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Influence of temperature variation on embryonic and early larval development of a commercially important tropical sea urchin *Tripneustes gratilla* (Linnaeus, 1758)

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ABSTRACT

The present study investigated the influence of different temperature levels (16, 19, 22, 25, 28, 31 and 34°C) on embryonic and early larval development of the tropical sea urchin, *Tripneustes gratilla* (Linnaeus, 1758) in a controlled laboratory condition. The critical lower and higher temperature for embryonic development was found to be 16 and 34°C, respectively. Embryos reared in these temperatures exhibited 100% abnormality within 48 h post-insemination. The time required to reach embryonic and larval stages was increased with temperature from 28°C followed by 31, 25, 22 and 19°C. The developmental time of 2-cell to 4-arm pluteus larvae showed significant ($p<0.05$) differences. The survival (%) of larvae at the prism, 2-arm and 4-arm stages were observed as dissimilar from 22 to 34°C, and the highest values (100% or near 100%) were found at 25 and 28°C. The morphometric measurements from prism to 4-arm pluteus larvae at different temperatures differed significantly ($p<0.05$). However among the temperatures evaluated, 28°C was found as the best temperature for better growth and development of larvae at all stages. The findings of the study would help to develop captive breeding and seed production programmes for commercial aquaculture of the species.

Keywords: Development, Embryo, Larva, Temperature, *Tripneustes gratilla*

Introduction

The average global temperature has already increased by 0.74°C in the last century and is estimated to increase by another 2-4°C by the year 2100 (IPCC, 2007). Thus, increasing temperatures have already caused several direct and indirect ecological changes including species range shifts, reduced recruitment, mass community mortalities, changes to breeding seasons and enhanced establishment of invasive species (Southward *et al.,* 1995; Walther *et al.*, 2002; IPCC 2007; Przeslawski *et al.,* 2008; Gambainani *et al.,* 2009, Graham *et al.,* 2009; Johnson *et al.*, 2011; Wemberg *et al.*, 2011). In the past 60 years, the surface temperature in Australia has risen by 2ºC and is likely to further increase by another 3ºC by 2070 (Poloezanska *et al.*, 2007; Figuera *et al.*, 2010). However, ocean warming is considered to be of fast concern for south-eastern Australia.

Tripneustes gratilla (Linnaeus, 1758) (Echinodermata: Tripneustidae) or collector sea urchin, one of the commercially important regular echinoids, has a circumtropical distribution extending into the subtropics (Lawrence, *et al.,* 2001a). It occurs most abundantly throughout the Indo-West Pacific, where it can be found from East Africa (Red Sea to Natal), the South Sea islands (from the Norfolk and Kermadec Islands to the Marquesas and Hawaii) and from Australia (to Port Jackson on the east coast and Sharks Bay on the west) to Southern Japan (with the Bonin Islands) (Lawrence *et al.,* 2001a, b). It can also be found in the warm tropical regions including Pulau Bum near Semporna, between Sabah and Philippines (Parvez *et al.*, 2016a, b). It is most common in shallow water habitat on a variety of hard substrates and is found at depths from 2 to 30 m (Lawrence *et al.,* 2001a).

The increasing sea surface temperature affects early life stages of broadcast spawning marine invertebrates (Byrne 2010, 2012). Sea urchin is widely distributed throughout the Indo-Pacific (Lawrence *et al.*, 2007). In addition, fertilisation and larval development of sea urchin occur within distinct temperature ranges for some species

(O'Connor *et al.,* 1977, Mita *et al.,* 1984; Sewell *et al*., 1999). So, ocean warming is likely to be harmfully affecting these life stages.

T. gratilla is commercially important due to presence of highly rich polyunsaturated fatty acids (PUFAs). In Malaysia, sea urchin gonads are used for preparing different types of food *viz.,* Oku-Oku or Ketupat tehe-tehe during special occasions *i.e.,* Lepa-Lepa festival, wedding ceremony and also other events (Parvez *et al.,* 2016b). It is also considered important for the treatment of a number of diseases such as arrhythmia, cardiovascular diseases, cancer, tumor development and light sensitivity diseases (Britton, 2004; Pulz and Gross, 2004). It is also used for improving sexual potency of human, especially the middle aged (Seifulla *et al.,* 1995; Yur'eva *et al.,* 2003). *T. gratilla* is also important ecologically, especially in sea grass habitats and is a food source with good potential for aquaculture (Juinio-Menez *et al.,* 1998; Dworjanyn *et al.,* 2007; Lawrence *et al.,* 2007; Unsworth, 2010). Early life stages (Hart, 2002) of sea urchins, in particular, are known to be highly sensitive to a wide range of environmental contaminants and stressors (Dinnel *et al.,* 1989) which is making them ideal organisms for assessing impacts of climate change. Its life cycle has a planktonic period of days or weeks in the water column and seawater chemistry and temperature have major impacts on the development. Temperature has a major influence in shortening the planktonic period, an effect that decreases predation pressure and also alters connectivity between populations (O'Connor, 2007; Byrne *et al*., 2010).

