



Combined Effects of Temperature and Salinity on Hatching and Larval Survival of Commercially Important Tropical Sea urchin, *Tripneustes gratilla* (Linnaeus, 1758)

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Authors' contributions

The work was carried out in collaboration among all authors. All authors read, reviewed and approved the final manuscript.

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ABSTRACT

The combined effects of temperature and salinity on percent hatching, normal larval rate at hatching, and survival of fasting larvae after hatching (survival activity index; SAI) of the commercial species of collector sea urchin, *Tripneustes gratilla* were investigated in a captive laboratory condition. The study was conducted by setting different levels of temperatures (24°C to 36°C) and

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salinities (38‰ to 23‰). Within the range of temperature from 24 to 36°C and at 32‰ salinity, hatching and normal larval rates, and SAI values were highest at 24 and 27°C. The highest hatching and normal larval rates were found at 35 and 38‰ within the salinity range of 23-38‰; however, SAI value was the highest at 26‰. The results of the experiments in each level of temperature (24, 27 and 30°C) with each salinity (32, 35 and 38‰) indicated interactive effects of temperature and salinity, and within the experimental protocols of 24°C at 38‰ gave an optimal combination for highest hatching and survival of *T. gratilla*. The findings obtained from the present research would not only be immensely helpful towards the understanding of the suitable temperature-salinity interactions but also facilitate the development of captive breeding, larval raising and mass seed production of this high-valued sea urchin for commercial aquaculture.

Keywords: *T. gratilla*; temperature; salinity; hatching rate; normal larval rate; survival activity index.

1. INTRODUCTION

Among the environmental factors, seawater temperature and salinity are the critical abiotic factors that have the greatest effect on the whole life history of fishes [1,2] and echinoderm sea urchins (see the references below). In particular, at embryonic and early larval stages, water temperature and salinity independently and/or interactively affect survival by influencing the physiological states [3,4]. Among the abiotic factors, temperature and salinity are considered as the most vital factors in embryonic development of purple sea urchin, *Paracentrus lividus* [5]. Besides that, several former studies showed that the salinity has significant effects on survival, embryonic and larval development of sea urchins [6,7,8]. Kashenko [9] stated that increasing salinities have affected the time needed for embryonic development of *Echinocardium cordatum* in the same temperature. Metaxas [7] observed that decreasing salinities slowed larval development of *Echinometra lucunter*. Allen and Pechenik [10] reported that fertilization envelope of eggs rarely rises and even successfully fertilized eggs do not cleave after presenting to low salinity seawater. In regards of larvae, the range of salinity tolerance can be broader or narrower than their adults. The larvae of Atlantic sea urchin (*Echinometra lucunter*) are more sensitive to salinities and can tolerate narrower ranges of salinities than those of their adults [7]. The low salinity condition reduces feeding rate, decreases growth performance and consequently limits the size of ectoderms [9]. Decreasing salinity caused the reduction of viability and also yielded mass mortality of adult sea urchin, *Lytechinus variegatus* at Florida [11].

Early life stages of broadcast-spawning marine invertebrates from the phylum Echinodermata has been used to assess the effects of increasing surface temperature on marine biota

[12,13]. Over the past century, the fertilization of eggs of echinoderms was well categorized due to use in comparative embryological research [14]. Early life stages of echinoderms, especially sea urchins are also recognized to be highly sensitive to a wide range of environmental contaminants and stressors [15], and thus making them as an ideal state for assessing impacts of climate-change. In addition, the egg fertilization and larval development of sea urchin have previously been shown only to happen within distinct temperature ranges for some species [16,17]. So, the ocean warming largely affects these life stages.

The heart-shaped irregular sea urchin, *Echinocardium cordatum* (Pennant) occurs in temperate latitudes of the Pacific and the Atlantic [18,19] and also the Peter Great Bay in Russian waters [20]. According to Kashenko [21], the lower limit of salinity tolerance of the adult heart-shaped sea urchin was 28‰, which corresponded to its regular habitat in such a depth of the marine ecosystem. The animals those were placed on firm substrate without having any opportunity to burrow, could even survive for 3 days within the salinity levels of 28–33‰, but all of them were dead at the end of the 8th day, however, upon a salinity drop to 20‰ or lower, all of them were dead within a day.

