the event of spawning during transport. They were taken to the laboratory of Ecología del Desarrollo of the Universidad del Mar in Puerto Ángel and maintained under controlled conditions in a 90 l container (constant aeration, Salinity 35, temperature ~28 °C, pH 8.1, feeding *ad libitum* with small rhodoliths).

2.3. Laboratory work

2.3.1. Experimental design and spawning induction

For the experiment, three water baths (Grant W14) were used, which were filled with distilled water and programmed at three temperatures (28, 30 and 32 °C), considering the first as the control (average sea water temperature in the area and predominant temperature during the spawning season of several echinoderms) and the following, two and four degrees above that average. It was also taken into account the increase predicted by the IPCC for the region by 2100, which would be in the most extreme scenario of up to 4 °C above the average. To avoid significant fluctuations in the programmed temperature of each bath, the equipment was kept inside the laboratory with the air conditioning turned on to keep the room temperature at 20 °C. Three 500 ml beakers were placed inside each water bath, filled with filtered seawater (FSW), consisting of water extracted from the sea that was mechanically filtered up to 1 μm and irradiated with UV light, after which they were sealed. This procedure was done one day before

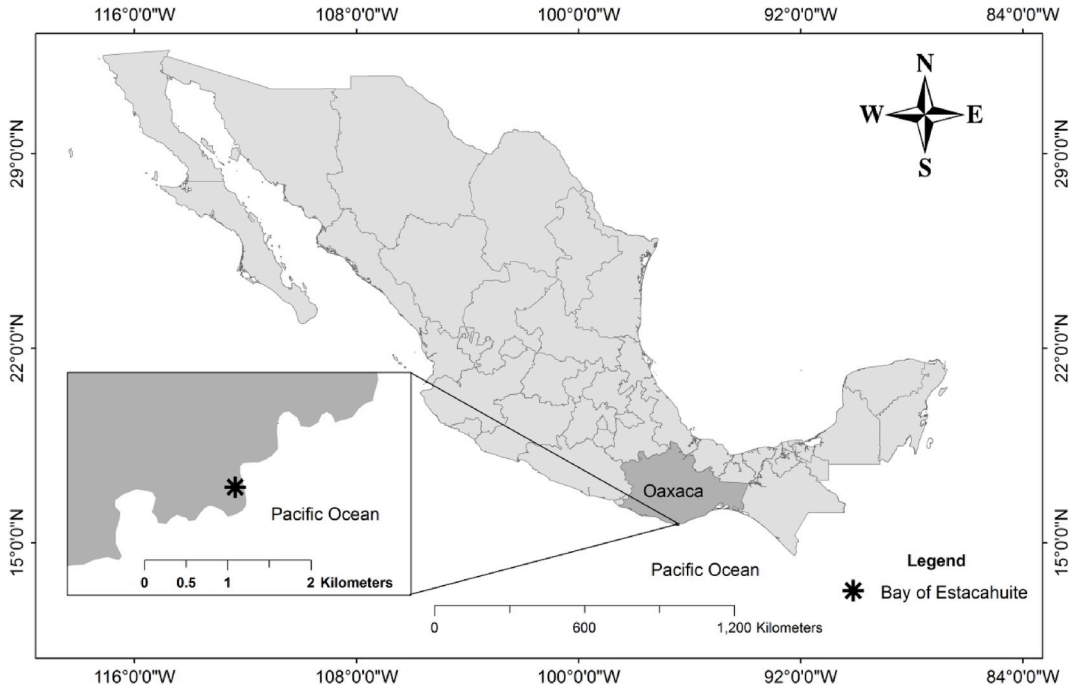

Fig. 1. Map showing the Bay of Estacahuite in the Mexican south Pacific, where the organisms were collected.

performing the experiment, to facilitate a stable temperature in each water bath so that the gametes were always in water at the temperature of each treatment. The experiment was carried out on three dates (April 13th, June 28th and September 2nd, 2016) with different sets of parents and sea water temperature fluctuated from 28 to 29 °C during this period. The trials with each set were characterized as experiment 1 (E1), experiment 2 (E2) and experiment 3 (E3). The experiment consisted of evaluating the fertilization and larval survival percentages in response to temperature, applying three treatments of temperature to three subjects (male-female pairs) on three separate occasions. Before the spawning induction, the organisms were washed with FSW to eliminate epibionts and the remains of organic matter adhered to their spines.

