Aquaculture Studies, 24(5), AQUAST1452
http://doi.org/10.4194/AQUAST1452

RESEARCH PAPER

Gamete Viability, Fertilization, and Embryonic Development of Gracious Sea Urchin *Tripneustes gratilla* Under Varying Temperature Ranges

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How to Cite

Rosal, M.S.S., Bitacura, J.G. (2024). Gamete Viability, Fertilization, and Embryonic Development of Gracious Sea Urchin *Tripneustes gratilla* Under Varying Temperature Ranges. *Aquaculture Studies*, *24(5), AQUAST1452*. http://doi.org/10.4194/AQUAST1452

Article History
Received 14 April 2023
Accepted 30 April 2024
First Online 14 May 2024

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Keywords
Echinoderm culture
Climate change
Global warming
Invertebrates
Reproduction

Abstract

The Gracious Sea Urchin *Tripneustes gratilla* is a model organism for invertebrate marine animals and an economically important marine resource. This study investigated the effects of varying temperature ranges 29-30°C, 34-35°C, and 39-40°C on the reproductive capacity of *T. gratilla*. Under these temperature treatments, gamete viability was determined through the resazurin reduction test and quantified through resazurin absorbance at 630 nm, while fertilization and embryonic development were investigated through *in vitro* experiments. With increasing temperature, there was a significant decline in the viability of both sperm and egg cells. Gamete death was highest at 39-40°C followed by 34-35°C temperature ranges. Successful fertilization was highest at 29-30°C (92.26%) followed by 34-35°C (20.83%) and 39-40°C (11.05%) temperature ranges for the two batches. Normal embryonic development from 2 cells up to 2 arm echinopluteus was observed at 29-30 °C while abnormalities including apoptotic body formations, cell fragmentation, cell lysis, and embryo arrest were observed at 34-35°C and 39-40°C temperature ranges. This study confirmed the deleterious effect of increased temperature (34-40°C) on the reproductive potential of *T. gratilla*.

Introduction

The continuous change in the world's climate has been one of the major crises that greatly distress the marine ecosystem (Doney et al., 2012). The alteration in the world's climate due to anthropogenic activities such as combustion of fossil fuels (Höök & Tang, 2013) leading to elevated greenhouse gases is causing the oceans to warm, becoming acidic and more saline (García Molinos et al., 2016). Comiso et al., (2015) reported that the average sea surface temperature (SST) of the Philippines was about 29°C, based on extensive data from the 1980s to the 2000s, but the highest recorded SST from 1981 to 2014 in the Warm Pool Region was 30.1°C in November 2013, the time when Visayas was devastated by Typhoon Haiyan. Moreover, climate change scenarios for 2050 suggest surface temperatures worldwide will likely increase by 1 to 3°C (Weinmann et al., 2013), and up to 6°C by 2100 (Bernstein et al., 2008).

With the ocean temperature increasing, it is predicted that many processes in living organisms, such as reproduction and physiological performance, will be affected (Pörtner, 2008). Reproduction of individuals is vital for stabilizing the population. It is an intricate process that involves different life stages and is controlled by highly balanced mechanisms (Boegner et al., 2013). Consequently, climate change is foreseen to

affect the reproductive performance of many organisms and it could be the main factor threatening population decline in marine organisms (Miller et al., 2015). There is sufficient evidence that temperature surges have resulted in several direct and indirect ecological alterations that include mass community mortalities, shifts in species range, breeding season changes, decreased recruitment, and enhanced establishment of invasive species globally (Bernhardt et al., 2020).

Echinoderms such as sea urchins are extensively utilized in research regarding fertilization and development (O'Donnell et al., 2010; Hernroth et al., 2011; Karelitz et al., 2017; Lenz et al., 2019; Wessel et al., 2021; Limatola et al., 2022). These organisms are ideal model systems because they exhibit simple early development and they can be easily studied in the laboratory (McClay, 2011; Ettensohn, 2017; Adonin et al., 2021). Because of this, several species of sea urchins have been studied for their fertilization and reproduction efficiencies as affected by major physico-chemical parameters such as temperature and pH (Byrne et al., 2013; Delorme & Sewell, 2013; Mejía-Gutiérrez et al., 2019).

The echinoderm *Tripneustes gratilla,* locally known as "swaki", is a high-value sea urchin in the Philippines. This species is an important grazer in the tropical sea grass ecosystem (Klumpp *et al*., 1993) and a preferred source of high-quality roe over other urchins *(*Regalado et al., 2010). Due to its commercial demand, *T. gratilla* has been overharvested in many areas of the country. Hence, several techniques to culture it have been initiated in the past years (Juinio-Meñez et al., 1998). The threat of increasing sea surface temperature and the development of better culture conditions necessitate an investigation of the effect of increased temperatures, as simulated thermal stressors, on the gamete viability, fertilization, and embryonic development of *T. gratilla in* a laboratory setting.