From the fertilization to formation of the pluteus larval stage, the early development of heart-shaped sea urchin has been described in detail, however, the times needed for reaching different developmental stages varied to some extent according to the studies of different authors [22]. The sequence of the developmental events of the sea urchin, inhabiting the Vostok Bay in the Sea of Japan, was determined by Kashenko [23]. In discrepancy to the bottom-dwelling adult sea urchin, the early development occurs in the water column. During the monsoon weather, the suddenly fluctuating temperature and salinity of

sea water could terribly impact on the larvae of marine invertebrates.

To determine the survival rates and development of larvae of marine benthic invertebrates, the importance of temperature and salinity have not been completely characterized. Several previous studies have done on different invertebrate taxa and found that deviations in temperature and salinity from ambient values had caused increased mortality and/or delayed development (e.g. barnacles: [24,25]; bivalves: [26,27]; echinoderms: [6]; polychaetes: [28,29]), while others observed that salinity is not an important factor in determining larval survival [30-32].

In addition, data on environmental control for the improvement of the hatching rate and larval development will potentially be applicable to high density intensive egg management systems for mass larval production. Therefore, our study aimed to contribute information on the suitable egg incubation and early larval rearing environment for the development of sea urchin aquaculture technology as well as to obtain appropriate information on sea urchin, *T. gratilla* early life history. This study examined the rates of hatching and normal larval development, and the survival activity index (SAI) of *T. gratilla* larvae by rearing under various temperatures and salinities after the blastula stage. SAI values can be used as a practical indicator to evaluate

the larval tolerance to varying environmental conditions [33,34].

2. MATERIALS AND METHODS

2.1 Sample Collection and Conditioning

Around 56 matured adults of *T. gratilla*, weighing from 165 to 256 g in live weight and 84 to 122 mm in test diameter, were collected from Bum Bum Island (5°66'N, 100°28'E), Semporna, Sabah, Eastern Malaysia (Fig. 1) at low tide during their natural breeding season from January to May, 2016. The specimens were then transferred with aerated plastic bucket to the laboratory of the Institute of Bioscience, Universiti Putra Malaysia (UPM), where they were maintained in an outdoor tank with flow-through seawater and fed with a diet of brown macroalgae (*Sargassum* sp.).

2.2 Spawning and Fertilization

Most of the urchins were used for this experiment within a week after collection. The Aristotle's lantern was removed from the healthy specimens by using scissors and forceps, and then rinsed thoroughly with sterilized filtered sea water (SFSW), which was first prepared on Advantec qualitative filter paper (Toyo Roshi Kaisha, Japan) and then autoclaved 10 minutes for sterilization. Gametes were obtained from each



Fig. 1. Sampling area of *T. gratilla* in Pulau Bum Bum (Bum Bum Island), Semporna, Sabah, Eastern Malaysia

sea urchin after the injection with 2-3 ml of 0.5M KCl solution into the coelomic cavity. Eggs were collected by inverting the gravid female urchin on a glass beaker filled with SFSW. "Dry" sperm (in the most concentrated form of sperm, released at the time of spawning using the above KCl method) were then pipetted off the genital pores and kept in a refrigerator at 4-5°C for not more than 3-4 hours, while the eggs were placed in a glass beaker containing SFSW and maintained at normal room temperature (28-30°C).

Fertilization was done by adding two drops of diluted sperm into a petri dish containing 15 mL egg suspension (300 eggs/mL). Sperm concentration was maintained at 10^{-5} dilution of "Dry" sperm [35,36]. The sperm was kept with eggs for 5-10 minutes and then sperm in excess was cleaned by 3-4 successive washes with SFSW [35,36]. Three replicate fertilization experiments were performed using fresh gametes from new specimens in each time.