Very few systematic works have been done on the abundance, distribution, breeding, development and population growth patterns of some non-commercial tropical species of echinoids (*e.g., Diadema setosum* and *Salmacis sphaeroides*) in Peninsular Malaysia (Rahman *et al.,* 2012a, b) but no published information is available on the breeding, nursing, seed production and culture techniques of the high-valued sea urchin species, *T. gratilla* (Linnaeus, 1758). Due to the higher biological, ecological, aquacultural and nutritional importance of *T. gratilla*, it is urgently needed to develop induced breeding, larval rearing and seed production protocols in captivity (Poloczanska, 2007). Fertilisation in *T. gratilla* and other echinoids is robust to climate change stressors (Byrne, 2010; Byrne *et al*., 2011). In the present study, an attempt has been made to assess the effects of water temperature and its optimum level for embryonic and early larval development of *T. gratilla* in a controlled rearing system.

Materials and methods

Sample collection and conditioning

In total, 50 mature adults of *T. gratilla* weighing 160 to 260 g and measuring 80 to 112 mm in diameter, were collected from Bum Bum Island ($4^0$27′55.08″N; $118^0$40^{0}94″E) in Semporna, Sabah, Malaysia (Fig. 1), at low tide during their natural breeding season from January to May, 2016. The collected specimens were then transferred live to the Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia (UPM), maintained in aerated closed aquaria and were used for the experiments within 3-4 days of collection.

Spawning

A total of eight pairs of sexually mature adult specimens of *T. gratilla* were used for spawning. Captive

Fig. 1. Map showing sampling area of *T. gratilla* in Bun-Bun Island, Semporna, Sabah (Source: Parvez *et al*., 2016b)

breeding was attempted by adding 2 ml of 0.5M of KCl into the coelomic cavity of both male and female sea urchins (Rahman *et al.,* 2000; 2005; 2012b). Later on, the eggs were collected by inverting the female to a glass beaker filled with 2 µm filtered seawater (FSW). Soon after collection, the maturity and condition of eggs were checked under a compound microscope (Zeiss Axioskop 2). Good quality eggs having uniform shape and distinct nucleii were used for fertilisation experiment (Rahman and Uehara, 2004). The eggs were then washed consecutively with FSW 3-4 times in order to remove the debris and immature eggs by sucking out the supernatant seawater. Sperm from each male sea urchin were observed under a compound microscope to determine their motility (Rahman and Uehara, 2004). Only highly motile sperms were used for fertilisation trials in order to assure good fertilisation rate.

Temperature trials

The effect of temperature on embryonic and early larval development of *T. gratilla* was investigated at seven different temperature levels between 16 and 34°C. Water baths (Thermal Robo TR-1A) equipped with cooler (TRL107NHF) and normal temperature with water flow tank in the open environment and also at room temperature were used for this experiment. Plastic falcon tubes (50 ml) were used for the culture of embryos and for growing larvae at different temperatures. The plastic tubes were allowed to float in the water bath to make sure that the seawater inside has movement in order to prevent embryos from accumulating at the bottom.