Materials and Methods

Collection of *T. gratilla*

Approximately 90 matured adult life stage of sea urchin *T. gratilla* with a test diameter greater than 65 mm were collected to ensure the presence of male and female urchins (Dworjanyn & Byrne, 2018). Sampling was done in Lawis, Punta, Baybay City, Leyte, Philippines (Figure 1). Matured *T. gratilla* were collected by free diving at the subtidal zones between 5 to 10 m depth (Pecorino et al., 2012). The collection was conducted 2-3 days before the full moon since sea urchins follow lunar rhythms (Pearse & Eernisse, 1982). Collected urchins were immediately transported to the laboratory and were kept maintained in a large bucket with seawater at 29-30°C temperature range, ph 8, and 35 ppt salinity. These urchin samples are the source of gametes for the gamete viability assay and *in vitro* fertilization and embryonic development experiments (Figure 2).

Filtered Seawater and Acid Wash Preparation

All glassware used in the experiments was acid-washed to eradicate contaminants that could negatively affect the study results. In preparing the acid bath (10%), hydrochloric acid (HCl) was diluted with distilled water. Glassware was first washed with soap and water to remove dirt. Then, all the glassware was placed into a container with 6 L of 10% HCl and they were left for 3-4 hours. Afterward, the glassware was washed with running distilled water 2-3 times. They were sterilized in an autoclave at 121°C, 15 psi for 40 min (Tualla & Bitacura, 2016). Furthermore, seawater filtration was accomplished by setting up a filter flask (containing a 47mm Whatman glass GF/A microfiber filter with a pore size of 1.6 µm) which was attached directly to the vacuum pump. The filtered seawater was stored in a sterile acid-washed container.

Gamete Collection

An induced spawning method was used to obtain the gametes of *T. gratilla* based on the method of Tualla and Bitacura (2016). Before the induced spawning process, one molar of potassium chloride (KCl) solution was prepared by dissolving 74.55 g of potassium chloride crystals into one litre of distilled water. A 0.2 ml of 1M KCl was injected inside the sea urchin using a 1 ml syringe with a 0.4 mm x 13 mm needle. The needle was directed over the top of the teeth to inject KCl solution into the body cavity. The sea urchins were shaken gently for a few seconds to mix the KCl solution inside them to allow them to spawn. After less than 5 minutes, the sea urchin released gametes through the pores at the top of their tests or shell. The sex of the sea urchin was determined through the colour of their spawn. Gonads in yellowish to orange indicated females, while whitish gonads were males. Further confirmation of the type of gametes released by the urchins was done by looking at their cellular morphologies under a compound light microscope.

Pure concentrations of gametes were collected to be used for gamete viability assay. It was done by inverting the sea urchins in sterile acid-washed containers. The containers containing pure concentration of gametes were covered with aluminum foil and these were used in the experiments right away. For the gametes to be used in fertilization and embryonic development assays, the sea urchins were placed upside down in a sterile acid-washed beaker filled with filtered seawater, and eggs were collected at the bottom of the beaker. Conversely, dry sperm was collected directly from the genital pore of the male sea urchin. All collected egg and sperm cells were stored at 4-5°C for 3- 4 hrs.

Figure 1. Map of Punta in Baybay City showing the area where the *T. gratilla* samples were collected.

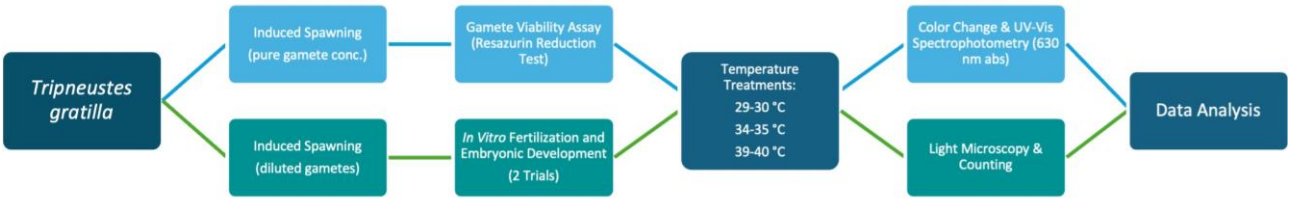

Figure 2. Schematic diagram of the experimental design.