2.3 Experimental Procedure

In this experiment, near about one thousand fertilized eggs from the incubation containers, were transferred to 1 L beakers filled with 800 mL of filtered seawater and maintained at the experimental temperatures and salinities. In regards of the single factor experiment studying the effect of temperature, treatments were adjusted to 24, 27, 30, 33, and 36°C with a salinity level of 32‰ (approximately equal to the spawning salinity at the Laboratory of Marine Biotechnology, Institute of Bioscience, UPM). For salinity trial, the treatments were adjusted to 23, 26, 29, 32, 35 and 38‰ with a temperature of 30°C (approximately equal to the spawning temperature at the same laboratory). In respect of the combined two-factor treatment with water temperature and salinity, three different temperatures of 24, 27 and 30°C were combined with three different salinities of 32, 35 and 38‰ for incubation. The experimental treatments were conducted in three biological replicates, corresponding to the three different spawning trials. All single and two-factor experiments were carried out with three replicates for each. Water was not changed and aeration was not provided in beakers during the experiment. Each experimental temperature was maintained in a water bath using heaters (Seapalex300, Nisso, Japan) and chillers (DSHP-4-WC, Aqua Logic Inc., USA). Each salinity trial was maintained by using mixtures of artificial sea salt powder (Sea life, Marine Tech Co. Ltd, Japan) and

groundwater (salinity level $\leq 0.2\%$). The dissolved oxygen contents in the treated water were measured at the start of egg stocking and after removing all dead larvae; the values (mean \pm SD; $n = 3$) for these stages were $83.76 \pm 4.20\%$ and $83.10 \pm 1.50\%$, respectively.

2.4 Data Calculation

In regards to the estimation of larval survival activity index (SAI), all the surviving gastrula larvae after hatching were used to obtain the SAI value in the same beakers of the incubation trials. From one day after hatching (DAH), all the dead larvae and larvae with morphological abnormalities were carefully removed daily from each beaker using a pipette and their number was counted. The hatching rate, normal larval rate, and SAI were calculated by the following equations [37]:

$$\text{Hatching rate (\%)} = \frac{N}{N+UE} \times 100 \quad (\text{i})$$

$$\text{Normal larval rate (\%)} = \frac{N-M}{N+UE} \times 100 \quad (\text{ii})$$

$$\text{SAI} = \frac{\sum_{i=1}^k (N-h_i) X_i}{N} \quad (\text{iii})$$

Where,

- N = Total number of larvae,
- UE = The number of unhatched eggs at 14 h after the start of hatching,
- M = The number of morphologically abnormal larvae,
- h_i = The accumulated mortality by the i -th day and
- k = The number of days elapsed until total larval mortality under fasting conditions.

2.5 Statistical Analyses

The percentage data are presented as mean \pm SD ($N = 3$) in the figures and tables. For statistical analysis, all percentage data from each experiment were arcsine transformed [36]. Hatching rates, normal larval rates, and SAI values obtained from the single factor experiments were statistically analyzed by one-way analysis of variance (ANOVA), while the values from the two-factor experiment were analyzed, using a two-way ANOVA. Where significant differences ($p < 0.05$) were found using Levene's test for homogeneity of variance in any experiment by one-or two-way ANOVA, the values of one-factor experiments were tested

post hoc by the Tukey test ($p < 0.05$). If a significant interaction ($p < 0.05$) between temperature and salinity by two-way ANOVA was observed, the simple main effect in each factor was then analyzed to determine the individual mean differences by the Tukey test ($p < 0.05$). All statistical analyses were carried out using the computerized SPSS version 20 (IBM SPSS Inc., Chicago, USA) for Windows 10.

3. RESULTS

In the single factor experimental treatment with temperatures (conducted in the laboratory at 32‰ salinity), the significantly highest ($p < 0.05$) rates of hatching, normal larval development and SAI of *T. gratilla* were obtained at 24°C and 27°C than those at the spawning temperature (30°C), while the values were the lowest at 36°C (Table 1).

While examining the effects of salinity in the single factor experiment (conducted at 30°C temperature), salinities 35‰ and 38‰, among the treatments, exhibited the significantly highest ($p < 0.05$) hatching success of 84.97±4.07% and 96.39±2.68% and the normal larval survival of

78.91±3.78% and 92.97±2.59%, respectively. However, the hatching and normal larval rates of 46.44-58.85%, and 10.68-43.9% at the salinity levels of 23-32‰, respectively were considerably low compared to the above values (Table 2). Furthermore, the SAI value was significantly higher ($p < 0.05$) at 26‰ (17.28±0.50%) than other salinities tested (≤11.70±0.68%).