The gametes were obtained according to the method described by Strathmann (1987). Of the 10 organisms collected, generally five or six were induced, which spawned, and then the gametes of the male-female pair that showed the best quality were used. When the spawning occurred, the males were put in small containers without water to obtain "dry" sperm and females were left to release eggs into the FSW. Once the viability of the gametes was corroborated (breakdown of germinal vesicle in eggs and sperm motility), a fertilization test was carried out: In a Petri dish with FSW, a sample of eggs was placed and 50 µl of "dry" sperm were added and left 15–20 min for fertilization to occur. Once this time passed, a sample was taken and observed under a microscope and a rapid count was made of the proportion of fertilized eggs (which were characterized by presenting the fertilization membrane).

2.3.2. Effect of temperature on fertilization

Once fertilization was corroborated under normal conditions, the eggs (diameter 105 ± 2.72 µm) were placed in a sieve of 35 µm mesh and washed with FSW. Once clean, they were placed in a jar and the total volume was brought to 4l with FSW. From this suspension of eggs, 500 ml were taken for each treatment and each portion was sieved to keep only the eggs, which were then placed in one of the beakers that was inside each water bath and that corresponded to each temperature (28, 30 and 32 °C). Subsequently, counts were made at each temperature and a final suspension of 25 eggs/ml was obtained for each one.

For each temperature, with the help of a syringe, three 19-ml portions of egg suspension were taken and placed in 20 ml vials and 1 ml of sperm was added to each one, which was taken from a previously prepared stock, which consisted of putting 100 µL of "dry" sperm in 9.9 ml of FSW (~1550 sperm.ml^{-1} as a final concentration).

After adding the sperm to each vial corresponding to each temperature, they were allowed to stand for 15 min in the water baths, and once this time had passed, all the samples were fixed with 1 ml of 36% formalin. From each vial, 100 eggs were counted under a compound microscope (Zeiss Primo Star), those that presented the fertilization membrane were considered fertilized and those that lacked it were considered unfertilized.

2.3.3. Effect of temperature on embryonic development

Once the gametes were obtained and tested for viability, the eggs were placed in a 35 µm mesh sieve and washed with FSW, then placed in a jar and brought to a total volume of 4l with FSW. 1 ml of "dry" sperm was added to the jar, homogenized and left to rest for 15 min. Once the fertilization membrane was visible, the content of the jar was placed on a 35 µm mesh sieve and washed to remove excess sperm. The clean zygotes were divided into three portions, which were each placed in one of the beakers with FSW that were at each temperature. As soon as the zygotes were placed into each temperature treatment, time was recorded. Subsequently, counts were made at each temperature and a final suspension of 25 zygotes/ml was obtained in each one.

With the help of a syringe and continuous homogenization, samples of the suspension of zygotes form the beaker were taken and placed in each of fifteen 20 ml vials and located inside the water bath, and this procedure was repeated for each temperature. Three vials were extracted for each temperature every 6 h and the content of each vial was fixed with 1 ml of 36% formalin until completing 30 h (the experiment was suspended at this time to prevent the prolonged permanence of the embryos in a small volume, as this would affect the oxygen consumption which would become a stress factor parallel to temperature). In each sample (vial), 100 embryos were counted.

2.3.4. Effect of temperature on larval survival

The same procedure mentioned above was performed with the gametes, until obtaining zygotes in suspension in a jar with 4 l of FSW.

The zygotes were left at room temperature (water at approximately 28 °C) to continue their development until they became two-armed pluteus larvae (around 36 h). Once the presence of the two-armed pluteus larvae was identified, they were rinsed with FSW and divided into three portions, which were placed in each of the beakers that were in the water baths. After the larvae were placed at each temperature, time was recorded.