Gamete Dilution

The sperm dilution set-up was prepared by collecting 200 μL of sperm cells from the beaker of stored dry sperm and transferring them into another beaker containing 100 ml filtered seawater which was covered with aluminum foil to serve as the stock sperm suspension. Before the preparation of egg dilution set-up, the removal of jelly coats was initiated following the method by and Tualla and Bitacura (2016). After the release of gametes by the female gracious sea urchin, the spawn was allowed to settle at the bottom. The eggs were then pooled in a 500 ml beaker and settled for 10 minutes. Decanting the overlying seawater removed the jelly coat that naturally covers the egg. Then, the sterile acid-washed beaker containing the eggs was added with 400 ml filtered seawater and it was slowly stirred for a minute. The beaker containing the urchin eggs was rotated by hand at three revolutions per second for a minute. With this process, the jelly covering of the egg was slowly removed. About 10 ml of egg suspension was obtained and added to a beaker containing 100 ml of filtered seawater. The beaker was then covered with aluminum foil to be used in the *in vitro* fertilization assay.

Temperature Treatments

Experimental temperature ranges were set at 29-30°C, 34-35°C, and 39-40°C. The 29-30°C temperature is

based on the average sea surface temperature of the country (Comiso et al., 2015), while 34-35°C and 39-40°C represent the drastic upper thermal stressor as predicted sea surface temperatures in 2100 and 22nd century, respectively (Bernstein et al., 2008; Weinmann et al., 2013). No treatment below the normal temperature range was tested since only the future scenarios for a tropical setting were considered. The fertilization test of the *T. gratilla* was done in a sterile acid-washed 150 ml beaker and it was subjected to three varying temperature range conditions of filtered seawater. Three water baths with filtered seawater at 29-30°C, 34-35°C, and 39-40°C were prepared under 35 ppt and pH 8.0 each (Tualla & Bitacura, 2016).

Gamete Viability Assay

Resazurin Reduction Test (RRT) was employed to test the viability of *T. gratilla* gametes under various treatments. Resazurin dye is widely used as an indicator of cell viability in several tests for evaluating the biocompatibility of dental and medical materials. Mitochondrial enzymes transfer electrons from NADPH + H^+ to resazurin, which becomes resorufin (Borra et al., 2009). Before the assay, resazurin sodium salt solution was prepared first by dissolving 0.10 mg resazurin powder into 20 ml sterile distilled water to obtain 0.5% concentration. The prepared resazurin solution was appropriately sealed to avoid contamination and it was stored at 4°C (Bitacura, 2018).

For temperature treatment of the gametes, fifty microliters (50 μL*)* of concentrated egg or sperm from each suspension were pipetted on the Eppendorf tubes and they were added with filtered seawater in a total reaction of 100 μL per well. The Eppendorf tubes were inserted in tube floaters and placed in water baths with the designated temperatures. The water baths were equilibrated for 30 minutes before the placement of the floaters. After 4 hours, the gametes were transferred in a sterile 96-well microplate and added with 10 μL of the previously prepared resazurin solution. The treatments considered were: Media control (filtered seawater alone), positive control (gametes suspended in filtered seawater treated with 3% hydrogen peroxide [H_2O_2]), negative control (untreated gametes in filtered seawater), and the gametes in filtered seawater treated with varying temperature levels: [T_1 (29-30°C), T_2(34-35°C) and T_3(39-40°C)]. The treatments were done in triplicates.

All microplates were then covered with aluminum foil and kept at room temperature. Colour changes were observed and compared. Reducing the blue resazurin into pink resorufin is an indirect method of determining the gamete viability (Tualla & Bitacura, 2016). For data gathering, the treatments were first compared based on a colour shift from blue to pink. The treatments that remained blue indicated the death of the gametes while those that turned from purple to pink implied the gametes were still alive. Secondly, quantitative analysis was done by measuring the treatment absorbance at 630 nm, which quantified the amount of resazurin present in the treatment wells.

In vitro* Fertilization of *T. gratilla

The fertilization experiment was done using the methods of Tualla and Bitacura, (2016) with modifications. To start fertilization, 1.8 mL of sperm suspension was seeded to the 90 mL treatment solution previously added with 9 mL of egg suspension. The solutions were mixed slowly, after which the beakers containing the solutions were placed in water baths with filtered seawater at 29-30°C, 34-35°C, and 39-40°C under 35 ppt and pH 8.0 each. To differentiate the fertilization success of *T. gratilla* under the varying treatments, three 20 μL aliquots were collected from each of the three replicate treatments after 30 min and they were viewed under a compound light microscope. The numbers of fertilized, unfertilized, and deformed eggs were counted. The presence of a fertilisation membrane indicated the presence of a fertilized egg.