Early development

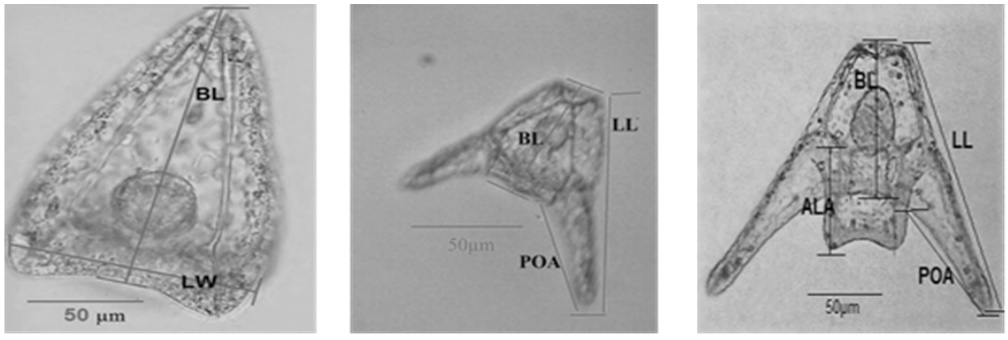
Eggs were allowed to remain with sperm for about 20-25 min for fertilisation at ambient temperature (28°C) and transferred to 50 ml plastic falcon tubes (18 mm dia, 175 mm depth) at a concentration of approximately 400-500 zygotes/tube. The plastic tubes were then placed in water baths, which were pre-set at different temperatures *viz*., 16, 19, 22, 25, 28, 31 and 34°C. Observations were made at: 4, 8, 12, 24 and 48 h post-insemination. At each observation time, the percentage of each embryonic stage at different temperatures was estimated under a compound microscope at 10x magnification. Observations were also made at every 30 min interval. The time taken for 50% of the embryo and larva to reach each progressive stage was recorded (Fujisawa, 1993; Rahman *et al.,* 2002). Development stage(s) were assessed for the first 50 embryos observed under a compound light microscope at 10x magnification. Embryos were scored as Abn = abnormal; Deg = degenerate; 1 = fertilised eggs; 2 = 2-cell stage; 4 = 4-cell stage; 8 = 8-cell stage; 16 = equal to or more than 16-cell; 32 = equal to or more than 32-cells; B = blastula; G = gastrula; Pr = prism; 2P = 2-arm pluteus; 4P = 4-arm pluteus.

Survival

An experiment to assess the survival of different larval stages (*i.e.,* prism, 2-arm and 4-arm) was conducted in falcon tubes. The water bath was set at desired temperature levels (22, 25, 28, 31, 34, 37, 40 and 43°C). Approximately, 20-25 nos. of larvae were placed in each tube with 40 ml FSW for 2 h at each experimental temperature. Larvae were cultured first at 28°C and transferred to different temperatures for 2 h. At the end of the trial, each sample was examined under a dissecting microscope and the larvae were scored as swimming or dead. Larvae that were lying on the bottom of the container, but capable of swimming if distributed, were scored as being alive. Stages tested were: swimming blastula, gastrula, prism, 2-arm pluteus and 4-arm pluteus.

Larval measurements

Morphometric characteristics of larvae were checked at regular intervals and also measured and compared among the temperature treatments. Morphometric measurements taken were: larval length (LL), larval width (LW), body length (BL), post-oral arm (POA) and anterolateral arm length (ALA) (Fig. 2). All measurements



(a) (b) (c)

Fig. 2. Morphometric measurements of early larval stages of *T. gratilla* under Keyence digital microscope
a) Prism. b) 2 arm pluteus and c) 4 arm pluteus stages. LL= larval length, LW= larval width, BL= body length, POA= post-oral arm length and ALA= antero-lateral arm length

were made on freshly prepared specimens of larvae (within 1-2 h), following the techniques described previously by McEdward (1985) with some modifications. Larvae were fixed in 10% buffered formalin in FSW and then concentrated by allowing them to settle on the bottom of a watch glass. Subsequently, they were placed on microscope slides with a cover slip for final measurements and for taking photomicrographs under a compound microscope (Keyence VH-S30K).

Data analysis

Data were arcsine transformed before statistical analyses. All data collected from the fertilisation trials as well as larval development and growth trials were analysed by one-way analysis of variance (ANOVA), followed by Duncan's new range test (Duncan, 1955) using the statistical software "SPSS" version 20 and the significance level was set at 0.05.

Results

Hatching rate

The hatching rate of *T. gratilla* embryos was found to be correlated with time at different experimental temperatures (16-40°C) (Fig. 3). The maximum hatching rates were observed at 22 to 28°C where hatching times varied from 12 to 6 h, respectively. However, the highest hatching success (100%) was found with lower hatching duration at 28°C.