To obtain a density of 6 larvae/ml, the same counting process described for the fertilization effect experiment was carried out. Once the final suspension of 3000 larvae in 500 ml was obtained, samples of the suspension from the beakers were taken with the aid of a syringe and continuous homogenization, and placed in each of the 20 ml vials (three vials for each temperature). The vials with the larvae were left at each temperature for 24 h and after this time the *in vivo* count of the samples was performed in order to obtain the average percentage of live larvae at each temperature.

The content of every vial was stirred and then poured into a counting chamber. At least 100 larvae were counted in each vial, of which it was considered that the live ones were those that were swimming in the water column, exhibited a transparent aspect and the movement of the internal organs was observed. The dead ones were those that were observed sunken and motionless at the bottom of the counting chamber, showing a dark greenish color and with no movement detected in the internal organs. All counts were observed at 4x, 10x and 40x, and photographs were taken with the help of the ZEN 2012 (Blue edition) program.

2.4. Data analysis

2.4.1. Fertilization

To evaluate the normality and homogeneity of the variances of the data, the Shapiro-Wilk and Levene tests were applied respectively. Analyzes were carried out using the STATISTICA 7.0 software. As the fertilization data passed both tests, a matched-samples ANOVA ($\alpha = 0.05$) was applied to evaluate the effect of the temperature on the percentage of fertilization of the three male-female pairs (E1, E2 and E3).

2.4.2. Embryonic development

To determine the existence of spatial patterns that graphically showed the differences between the three temperature treatments and their effect on the embryonic development, a spatial ordering technique was applied using PRIMER 6: non-metric multidimensional scaling (nMDS) from a resemblance matrix created with the Bray-Curtis Index (Clarke and Warwick, 2001).

The existence of significant differences between the three temperature treatments and their effect on embryonic development was evidenced by a two-way similarity analysis (ANOSIM), considering the differences in the temperature factor (28, 30 and 32 °C), and between male-female pairs (E1, E2 and E3). The analysis also uses the Bray-Curtis similarity matrix.

2.4.3. Larval survival

The larval survival data did not fulfill the assumptions of normality and homoscedasticity, for which a non-parametric test was applied. The Friedman's test was performed to show the effect of temperature on the percentage of larval survival of the three male-female pairs (E1, E2 and E3).

3. Results

3.1. Effect of temperature on fertilization

The comparison of fertilization percentage between experiments showed that the temperature had a significant effect on the fertilization of eggs of *Toxopneustes roseus*, (matched-samples ANOVA $F_{(2,16)} = 4.191$, $p < 0.05$). Fig. 2 shows that there were no significant differences between 28 and 30 °C, but between 28 and 32 °C and between 30 and 32 °C there were significant differences.

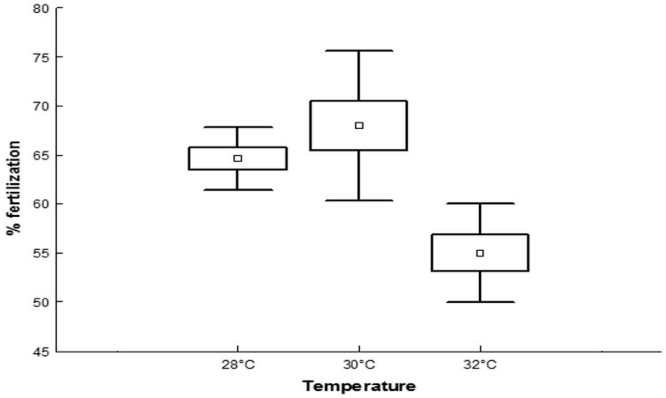

Fig. 2. Percentage of fertilization (average ± SD) of gametes of *Toxopneustes roseus*, comparing the three temperature treatments (28, 30 and 32 °C).

3.2. Effect of temperature on embryonic development

During the experiment, at 6, 12, 18, 24 and 30 h post-fertilization, seven stages of embryonic development were observed: early blastula, late blastula, early gastrula, gastrula, prism, early pluteus (with arms just starting projecting and stomach not fully formed) and two-armed pluteus (arms clearly visible and stomach fully formed), as well as embryos that did not develop (abnormal) (Fig. 3).