From the data gathered, the percent successful fertilization under various temperature ranges was computed using equation 1. Additionally, percent fertilization inhibition and embryo deformity were calculated using equations 2 and 3, respectively.

$$\text{Percent Successful Fertilization} = \frac{\text{no. of fertilized eggs}}{\text{total no. of eggs}} \times 100 \quad (1)$$

$$\text{Percent Inhibition} = \frac{\text{no. of unfertilized eggs}}{\text{total no. of eggs}} \times 100 \quad (2)$$

$$\text{Percent Deformity} = \frac{\text{no. of deformed eggs}}{\text{total no. of eggs}} \times 100 \quad (3)$$

Where:

Total no. of eggs = No. of fertilized eggs + No. of unfertilized eggs + No. of deformed eggs

Observation of Embryonic Development of *T. gratilla*

The protocol described by Tualla and Bitacura (2016) was utilized to observe embryonic development. Observations were made every 30 min, 3, 6, 12 and 24 hours after adding the sperm cells into the beakers without aeration. For every observation time, three 20 μL aliquots from each treatment were mounted into a depression slide and they were observed for the occurrence of the following: (EPC) embryos with pigmented cells; (SCD) successful cell division on each cell stage; (DD) delayed development; (NFD) no further development and (DE) deformed embryos.

Data Analysis

Gamete viability assay was laid out in a completely randomized design (CRD) and a randomized complete block design (RCBD) for the fertilization test. The significant differences among the treatments for the

gamete viability test were determined using Analysis of Variance (ANOVA). Moreover, a post hoc comparison of treatment means was done using a homogenous subset of the Duncan Multiple Range Test. All the analyses were carried out using SPSS v.22

Results

Gamete Viability of *T. gratilla* Under Varying Temperature Ranges

The average absorbance at 630 nm of the wells containing the temperature-treated gametes is shown in Figure 3a. On the other hand, Figures 3b and 3c show the Resazurin Reduction Test results for the adverse effects of increased temperatures in *T. gratilla* gametes, displaying colour shifts between sperm and egg cells. There was a significant difference ($P<0.05$) in the absorbance values among the treatments at 630 nm, the peak absorbance of resazurin. The highest absorbance was recorded for the gametes under 39-40°C temperature treatment, followed by the gametes under 34-35°C and the positive control, and the least are the gametes under 34-35°C and negative control (Figure 3). As seen in treatment wells, no colour changes (blue) were observed in the gametes exposed to 34-35°C, 39-40°C, and positive control indicating gamete mortalities under these treatments. Conversely, transformations in colour from blue to pink were observed in gametes subjected to 29-30°C and negative control, indicating the viability of the gametes under these treatments. Not shown in the figure is media control since it has no gametes present, therefore obtaining different absorbance values.

Fertilization of *T. gratilla* Under Varying Temperature Ranges

Figure 4 shows that the percent successful fertilization, inhibition, and deformity of *T. gratilla* under increasing temperature for batch one (Figure 4a) and two (Figure 4b) are significantly different ($P<0.05$). For batch one, gametes subjected to 34-35°C and 39-40°C had ~5 and ~8-fold lower percentages of successful fertilization, respectively, compared to the 29-30°C temperature treatment. For batch two, gametes exposed to 34-35°C and 39-40°C had ~4-fold and ~8-fold lower percentages of successful fertilization, respectively, compared the 29-30°C temperature level. Both batches with temperature treatment levels of 29-30°C and 39-40°C displayed a decreasing trend in percent successful fertilization and an increasing trend in deformed eggs. It further showed that successful fertilization occurred at the lowest temperature range set. The formation of the fertilisation envelope indicated successful fertilization. Gametes exposed to 34-35°C and 39-40°C had significantly lower ($P<0.05$) percentages of successful fertilization than the 29-30°C temperature range.

Embryonic Development of *T. gratilla* Under Varying Temperature Ranges

The overall observations of the embryos treated at various temperatures are summarized in Table 1. The results showed that the temperature range 34-35℃ and 39-40℃ revealed no further development starting at the 2-cell stage. The increased temperatures halted the embryonic development of *T. gratilla* at the 2-cell stage, leaving the embryos exposed to 29-30℃ temperature range to develop into a normal echinopluteus.

Figures 5a to 5e show the normal development of embryos exposed to 29-30℃. Normal development of the embryos was observed from the 2 to 4-cell stage up to the formation of 2-armed echinopluteus. Moreover, the embryos exposed to the other two warmer temperatures were malformed throughout the experiment, as shown in Figures 5f to 5o. The two highest temperature levels also displayed deformities such as cell fragmentation, formation of apoptotic bodies, blebbing, cytolysis, and uneven cell division (Figures 5f – 5o).