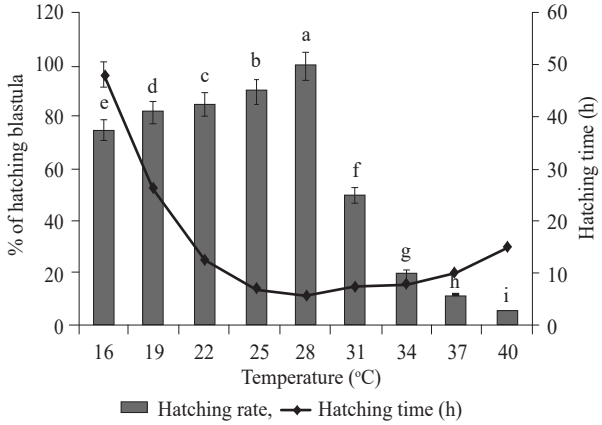


Fig. 3. Hatching time and percentage of hatching of *T. gratilla* embryos at different temperatures

Incubation and early development

The effects of different temperatures on embryonic and early larval development of *T. gratilla* are shown in Table 1a and b. At 16°C, nearly 52.92 and 47.08% attained fertilised eggs and 2-cell stage respectively, whereas about 93.71% embryos reached 4-cell stage at 8 h of incubation (Table 1a). On the other hand, 96% embryos had touched the 16-cell stage in 12 h whereas 49.95 and 29% embryos reached blastula and 32-cell stage respectively at 24 h of incubation (Table 1a). It was also observed that 15.55% embryos degenerated during the same time of incubation. Nevertheless, 75.65% swimming and 20.5% abnormal

Table 1a. Effects of temperature (16-22°C) on early development of *T. gratilla.* Data are expressed as % and denote mean values with five replicates

Temp (°C)	Time (h)	Degen*	Abn**	% of development stages										
				1	2	4	8	16	32	B	G	Pr	2P	4P
16	4			52.92	47.08									
	8				6.29	93.71								
	12						3.93	96.07						
	24	15.55						5.5	29	49.95				
	48		20.5(B)						3.85	75.65				
	72		49.53(B)							50.47				
19	4			43.26	41.42	11.85	3.47							
	8						11.18	88.82						
	12								100					
	24	18.47	2.05(B)							79.48				
	48		15.53 (Pr)							9.75	36.65	38.08		
	72		38.48(pl)										61.52	
22	4				13.85	55.83	30.32							
	8								100					
	12										93.29	6.71		
	24	6.03									87.32	6.6		
	48										41.88	6.9	51.21	
	72											14.88	85.12	

Embryos were scored as Abn = abnormal; Deg = degenerate; 1= fertilised eggs; 2 = 2-cell stage; 4 = 4-cell stage; 8 = 8-cell stage; 16 = ≥16 cells; 32 = ≥32 cells; B = blastula; G = gastrula; Pr = prism; 2P = 2-arm pluteus; 4P = 4-arm pluteus

blastula were observed at 48 h incubation. However, 100% embryos attained the blastula stage in which 50.47% were swimming and 49.53% were abnormal at 72 h of incubation (Table 1a).

At 19°C, nearly about 43.26 and 41.42% embryos attained the fertilised eggs stage, and 2-cellstage respectively at 4 h incubation, whereas 100% embryos were found to be at 32-cell stage at 12 h of incubation. On the other hand, around 88.82 and 79.48% embryos were observed at 16-cell and swimming blastula stages at 8 and 24 h incubation respectively (Table 1a), whereas some larvae appeared to show abnormal (2.05%) growth of cells in the blastocoels and also 18.47% were found degenerated. At 48 h of incubation, nearly 84.72% embryos had reached gastrula stage whereas about 15.28% were still in prism stage and were observed to be abnormal. About 61.52% embryos at 2-arm pluteus stage were found to be swimming, whereas 38.48% of 2-arm pluteus stage were found in abnormal condition (Table 1a).

At 22°C, nearly about 55.83% embryos reached 4-cell stage in 4 h of incubation, followed by 30.32 and 13.85% embryos reached 8-cell and 2-cell stages respectively, whereas 100% embryos were found at 32-cell stage at 8 h of incubation (Table 1a). It was also observed that 93.29% embryos reached gastrula stage at 12 h incubation, whereas, 87.32% embryos attained the same stage at 24 h of incubation. About 6.03% embryos were found degenerated. At 48 h of incubation, 51.21 and 41.88% embryos were found at 2-arm and gastrula stage respectively whereas 6.9% were found at prism stage. At 72 h of incubation, 85.12% embryos were at 2-arm stage whereas, 14.88% were at prism stage (Table 1a).

In 4 h incubation at 25°C, 100% embryos reached 32-cell stage, whereas nearly about 95.25% embryos reached swimming blastula stage. In addition, it was also observed that 65.67 and 34.33% embryos had extended swimming blastula and gastrula stages respectively at 12 h of incubation whereas 94.48, 94.26 and 100% embryos reached prism, 2-arm pluteus and 4-arm pluteus stages at 24; 48 and 72 h of incubation respectively (Table 1b).