Since the first hours of sampling, high percentages of normal embryos were obtained at 28 and 30 °C and a small percentage of blastulae at 32 °C. As the development process continued, similar stages of development predominated at every time point at 28 and 30 °C. However, continuous exposure to a temperature of 32 °C turned out to be deleterious for *T. roseus*, with more than 90% of abnormal development of the embryos (Table 1, Fig. 4).

The MDS analysis showed a grouping pattern with the samples of 32 °C separated from the others. At the same time, it can be observed that between the groups of 28 °C and 30 °C there is a high similarity at every time point. The stress level was 0.1, indicating that it was a reliable ordering (Fig. 5).

The differences observed in the samples according to the different temperature treatments were statistically significant (ANOSIM: $R = 0.42$, $p < 0.05$). The paired tests showed that the replicates exposed to 28 and 30 °C did not show significant differences ($R = 0.059$, $p > 0.05$), while between 28 and 32 °C there were differences ($R = 0.645$; $p < 0.05$) as well as between 30 and 32 °C ($R = 0.584$, $p < 0.05$). When comparing between experiments (E1, E2 and E3), the ANOSIM indicated that the differences are not statistically significant ($R = 0.04$, $p = 1$).

3.3. Effect of temperature on larval survival

The highest percentage of survival occurred in larvae exposed to 30 °C with 94.2%, followed by the temperature of 28 °C with 81.4% and the lowest percentage corresponded to the larvae exposed to 32 °C (69.5%). Friedman's test showed that temperature had a significant effect on larval survival ($X^2 = 16.22$, df = 2, $p < 0.05$). In Fig. 6 it can be noticed that there were not significant differences between 28 and 30 °C, but between 28 and 32 °C and between 30 and 32 °C there were significant differences.

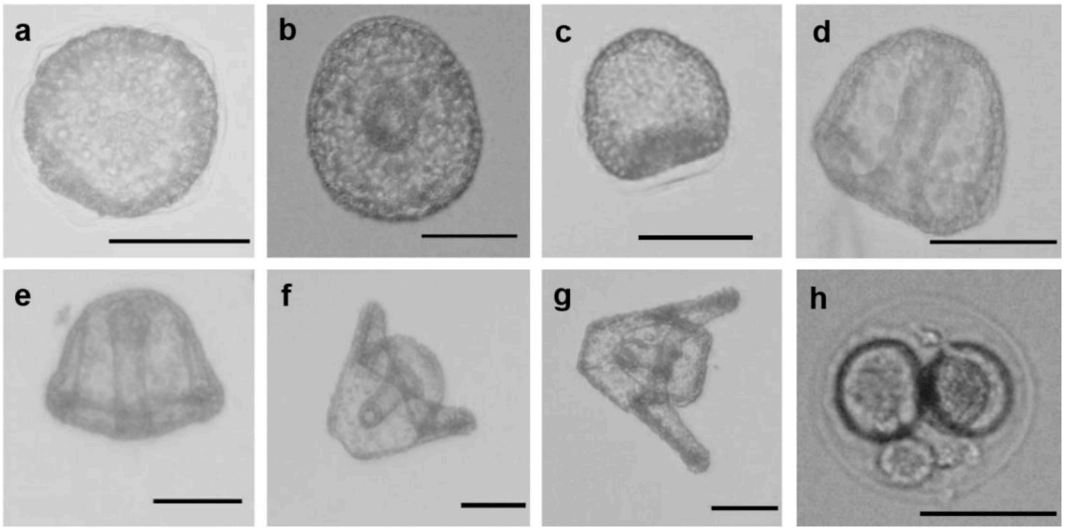

Fig. 3. Developmental stages of *Toxopneustes roseus* recorded during the experiments: **a** early blastula, **b** late blastula, **c** early gastrula, **d** gastrula, **e** prism, **f** early pluteus, **g** pluteus, **h** abnormal (asymmetrical cleavage). Scale bar 100 µm.

Table 1
Predominant developmental stages (> 50%) achieved of *Toxopneustes roseus* in experiments at 28–32 °C across time.