Discussion

Gamete Viability of *T. gratilla* Under Varying Temperature Ranges

The result of the Resazurin Reduction Test conducted confirmed the viability of the cells at 29-30°C since they successfully converted resazurin to resorufin. This temperature range is the upper thermal limit for gamete viability based on the compared temperature ranges. Furthermore, these reactions were linked to the temperature treatments since no colour changes were detected on the control media (not shown), which indicated the filtered seawater used presented zero cells that could convert resazurin to resorufin. On the other hand, the sperm and egg cells that were exposed to 34-35°C and 39-40°C temperature ranges showed no colour changes same as the positive (cytotoxic) control. This indicates the deleterious effects of the increased temperature levels on *T. gratilla* gametes.

Measuring the absorbance at 630 nm quantified the amount of resazurin present in the treatment wells. The result suggests that the more viable cells are, the more they can reduce blue resazurin to pink resorufin, resulting in a low absorbance at 630 nm. Cells exposed to a lethal treatment condition have a poorer ability to convert resazurin to resorufin, resulting in a higher absorbance at 630 nm due to cell mortality. As a result, it is assumed that the highest absorbance will be obtained during treatments in which cells are still in the apoptotic phase of cell death. After which, the absorbance will begin to decline as cells enter the necrotic phase as suspected by Tualla and Bitacura (2016). In this study, the cell necrosis is most probably due to the higher levels of the temperature treatments. This is especially true in the case of the sperm cells. A