At 28°C in 4; 8 and 12 h incubation, 100% embryos reached 16-cell, 32-cell and blastula stages respectively whereas, 94.3 and 94.67% embryos reached prism and 4-arm pluteus stages in 24 and 48 h incubation respectively (Table 1b).

At 31°C, 96.5%, embryos attained 32-cell stage in 8 h of incubation. On the other hand, nearly about 94.7 and 49.18% embryos attained swimming blastula stage in 8 and 12 h of incubation respectively, while 50.82% embryos reached gastrula stage in 12 h incubation. In 24 h incubation, 88.85 and 11.15% embryos attained

Table 1b. Effects of temperature (25-31°C) on early development of *T. gratilla.* Data are presented as % and denote mean values with five replicates

Temperature (°C)	Time (h)	Deg.*	Abn**	% of development stages										
				1	2	4	8	16	32	B	G	Pr	2P	4P
25	4							100						
	8							4.75	95.25					
	12								65.67	34.33				
	24	3.73	0.42								2.12	93.88		
	48	2.17	0.65										12.38	84.8
	72												100	
28	4							100						
	8								100					
	12									100				
	24	5.65									5.7	88.65		
	48	0.8											5.83	93.37
	72												100	
31	4							3.5	96.5					
	8								5.3	94.7				
	12	5.04								49.18	45.78			
	24	42.88	12.43(Pr)									44.69		
	48	100												
	72	100												

Embryos were scored as Abn = abnormal; Deg = degenerate; 1= fertilised eggs; 2 = 2-cell stage; 4 = 4-cell stage; 8 = 8-cell stage; 16 = ≥16 cells; 32 = ≥32 cells; B = blastula; G = gastrula; Pr = prism; 2P = 2 arm pluteus; 4P = 4 arm pluteus

2-arm pluteus and prism stages respectively whereas, 66.58 and 33.42% embryos attained 4-arm and 2-arm pluteus stages in 48 h incubation respectively. However, at this temperature, embryos terminated to develop further from 2-arm pluteus stage (Table 1b).

Effects of temperature on the duration of development

The effects of different temperatures on development time of *T. gratilla* are summarised in Table 2. The 2-cell stage occurred within 5.28±0.02, 3.10±0.05 and 2.46±0.04 h after fertilisation at 16, 19 and 22°C, respectively. When seawater temperatures were set at 25 and 28°C, the 2-cell stage reached at 1.38±0.02 and 1.22±0.01 h, whereas the hatching blastula developed within 10.69±0.09 and 7.81±0.08 h after incubation, respectively (Table 2). At 34°C, embryos attained the swimming blastula stage but showed abnormal growth after 7 h post-incubation (data not shown). At 31°C, the developmental time of 2-cell up to 4-arm pluteus stage was comparatively shorter than those of other temperatures (Table 2). On the other hand, the survival (%) of larvae at prism, 2-arm and 4-arm stages were observed to be dissimilar at different temperatures (22 to 34°C), but the highest values (100% or near 100%) were always found at 25 and 28°C (Fig. 4).

Development times of 2, 4, 8 and 16-cell stages showed significant differences ($p<0.05$) among all the temperature levels tested. The development time of 2-cell stage decreased with increasing temperature (Table 2) and within the temperature range between 25 and 28°C, the required time was found to be almost similar. However, the development time of 2-cell stage decreased when temperature was set at >28°C. The hatching time decreased sharply from 16 to 22°C, but was nearly constant at higher temperatures (25-34°C). The developmental times from

Table 2. Time (h) (Mean±SD; n=10) taken by 50% of *T. gratilla* embryos to reach different developmental stages at different temperatures