Time point	Stage achieved		
Post-fertilization	28 °C	30 °C	32 °C
6 h	Early Blastula	Early Blastula	Abnormal
12 h	Early Gastrula	Early Gastrula	Abnormal
18 h	Early Gastrula	Gastrula	Abnormal
24 h	Early pluteus	Early pluteus	Abnormal
30 h	Pluteus	Pluteus	Abnormal

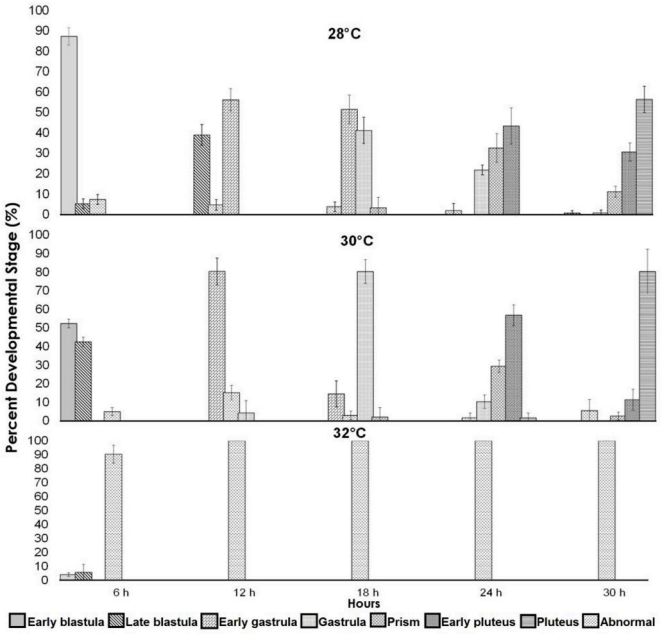

Fig. 4. Percentage (± SD) of developmental stages of *Toxopneustes roseus* achieved at every time point during the experimental period (30 h) at the three temperature treatments.

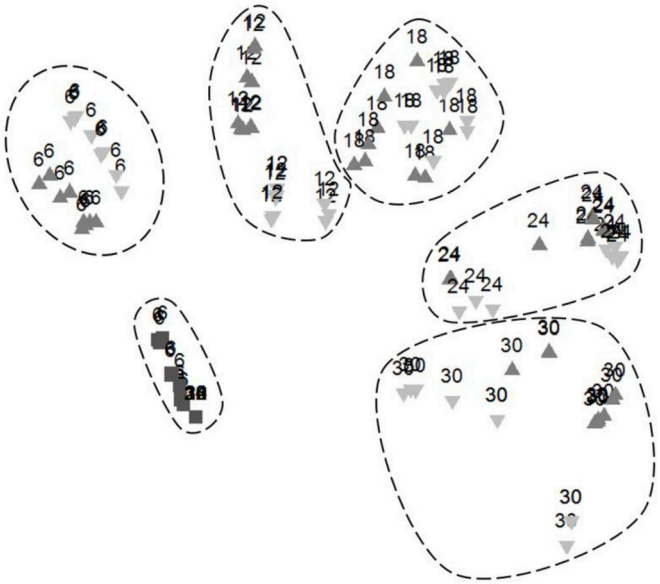

Fig. 5. Ordination constructed by the MDS from a resemblance matrix obtained with the Bray Curtis similarity, applied to the percentage of the different developmental stages sampled from each temperature treatment. Triangles represent those samples of embryos exposed to 28 °C, inverted triangles represent samples from 30 °C, and squares correspond to samples from 32 °C. The numbers refer to the time points (hours) when the samples were taken and the circles of dashed lines are grouping similar time points.

4. Discussion

A temperature of 2 °C above the average temperature (28 °C) of the area where the population studied inhabits, promoted a larger fertilization success, coinciding with the temperature that prevails during the year's warmest periods recorded in the study area (between May and September) when a number of marine invertebrates show a more intense reproductive activity (Benítez-Villalobos and Martínez-García, 2012; Benítez-Villalobos et al. 2013, 2015; Avila-Poveda, 2013; Alejo-Plata and Gómez-Márquez, 2015). In contrast, the temperature of 32 °C