In the two-factor experiment, the temperatures (24, 27, and 30°C) and salinities (32, 35, and 38‰) that showed higher hatching and normal larval rates in the single factor experiment, were selected for further study. In this trial, the highest rates of hatching (100.0±0.0%) and normal larval development (99.7±0.5%) were observed at 24°C and 38‰, among the combinations of the two factors evaluated (i.e. temperature and salinity) (Figs. 2 and 3).

Hatching began within 6 h post-fertilization in all water temperatures where >95% of eggs were hatched by 18 h at 27°C and 30°C, while >95% of the eggs hatched by 24 h at 24°C (Fig. 4). A significant interaction ($p < 0.05$) was observed between water temperature and salinity on the hatching of *T. gratilla* (Fig. 4).

Table 1. Hatching rate, normal larval rate (NLR), and survival activity index (SAI) of the tropical sea urchin (*T. gratilla*) at different experimental temperatures in the laboratory at 32‰ salinity

Water temperature	Hatching rate (HR %)	Normal larval rate (NLR %)	Survival activity index (SAI)
36°C	18.92±6.25 ^a (13.50-25.75)	1.95±0.64 ^a (1.39-2.65)	1.38±0.45 ^a (0.98-1.87)
33°C	43.17±8.13 ^b (35.5-51.75)	29.00±5.48 ^b (23.85-34.77)	10.20±1.93 ^b (8.38-9.98)
30°C	76.86±8.73 ^c (69.55-86.52)	69.98±7.94 ^c (63.33-78.78)	11.80±1.34 ^b (10.68-13.28)
27°C	96.06±2.95 ^d (92.75-98.55)	89.15±2.78 ^d (86.08-91.44)	19.31±0.60 ^c (18.65-19.81)
24°C	98.80±1.23 ^d (97.55-100)	96.03±1.19 ^d (95.2-97.2)	23.41±0.29 ^d (23.2-23.7)

All values represent mean ± SD with ranges in parentheses. Mean values in the same column with different superscripts are significantly different ($p < 0.05$)

Table 2. Hatching rate, normal larval rate (NLR), and survival activity index (SAI) of the tropical sea urchin (*T. gratilla*) under different salinities in the laboratory at 30°C temperature

Salinity	Hatching rate (HR %)	Normal larval rate (NLR %)	Survival activity index (SAI)
38‰	96.39±2.68 ^d (93.55-98.88)	92.97±2.59 ^f (90.22-95.36)	8.57±0.23 ^b (8.31-8.79)
35‰	84.97±4.07 ^c (80.54-88.55)	78.91±3.78 ^e (74.79-82.23)	9.80±0.47 ^c (9.34-10.28)
32‰	58.34±2.47 ^b (55.65-58.85)	42.31±1.79 ^d (40.36-43.90)	5.55±0.23 ^a (5.29-5.79)
29‰	49.20±2.88 ^a (46.52-48.48)	34.87±2.04 ^c (32.96-37.02)	11.70±0.68 ^d (11.05-12.41)
26‰	44.68±1.96 ^a (44.55-46.65)	20.92±0.92 ^b (20.86-21.84)	17.28±0.50 ^e (16.92-17.72)
23‰	49.23±3.09 ^a (46.44-52.56)	11.36±0.70 ^a (10.68-12.09)	8.97±0.56 ^b (8.46-9.57)

All values represent mean ± SD with ranges in parentheses. Mean values in the same column with different superscripts are significantly different ($p < 0.05$)