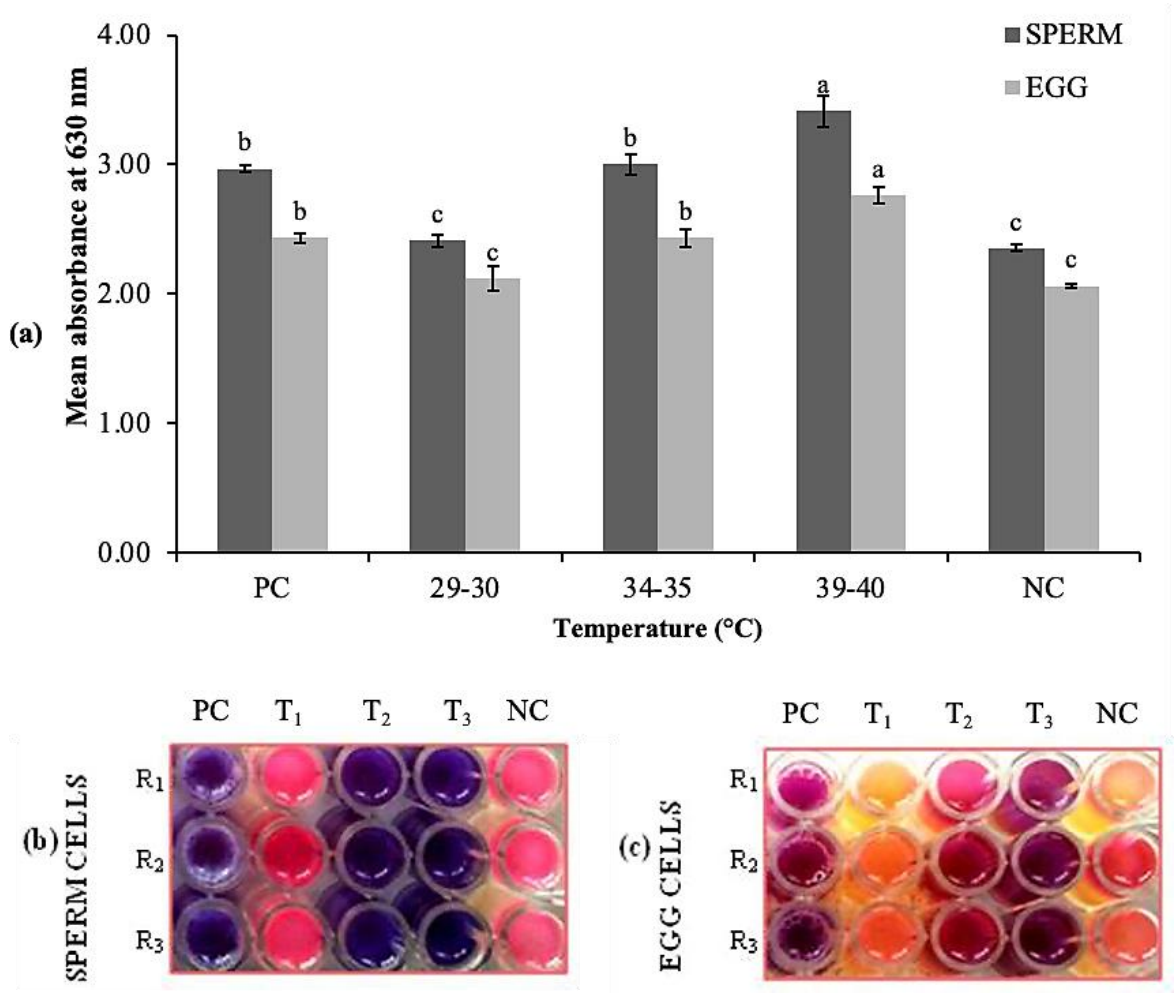

Figure 3. (a) Mean absorbance (±SE) at 630 nm of the wells containing *T. gratilla* gametes at different temperatures after 30 min of incubation with resazurin. (b) Results of Resazurin Reduction test performed on *T. gratilla* sperm cells subjected to varying temperatures and (c) egg cells exposed to the same temperature levels. Letters on the graph correspond to the post hoc comparison of means at 0.05 level of significance between temperature treatment mean absorbance at 630 nm for each experiment on sperm and egg viability. Abbreviations: PC (positive control), Temperature treatments: T_1 (29-30°C), T_2 (34-35°C), T_3 (39-40°C), and NC (negative control). R1–R3 are the replicate well.

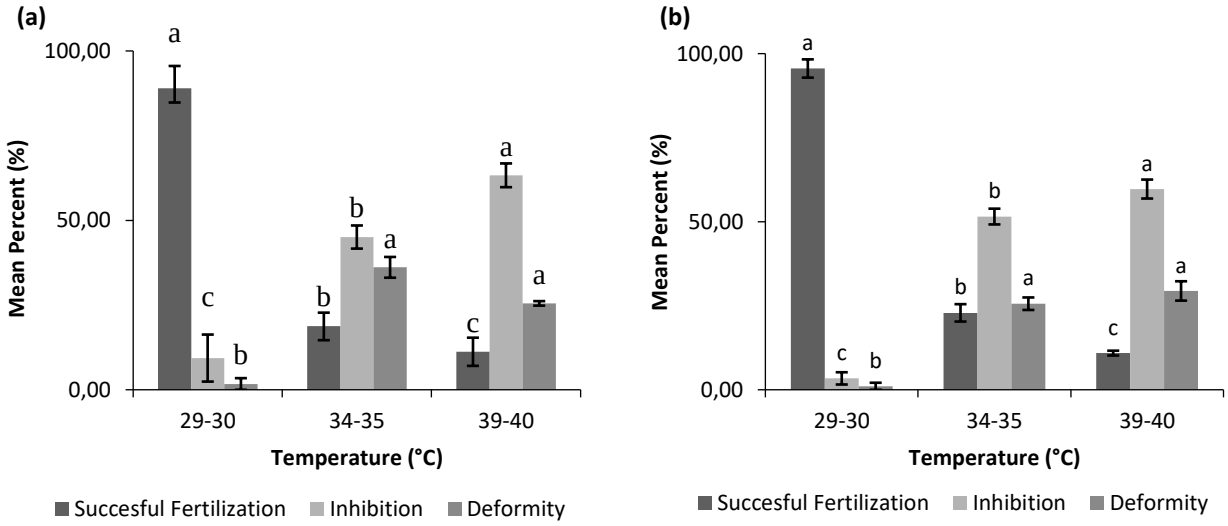

Figure 4. The mean percent (±SE) [(a) batch 1 & (b) batch 2] successful fertilization, inhibition, and deformity of the gametes exposed to varying temperature treatments after 30 min. Letters on the graph correspond to the post hoc comparison at 0.05 level of significance between temperature treatment mean percentage of successful fertilization, inhibition, and deformity.

Table 1. The morphology of embryos of *T. gratilla* exposed to different temperature ranges for 3, 6, 9, 12, and 24 h

Duration (hr)	Temperature (°C)		
	29-30	34 -35	39 -40
3			
2cells	SCD	NFD, DE	NFD, DE
4 cells	SCD	NFD, DE	NFD, DE
6			
8 to 16 cells	SCD	NFD, DE	NFD, DE
9			
32 to 64 cells	SCD	NFD, DE	NFD, DE
12			
Blastula	SCD	NFD, DE	NFD, DE
24			
Gastrula	SCD	NFD, DE	NFD, DE
2 -arm Echinopluteus	SCD	NFD, DE	NFD, DE
General observation	No abnormality	Blebbing embryos	Cell fragmentation

SCD = Successful Cell Division, NFD = No Further Development, DE = Deformed Embryos