Temperature (°C)	Duration (h) of cell developmental stages								
	2-cell	4-cell	8-cell	16-cell	Blastula	Gastrula	Prism	2-arm	4-arm
16	5.28±0.02^{a} (5.26-5.32)	7.07±0.02^{a} (7.03-7.09)	8.40±0.01^{a} (8.38-8.42)	9.93±0.04^{a} (9.88-9.99)	48.87±0.09^{a} (48.68-48.99)	*	-	-	-
19	3.10±0.05^{b} (3.05-3.18)	5.11±0.02^{b} (5.09-5.14)	5.93±0.09^{b} (5.78-6.03)	5.95±0.06^{b} (5.87-6.04)	23.86±0.08^{a} (23.68-23.96)	37.58±0.08^{a} (37.48-37.76)	51.97±0.08^{a} (51.88-52.11)	71.51±0.05^{a} (71.43-71.58)	*
22	2.46±0.04^{c} 2.39-2.50)	4.30±0.04^{c} (4.25-4.38)	4.91±0.08^{c} (4.78-5.03)	4.88±0.07^{c} (4.77-4.98)	11.80±0.09^{c} (11.67-11.98)	21.75±08^{b} (21.65-21.87)	27.83±0.08^{b} 27.65-27.91)	47.10±0.06^{b} (47.01-47.20)	71.04±0.08^{a} (79.89-71.12)
25	1.38±0.02^{d} (1.35-1.41)	2.36±0.08^{d} (2.23-2.45)	2.84±0.08^{d} (2.68-2.89)	3.19±0.08^{d} (3.08-3.36)	10.69±0.09^{d} (7.64-7.89)	18.81±0.07^{c} (18.68-18.89)	24.25±0.04^{c} (24.18-24.31)	34.88±0.04^{c} (34.80-34.95)	48.02±0.10^{a} (47.85-48.2)
28	1.22±0.01^{e} (1.21-1.24)	2.09±0.02^{e} (2.06-2.11)	2.76±0.10^{e} (2.65-2.92)	2.90±0.07^{e} (2.77-2.99)	7.81±0.08^{e} (7.64-7.89)	14.58±0.05^{d} (14.48-14.65)	22.25±0.06^{d} 22.18-22.35)	33.01±0.07^{d} (32.88-33.11)	45.22±0.05^{c} (45.12-45.30)
31	1.08±0.03^{f} (1.01-1.11)	1.94±0.04^{f} (1.89-1.99)	2.40±0.02^{f} (2.38-2.43)	2.80±0.09^{f} 2.67-2.98)	7.67±0.11^{ef} (7.52-7.86)	12.05±0.06^{e} (11.95-12.15)	16.91±0.06^{e} (16.78-16.98)	26.02±0.09^{e} (25.89-26.13)	41.28±0.05^{d} (41.17-41.33)
34	1.01±0.05^{d} (0.96-1.11)	1.82±0.03^{g} (1.78-1.87)	2.84±0.21^{g} (2.35-2.89)	2.61±0.08^{g} (2.48-2.76)	*	-	-	-	-

*All larvae showed abnormal development; Desired larval stage was not found and all died
Mean values in the same column with the same superscripts are not significantly different ($p>0.05$)

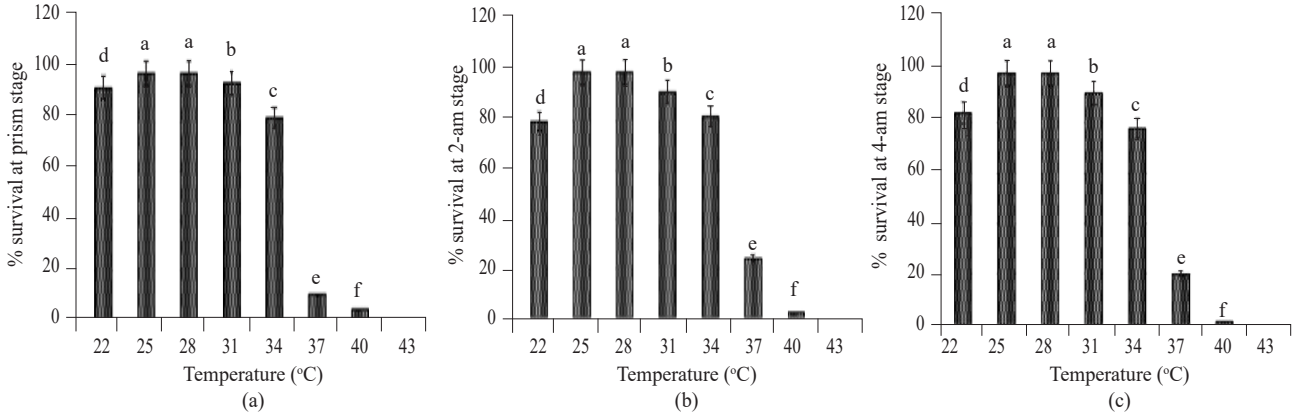


Fig. 4. Percentage survival of (a) prism, (b) 2-arm, (c) 4-arm pluteus stages of *T. gratilla* at different temperatures

early gastrula to 4-arm pluteus stages among all temperature treatments differed significantly ($p<0.05$).