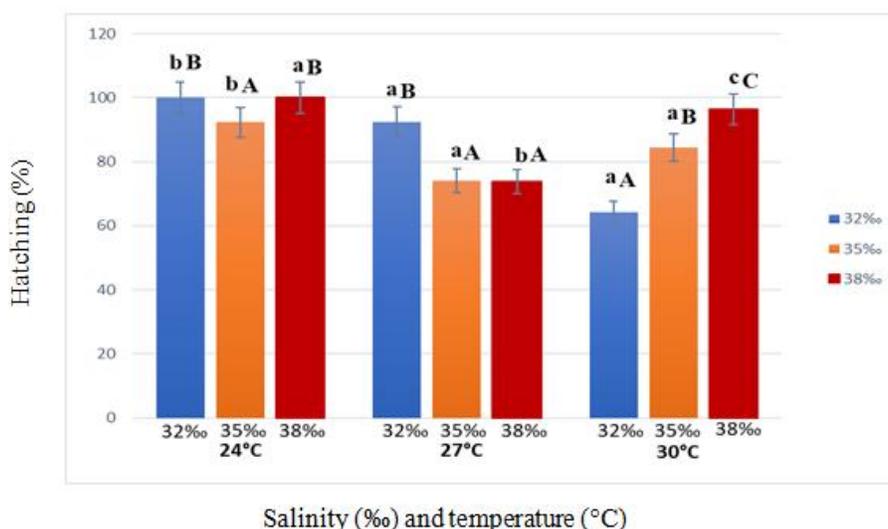


Fig. 2. Combined effects of temperature and salinity on the mean hatching (%) of *T. gratilla*. The bar on each diagram indicates standard deviation (\pm SD); n=3. The letters a, b and c show significant differences within the same temperature ($p < 0.05$), while A, B, C indicate significant differences among temperatures within the same salinity ($p < 0.05$)

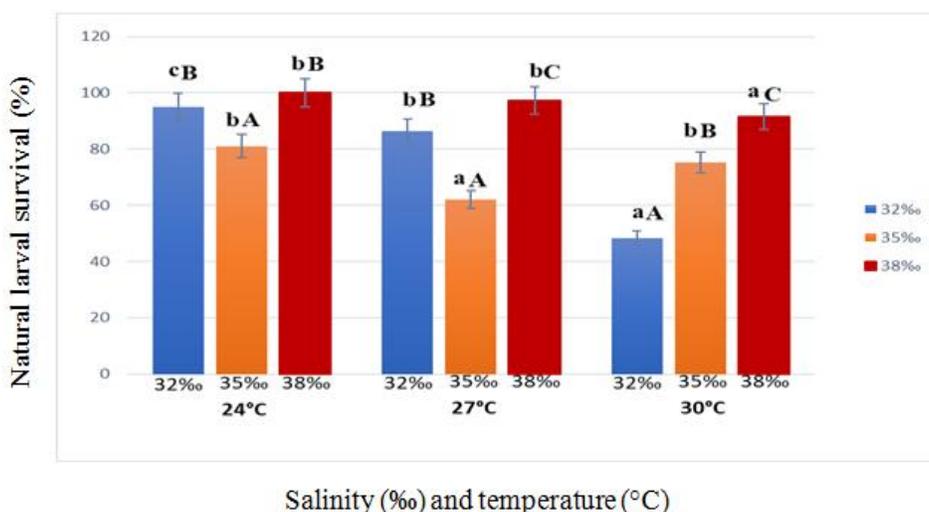


Fig. 3. Combined effects of temperature and salinity on the mean natural larval survival (%) of *T. gratilla*. The bar on each diagram indicates standard deviation (\pm SD); n=3. The letters a, b and c show significant differences within the same temperature ($p < 0.05$), while A, B and C indicate significant differences among temperatures within the same salinity ($p < 0.05$)

Table 3. The effects of combinations of salinity and temperature on the hatching rates of the sea urchin, *T. gratilla* by two-way ANOVA

Sources of variation	SS	df	MS	F-ratio	5% F-limit (or the tabulated value)
Temp (T)	2	1645.1283	822.56413	60.53	<0.0001
Salinity (S)	2	112.61616	56.308078	4.14	0.0331
T x S	4	2196.4201	549.10503	40.41	<0.0001