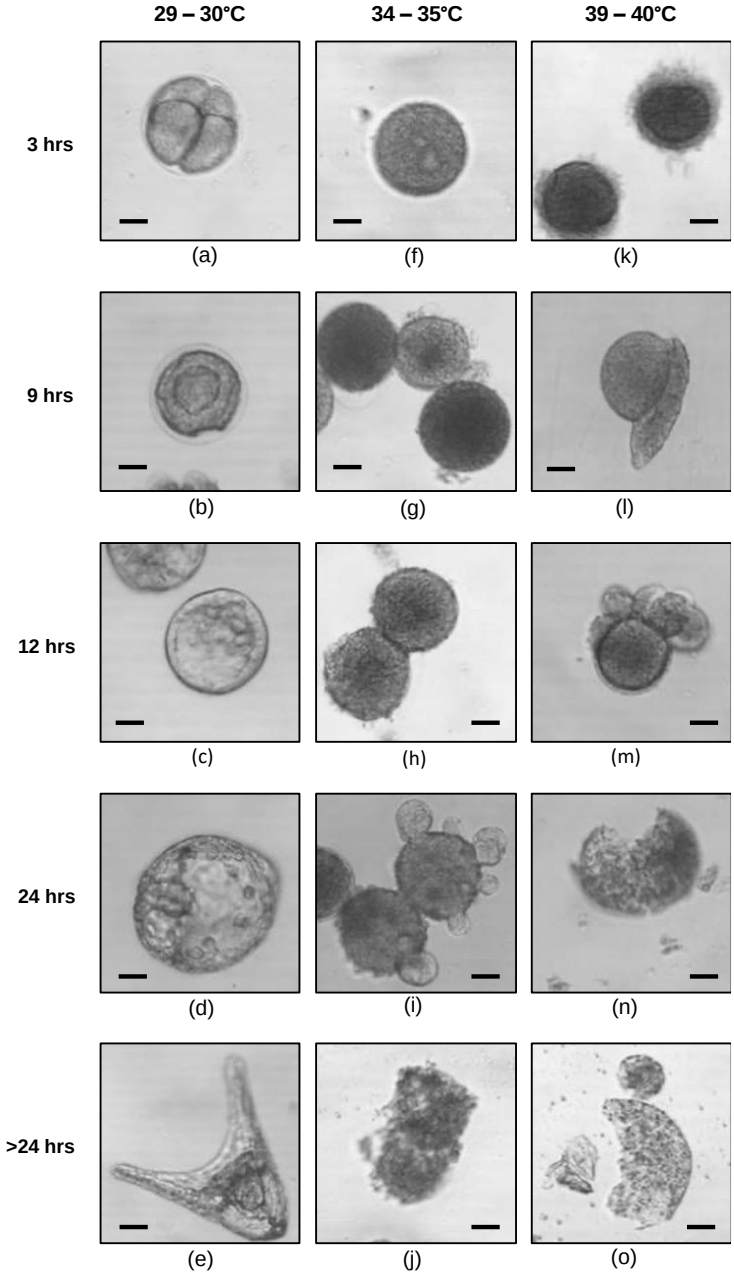

Figure 5. Embryonic development of *T. gratilla* at various thermal treatments (scale bars: 30 µm). (a–e) Normal development was observed from 2 cells up to 2-arm echinopluteus at 29-30 °C, and abnormalities in the embryos were observed under (f – j) 34-35°C and (k–o) 39-40°C.

slight difference in absorbance reading between the egg and sperm cells is attributed to the yolk giving the eggs a yellowish color. The peak absorbance at 630 nm implied the most extreme range level that induced apoptosis on the cell (Tualla & Bitacura, 2016).

Fertilization and Embryonic Development of *T. Gratilla* Under Varying Temperature Ranges

The results of the percent successful fertilization, inhibition, and deformity of *T. gratilla* under increasing temperature imply that *T. gratilla* has its upper temperature limits of around 29-30°C. Beyond this temperature, fertilization of *T. gratilla* will be arrested. Results also indicate that the zygote can hatch within 29-30°C temperature range. Embryos may hatch more slowly or could not cleave at all at temperatures above 29-30°C, possibly due to the lack of catalysis by enzymes required to disintegrate egg membranes during hatching (Ishida, 1936).

Rahman *et al.* (2009) indicated that *T. gratilla* zygotes could withstand a broad temperature level of 13–34°C. In their laboratory experiments, all zygotes had irregular cleavage at extremely low temperatures (13°C), whereas no cleavage occurred at extremely high temperature (34°C); these temperatures (13°C and 34°C) do not occur during the spawning season of this species (Rahman et al., 2009). This study showed arrested embryonic development of *T. gratilla* beyond 29-30°C while *Rahman et al*. (2009) showed that *T. gratilla* larvae developed abnormal morphology at lower temperature levels (14–19°C) in different developmental stages including hatching blastula, blastula, and prism.