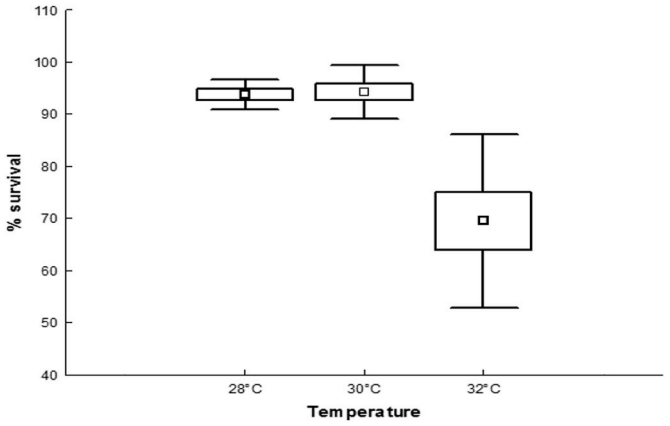

Fig. 6. Percentage of living larvae (average ± SD) of *Toxopneustes roseus* after 24 h of culture, comparing the three temperature treatments (28, 30 and 32 °C).

caused negative effects on the successful fertilization of *T. roseus*. However, the percentage of fertilization success obtained could be considered even relatively high (above 50%), therefore this not-drastic affectation could be owed to the presence of cellular mechanisms such as the accumulation of heat-shock proteins inherited from parents acclimated to warm environments, which provide protection to their gametes (Byrne et al., 2011; Hamdoun and Epel, 2007).

The negative effect that caused the increase in temperature (4 °C above average), can result in the embryos being unable to reach the subsequent stages (phase of skeleton formation) in a warmer ocean, and the few embryos that survive probably have an important metabolic wear and this could be reflected in the survival and development of larvae, as well as in the settlement (Delorme and Sewell, 2013). Byrne et al. (2009, 2011) studied the effects of temperature and pH on the embryonic development of *Heliocidaris erythrograma*, finding that at 4 °C above the average, development is compromised and the effect is lethal at 6 °C above the average. However, the embryos of other echinoderms (*Echinometra mathei*, *Hemicentrotus pulcherrimus*, *Asterias amurensis*, *Holothuria spinifera*) are highly thermotolerant, so it has been proposed that widely distributed species may be able to prevail in a warming ocean by means of dispersion of larvae with genotypes adapted to warmer environments (Foo et al., 2012). In contrast, in the case of *T. roseus* we observed that despite being a species that inhabits the entire tropical eastern Pacific, its embryos tolerate a relatively narrow range of increase in temperature, which could establish a critical point or bottle neck, which could propitiate the drastic diminution or disappearance of the populations. Although there were not specific experiments performed to test the effect of temperature over key developmental stages such as the early gastrulation, when patterns of gene expression change and there occurs the development of the gut and germinal layers, the time of development and features of gastrulae at 28 and 30 °C were similar to other embryos cultured independently at room temperature (27–29 °C) and 2 embryos/ml density (unpublished data). However at 32 °C the development of the embryos was totally abnormal, and only at the 6 h time point 4 and 5.5% of the recorded embryos were early and late blastulae respectively, which eventually underwent an abnormal development as time passed.

Our results show that *T. roseus* probably lives relatively near the upper thermal threshold for successful development, and future climate-driven ocean warming of its habitat could threaten the permanence of the species in the eastern tropical Pacific, or at least lead to contractions or fragmentation of its range limits. We conclude this as the population studied in this work is located in the warmer zone of the distribution range of the species, therefore if sea temperature rises globally, it could produce the disappearance of these populations that are living at the edge of their thermal tolerance, but for other populations located in more temperate latitudes, it could propitiate favorable conditions for fertilization and survival of embryos and larvae. This likely reduction of geographical distribution has been suggested for other temperate and tropical echinoid species (Byrne et al., 2011, Hardy et al. 2014); although it is compulsory to carry out experiments with other populations of *T. roseus* from different latitudes in order to test this hypothesis.