SS = Sum of squares; df = degree of freedom; MS = mean of square

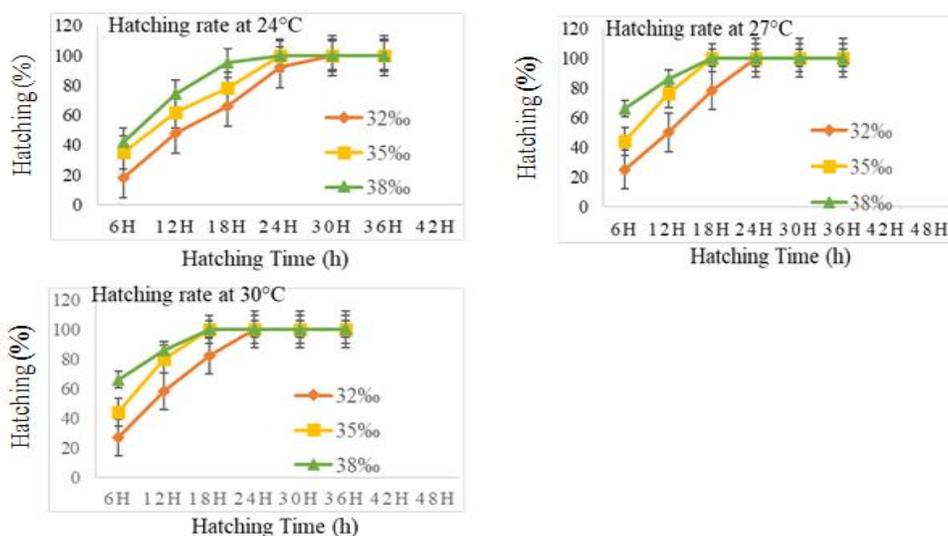


Fig. 4. Comparisons of hatching success of *T. gratilla* eggs at different temperatures under different salinity treatments

Table 4. The effects of combinations of salinity and temperature on the normal larval rates of the sea urchin, *T. gratilla* by two-way ANOVA

Sources of variation	SS	df	MS	F-ratio	5% F-limit (or the tabulated value)
Temp (T)	2	1785.4678	892.73388	71.45	<0.0001
Salinity (S)	2	2767.0298	1383.5149	110.73	<0.0001
T x S	4	2559.6509	639.91273	51.21	<0.0001

SS = Sum of squares; df = degree of freedom; MS = mean of square

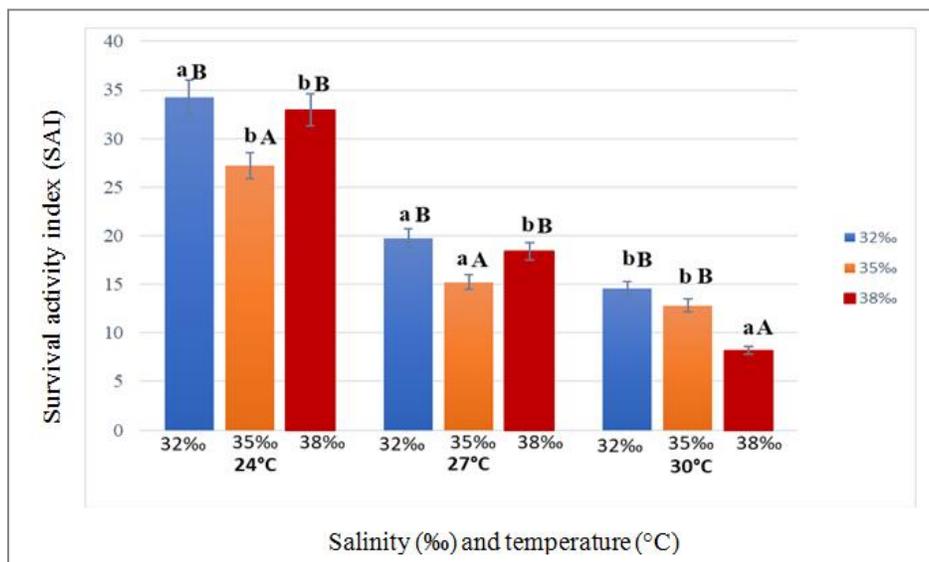


Fig. 5. Comparison of survival activity index (SAI) of *T. gratilla* larvae under different combinations of temperature and salinity. The bar indicates standard deviation (\pm SD); n=3. The letters a, b and c show significant differences within the same temperature ($p < 0.05$), while A, B and C indicate significant differences among temperatures within the same salinity ($p < 0.05$)