Larval growth and survival

Impact of temperature on early larval growth are presented in Table 3, 4 and 5. Only four temperature levels, *viz.,* 22, 25, 28 and 31°C, had development from 2-cell until 4-arm pluteus (Table 1). The morphometric differences of prism larvae at different temperature treatments were also investigated (Table 3). The highest larval length (LL) of 121.96±0.70 µm and larval width (LW) of 113.99±0.55 µm in prism stage were found at 28°C whereas, the lowest values of LL (91.48±0.05 µm) and WL (89.27±0.51 µm) were found at 22°C. The size of the larvae increased with increasing temperature from 22 to 28°C but reduced at 31°C. However, length and width of larvae differed significantly ($p<0.05$) among the 4 temperature levels examined. Morphometric differences of 2-arm pluteus larvae at different temperature treatments were also investigated (Table 4). In this stage, larvae attained maximum values for larval length (LL) (206.87±0.52 µm), body length (BL) (131.82±0.49 µm) and post-oral arm length (POA) (86.61±0.30 µm) at 28°C, whereas the minimum values for larval length (153.64±0.56 µm), body length (96. 28±0.37 µm) and post-oral arm length (59.05±0.356 µm) were observed at 22°C. However, body length and post-oral arm length among the four temperature treatments were significantly different ($p<0.05$). Comparisons among the four morphometric characteristics in 4-arm pluteus larvae of *T. gratilla* at different temperature levels are presented in Table 5. The results demonstrated that the 4-arm pluteus larva attained the highest larval, body, post-oral and anterior oral arm length of 250.42±0.60, 175.31±0.46, 133.32±0.47 and 92.35±0.34 µm at 28°C, while the lowest values of 186.09±0.46, 123.28±0.45, 68.03±0.30 and 40.50±0.48 µm, respectively were found at 22°C. However, similar to the 2-arm pluteus larvae, the morphometric measurements of 4-arm pluteus larvae in all temperature treatments differed significantly ($p<0.05$).

The survival (%) of larvae at prism, 2-arm and 4-arm stages were observed to be dissimilar at different

Table 3. Comparisons of two morphometric characters of the larvae of *T. gratilla* at prism stage under different temperature treatments. A total of 45 larvae were measured for each replicate in each treatment. All values represent mean±SE

Temperature (°C)	Measurement of morphometric characteristics (µm)	
	Larval length (LL)	Larval width (LW)
22	91.48±0.50^{a} (85.44-99.54)	89.29±0.51^{a} (83.86-97.85)
25	100.59±0.66^{b} (95.39-105.16)	95.19±0.47^{a} (91.17-98.85)
28	121.96±0.70^{d} (116.34-127.55)	113.99±0.55^{d} (102.58-119.98)
31	113.62±0.96^{c} (116.34-127.55)	110.36±4.74^{c} (101.85-119.36)

Mean values in the same column with the same superscripts are not significantly different ($p>0.05$)

Table 4. Comparisons of three morphometric characters of the larvae of *T. gratilla* at 2-arm stage under different temperature treatments. A total of 45 larvae were measured for each replicate in each treatment. All values represent mean±SE

Temperature (°C)	Measurement of morphometric characteristics (µm)		
	Larval length (LL)	Post-oral arm length (POA)	Body length (BL)
22	153.64 ± 0.56^{a} (145.37 - 164.10)	59.05 ± 0.36^{a} (53.67 - 62.08)	96.82 ± 0.37^{a} (92.82 - 102.24)
25	168.99 ± 0.53^{b} (164.45 - 178.38)	63.66 ± 0.41^{a} (58.50-69.68)	110.82 ± 0.50^{b} (104.96 - 116.67)
28	206.87 ± 0.52^{d} (199.96 - 210.89)	86.61 ± 0.30^{d} (80.57 - 90.48)	131.82 ± 0.49^{d} (126.02 -139.27)
31	177.17 ± 2.98^{c} (146.37 - 210.42)	73.88 ± 0.37^{c} (69.15 - 76.68)	121.40 ± 1.79^{c} (92.68 - 133.72)

Mean values in the same column with the same superscripts are not significantly different ($p>0.05$)

Table 5. Comparisons of four morphometric characters of the larvae of *T. gratilla* at 4-arm stage under different temperature treatments. A total of 45 larvae were measured for each replicate in each treatment. All values represent mean±SE