According to Voronina and Wessel (2001), abnormal morphology such as blebbing or protrusions of the cell membrane observed in the sea urchin embryos is caused by apoptosis, resulting in morphological aberrations, chromatin degradation, and activation of caspases. Additionally, Sasaki and Chiba (2001) noted that fragmentation is produced by the rearrangement of F-actin, which governs the membrane and embryo fragmentation via extrinsic or intrinsic processes. They discussed that the death of cells employing a death receptor is demonstrated in an extrinsic pathway wherein during egg cell death the caspase-3 proteases regulate the death of the sea stars' eggs. Additionally, apoptosis of cells follows the intrinsic pathway, also called the mitochondrial pathway.

The examination of synchronized or asynchronized larval development is another way to understand the nature of the sea urchin development (Rahman et al., 2007). They reported that embryos of *T. gratilla* developed synchronously from 25 to 29℃. The same with the study of Li et al. (2021) and Molle et al. (2022) who used 25 and ~26℃, respectively, for their fertilization and embryonic development experiments. Rahman et al. (2009) further reported that at 22℃, only a few larvae displayed asynchronous development in which all larvae had normal morphology. It was also discovered that after a temperature shift from 25℃ to 22 or 30℃, the gastrula larvae can progress into competent larvae without any signs of abnormalities. Still, the mortality rate was significantly higher at the above temperatures. The results are also in consonance with the findings of Sheppard Brennand et al. (2010). They reported that increased temperature (+3°C) stimulated the growth of *T. gratilla*, producing significantly bigger larvae in all their treatments up to a thermal threshold of +6°C. This finding reveals the temperature tolerance range for this species' larval development occurring during its natural habitat throughout the year. According to Fujisawa and Shigei (1990), the spawning season and embryonic temperature sensitivity of sea urchins are correlated. Fujisawa (1989) reported that the distribution of three sea urchin species, *Hemicentrotuspulcherrimus*, *Anthocidariscrassispina*, and *Hemicentrotusdepressus,* are determined by the embryo's temperature tolerance.

According to Rahman *et al*. (2009), the most favourable temperature conditions for development of *T. gratilla* are within the intermediate temperatures (about 19–29℃), which corresponds to the species' habitat temperature range. They discussed that both larvae and adults can survive at higher temperatures for short periods, indicating that they have adapted to tropical shallow-water environments. They further discussed that due to the water circulation from the open sea, habitat temperatures usually do not reach potentially damaging levels for this species. However, this is not the case for this study, the study's collection site is in Camotes Sea which is found in between Cebu and Leyte Islands and not directly exposed to the Pacific Ocean. Thus, the results of this study agree more with the findings of Parvez et al. (2018). They observed that the critical lower and higher temperatures for the embryonic development of *T. gratilla* were 16 and 34℃, respectively.

Reproductive responses of other sea urchin species were also reported to be negatively affected by increased temperature levels. For instance, a study by Delorme and Sewell (2013) indicated that increased seawater temperature (15-21°C) caused an increase in the development rate of New Zealand sea urchin, *Evechinus chloroticus*. However, beyond 21°C, its abnormal development reached ~ 30%. Similarly, Byrne et al. (2013), reported that warming-acidification treatments decreased the percentage of larval development of the sea urchin *Heliocidaris tuberculate.* Furthermore, Mejía-Gutiérrez et al. (2019) revealed that the sea urchin *Toxopneustes roseus* is significantly affected by elevated temperature in terms of its success of fertilization, development of embryos, and survival of larvae.

Conclusion

Results of this study revealed that both sperm and egg cells of *T. gratilla* become nonviable at 34-40°C. The percent successful fertilization in *T. gratilla* is highest at 29-30°C. Temperature range and subsequently favored embryonic development up to 2-arm echinopluteus larva. Finally, an increased temperature range at 34-40°C induced the malformation of embryos, cell fragmentation, and cell lysis and eventually arrested embryonic development of *T. gratilla*. This study showed that the reproductive capacity of the sea urchin *T. gratilla* is highly vulnerable to increased temperature.

Ethical Statement

Not Appicable.

Funding Information

The authors received no funding for this work.

Author Contribution

MSSR: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – Original Draft Preparation; JGB: Conceptualization, Methodology, Supervision, Validation, Writing – Review & Editing

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Asst. Prof. Ris Menoel R. Modina and Prof. Dr. Analyn M. Mazo for their valuable inputs to the study.

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