Table 5. The effects of combinations of salinity and temperature on the SAI of the sea urchin, *T. gratilla* by two-way ANOVA

Sources of variation	SS	df	MS	F-ratio	5% F-limit (or the tabulated value)
Temp (T)	2	1876.2774	938.13872	228.75	<0.0001
Salinity (S)	2	133.4977	66.748848	16.28	<0.0001
T x S	4	112.40877	28.102193	6.85	0.0016

SS = Sum of squares; df = degree of freedom; MS = mean of square

Temperature, salinity and their combined trials in this study confirmed to have significant effects ($p < 0.05$) on the hatching and normal larval rates by two-way ANOVA (Table 3 and 4). Temperature exclusively ($p < 0.000$) and combinations of both temperature and salinity have significantly ($p = 0.032$) affected the SAI values (Table 5). Furthermore, there were no significant ($p > 0.05$) differences recognized at 24°C among the examined salinities by simple main effect analysis (Fig. 5).

4. DISCUSSION

It has been established that larvae of many sea urchin species are stenohaline and their survival and growth are greatly affected by salinity changes [10,38,39]. In this experiment, the effects of environmental factors on the fertilization and embryonic development of the collector sea urchin within the ranges of temperature and the salinity were chosen that correspond to those in various habitats of this species, e.g., the surface and bottom water layers of the Indo-Pacific Ocean. Our research demonstrated that the fertilization was successful in *T. gratilla* within a wide range of temperatures from 24 to 36°C that met the temperature requirements of this species to start spawning. A decrease in salinity depressed the fertilization ability of the collector sea urchin, especially in combination with the lowest (24°C) and the highest (36°C) temperatures, while a salinity of 23‰ inhibited it. However, fertilization was still possible in this minimal salinity (i.e., 23‰) that matched with the lower limit of resistance of adult individuals of the collector sea urchin [40,41]. A similar phenomenon was also recorded in other echinoderms, such as cucumaria sea cucumber (*Eupentacta fraudatrix*), and sea stars (*Asterias amurensis* and *Asterina pecinifera*), although fertilization in these species was still observed at the minimum salinity levels (20‰ and 18‰, respectively), which were lower than in *T. gratilla* [42,43,44,45]. However, in the present study, the fertilization success of *T. gratilla* was normally occurred at the temperatures from 24°C to 36°C within a salinity range from 23‰ to 38‰.

After the completion of blastopore stage of many fish species including Pacific Bluefin tuna (PBT) *Thunnus orientalis* [46], eggs are reported to have a higher tolerance to variations in environmental conditions compared to the blastomere stage [45,47]. Though the effects of a sudden changes of environmental conditions on hatching and normal larval rates in each development stage of fertilized eggs of *T. gratilla* have not been detailed in the literature, the effects may be similar to the observed results in the congeneric scombrid species of PBT [48]. In a view for mass seed production of *T. gratilla*, the collection of blastula stage eggs from a sea urchin bloodstock tank or a net cage is considered to be difficult to enable various procedures (eliminate impurities, sterilization, rinse, removal of unfertilized eggs, counting, etc.) for egg management as *T. gratilla* eggs have a fast development speed. Therefore, the fertilized eggs in the blastula stage, which are assumed to have low environmental tolerance may be unsuitable for egg management procedures, and the obtained results using Kupffer's vesicle-disappearance stage of fertilized eggs would be more appropriate for various procedures towards the mass seed production of *T. gratilla*; however, this requires further investigations.