This is the first study on the effects of increase in temperature on larval survival in *T. roseus*, and the positive and negative effects of this factor are shown. Regarding the temperature interval we tested, the average temperature of the habitat (28 °C) and even an increase of 2 °C above that average turned out to be the optimum temperature in the survival of larvae, obtaining a percentage of 81.4 and 94.2% respectively. However, an increase of 4 °C had negative effects, obtaining a survival percentage of 69.5%. When compared to the results obtained in other studies, we found that Sheppard et al. (2010) in Australia reported that an increase of 3 °C above the habitat temperature (24 °C) resulted in faster development and growth in larvae of *Tripneustes gratilla*. Likewise, it was reported that this increase reduced the negative effects of the pH decrease. In their results they also showed a percentage lower than 30% in normal larvae at a temperature of 30 °C, which indicated the proximity to the tolerance limit of the species. It should be mentioned that *T. roseus* is a sea urchin that lives in the subtidal zone, and this probably explains the low thermal tolerance in the offspring, since the parents are not exposed to wide circadian temperature variations like other species that inhabit intertidal zones.

The results of our study provide a view of the probable response of the early stages of development of *T. roseus* to the increase in temperature in the ocean as a consequence of climate change, which could have a negative impact on these stages, with important consequences on the ecosystem. Likewise, it is suggested that embryonic development is the most sensitive developmental stage of *T. roseus* to the increase in temperature, unlike fertilization and larval survival, because our data point to an upper limit of thermal tolerance of 32 °C both for fertilization and for larval survival, while this temperature has a lethal effect on embryonic development. However, it is important to remark that our results regarding the fertilization experiments need to be taken with some caution, since fertilization rates were not examined across a range of sperm concentrations nor were fertilization kinetics examined over time, although the significant differences we found regarding the fertilization percentages achieved at 32 °C compared to those at 28 and 30 °C, evidenced that this increase in temperature has an important effect over the fertilization rates of the species.

It is important to emphasize that more studies are needed both of the effect of this factor (temperature) and others (pH, salinity, etc.) on the early stages of development for this species, as well as in adults. In a number of studies, the negative effect of ocean change stressors, especially warming and acidification has emerged as a significant impact of global change on sea urchin larvae (Hardy and Byrne, 2014; Hardy et al., 2014; Zhan et al., 2016, among others), although in some cases, the adaptive capacity can contribute to the resilience of the species in a changing environment (Foo et al., 2012) or even the effect of ocean warming could mitigate the effect of acidification (Byrne et al., 2013). Therefore, multifactor experiments of the effect of climate change on early development of *T. roseus* will show a more complete panorama of the response of populations of this species to the changing ocean across the eastern tropical Pacific.

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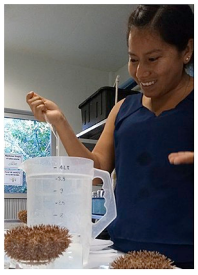

Leobarda Margarita Mejía Gutiérrez: She is a Master's student in Marine Ecology at the Universidad del Mar in Puerto Ángel, Oaxaca, Mexico. Her research project focuses on evaluating the influence of environmental variations on the seasonality of the reproductive patterns of the sea urchin *Toxopneustes roseus*. For the realization of her Bachelor thesis she worked under the direction of Dr. Francisco Benítez-Villalobos in the evaluation of the increase in temperature on the stages of ontogenetic development of *T. roseus*.

Dr. Francisco Benítez Villalobos: He obtained the degree of Doctor of Philosophy (PhD) in 2005 at the University of Southampton, England. His research interests consist of the evaluation of the influence of environmental variations on the seasonality of reproductive patterns and ontogenetic development, mainly of echinoderms, although he has also worked with corals fish and mollusks. He also has contributed to the characterization and evaluation of biodiversity for several populations and communities of echinoderms associated with coral reefs in the Mexican Pacific.

Julia Patricia Díaz Martínez: She is a PhD student in Marine Ecology at the Universidad del Mar in Puerto Angel, Oaxaca, Mexico. Her research project under the direction of Dr. Francisco Benítez-Villalobos and Dr. Eugenio Carpizo-Ituarte focuses on the characterization of the reproductive patterns and the ontogenetic development of the sea urchin *Arbacia stellata*, as well as the evaluation of the effect of temperature increase and acidification of the ocean on the embryonic development. She has collaborated in research projects to evaluate the influence of environmental variations on the seasonality of reproductive patterns and ontogenetic development of echinoderms.