The optimal temperature ranges for hatching success and normal larval development rate were reported to be 23-26°C in yellow fin tuna (YFT) by Harada et al. [49], who obtained the highest hatching rate ($\geq 78\%$, including dead and deformed larvae) and normal larval development rate ($\geq 58\%$) at a temperature range of 26.4-27.8°C without information on salinity. Although it is not possible to elucidate correctly the cause of this difference, it may be attributable to differences in the experimental methods, e.g., differences in the fertilization process (artificial and natural), stability of the treatment temperature, whether the beakers were aerated or not, the egg development stage at each treatment, and differences between the brood fish groups used (genetic, age, dietary factors, etc.). Regarding larval survival, the SAI values in our study were significantly higher at 24°C and

27°C than those in other higher temperature groups. The results of this study are similar to that obtained by Harada et al. [49].

In the present study, optimal range of hatching and normal larval development rates were observed within the salinity levels of 35‰-38‰ and was higher than that of the brood stock spawning. The previously observed positive effect of higher salinity during YFT embryonic development was related to the prevention of their sedimentation and better dispersion of floating eggs by increased buoyancy in the higher salinity water [50,51], which has a positive effect on the survival of embryos and larvae [52]. Adverse effects on hatching and normal larval development in the lower salinity water has been reported in many other species [53,54,55]. The salinity in the YFT's main habitats (fishing grounds) and spawning grounds in the Pacific and the Indian oceans have been known to range from 34.8 to 35.0‰ and 35.3 to 35.7‰, respectively [56,57]. These ranges are similar to the optimum range observed in this study.

In experiment with the combined effect of temperature and salinity, the hatching rate and normal larval rate of *T. gratilla* at 38‰ salinity consistently showed the highest values (96.39±2.68% and 92.97±2.59%, respectively) compared to those at 32‰ (58.34±2.47% and 42.31±1.79%, respectively) and at 35‰ (84.97±4.07% and 78.91±3.78%, respectively) regardless of the temperature treatment. The hatching rate (98.80±1.23%) and the normal larval rate (96.03±1.19%) at 24°C was significantly higher than that (18.92±6.25% and 1.95±0.64%) at 30°C. On the other hand, the SAI at 23°C showed the highest values in all the tested salinities. These results revealed that within the ranges tested, the combination of 38‰ and 24°C was found to be the most effective and optimal water temperature and salinity combination for improving/increasing the hatching and survival rates of the fertilized eggs of *T. gratilla*. Furthermore, the sensitivity to the temperature and salinity during the embryonic period and that for hatching larvae of *T. gratilla* seem to differ among each life stage. That is, in the embryonic period, combined effects of temperature and salinity occurred, while hatching larvae were affected solely by temperature (that is, larval stage had relatively higher salinity tolerance than the embryonic stage). In regards of the *T. gratilla* seed production, these results (preference for low water temperature) may be applied to the development of technology to

reduce occurrences of mass mortality during the early stage of sea urchin larvae [58].

Although the highest survival rate and smallest number of abnormal forms were recorded at 24°C, larvae reached to the hatching (gastrula) stage at normal salinity, while at 30°C to 36°C, they developed very slowly and only attained to the blastula stage. A decrease in temperature combined with a decrease of salinity delayed development and upon an increase of temperature, the range of salinity in which the larvae developed normally was narrowed. Thus, free swimming blastulae of the sea urchin, *T. gratilla* have adaptive abilities that enabled larvae to survive and develop for several days in the unstable condition of the surface water layer in a wide range of temperatures (from 24°C up to 36°C) and salinity (from 38‰ to 23‰). A sharp increase in the resistance of larvae at the stage of the free-swimming blastula with the variation of the environmental parameters was also the characteristics of other echinoderm species [42, 44,45,59]. Significant resistance of larvae to a decrease in salinity at various stages of development could be higher than adult individuals, which are also the characteristics of other invertebrates and most likely related to ecological features upon passing these stages [60,61,62,63].

5. CONCLUSION

From the present findings, it could be concluded that the significantly higher hatching and normal larval rate, and survival of fasting larvae after hatching (survival activity index, SAI) were observed at the lower experimental temperature and higher salinity, respectively. In respect of sea urchin hatchery production, the interactions of these environmental factors can be considered not only as the standard parameter for induced breeding and larval rearing of *T. gratilla*, but also will facilitate us to develop the appropriate techniques for mass seed production and commercial aquaculture of this important sea urchin fishery to a greater extent.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded

by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard, written ethical permission has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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