Temperature (^{0}C)	Measurement of morphometric characteristics (µm)			
	Larval length (LL)	Post oral arm (POA)	Body length (BL)	Anterior lateral arm (ALA)
22	186.09±0.46^{a} (180.04 - 189.85)	68.03±0.30^{a} (63.84-70.96)	123.28±0.45^{a} (118.27-128.32)	40.50±0.48^{a} (35.40-45.95)
25	197.99±0.40^{b} (192.68-202.72)	75.25±0.40^{b} (70.44-79.88)	134.96±0.61^{b} (127.78-139.86)	53.30±0.26^{b} (50.44-55.98)
28	250.42±0.60^{d} (244.27-256.38)	133.32±0.47^{d} (129.83-139.85)	175.31±0.46^{d} (169.98-179.02)	92.35±0.34^{d} (87.16-95.76)
31	238.51±0.49^{c} (232.55-244.25)	120.47±0.47^{c} (115.48-125.88)	147.48±2.26^{c} (117.82-77.58)	83.17±0.46^{c} (87.64-87.83)

Mean values in the same column with the same superscripts are not significantly different ($p>0.05$)

experimental temperatures (22 to 34°C), but the highest values (100% or near 100%) were always found at 25 and 28°C (Fig. 4).

Discussion

Critical limits of temperature on several sea urchin species have been experimented to assess the best temperature for optimum development and growth of embryo and larvae. Some studies concluded that temperature is one of the most limiting factors among the abiotic parameters that have influence on earliest stages of development such as fertilised eggs, zygotes and cleavage (Andronikov, 1975; Bressan *et al.,* 1995; Swewell and Young, 1999; Sarifudin *et al.,* 2016). Fujisawa and Shigei (1990) reported that temperature dependence is not universal but rather species specific, especially in early larval development stages. Nevertheless, our experiment represents the first-time investigation concerning the effects of various temperature levels on embryonic and larval development in short-spined collector sea urchin, *T. gratilla*. Results obtained from the present experiment revealed that embryos survived and developed through fertilisation within the temperature range from 19 to 31°C. However, at temperatures lower than 19°C or higher than 31°C, the embryos developed upto the swimming blastula stage and then became abnormal and ultimately died. At 19°C, the development of embryo continued until 2-arm pluteus stage but the larvae grew abnormally and died eventually. According to Andronikov (1975), environmental temperature is not only a limiting factor primarily in the earliest stages of development (from eggs to cleavage stages) but also in the distribution of a species. Therefore, the embryos and larvae will die if fertilisation takes place at temperatures beyond the limit for normal development. The results of our study demonstrated that 28°C was the best temperature in respect of highest growth, development and survival of larvae of *T. gratilla* compared to other temperature treatments tested. It can be explained that the maximum temperature limit for normal development is only 1-3°C higher than the temperatures encountered in the natural conditions (Andronikov, 1975; Sarifudin *et al.,* 2016). Slowest development occurred at 16°C, which was also observed in other sea urchin species (Sewell and Young, 1999; Rahman *et al.,* 2007; Sarifudin *et al.*, 2016). The findings of our study were also the same as Rahman *et al.* (2007), who found abnormality in the early development and larval growth of sea urchin (*Echinometra mathaei*) beyond the low (16°C) and high temperature (34°C) levels. Similar results were also observed in other temperate and tropical species of Indo-Pacific sea urchins (Matsumoto *et al.,* 1988; Fujisawa, 1993; Sarifudin *et al*., 2016).

Morphometric characteristics for prism larvae did not differ significantly among the temperature levels investigated. In our study, the highest length and width of larvae for prism, 2 and 4-arm pluteus stages were observed at 28°C. The lowest values of length and width were obtained at 22°C for prism until 4-arm pluteus stage. According to Rupp (1973) and Sarifudin *et al*. (2016), normal larval development of tropical sea urchins occurred only below 34°C. The synchronised development of larvae is another point to be considered in the effect of temperature on the mode of development. Fig. 2 clearly shows that embryos of *T. gratilla* developed synchronously in the temperature range from 19 to 28°C and asynchronously after 31°C. Most of the larvae were physically normal at this tested temperature (31°C), indicating a range of temperature tolerance of this species. Fujisawa (1989) and Rahman *et al.* (2007) stated that the tolerance characteristics of embryos and larvae could determine the distribution of sea urchins.

The average annual seawater temperature in Malaysia ranged between 27 to 31°C (Sarifudin *et al.,* 2016). The developmental speed of *T. gratilla* varies with the above seawater temperature fluctuations and also becomes lethal when the temperature is extremely low or high. Our study revealed that lowering the temperature decreases the embryonic and early larval developmental speed and *vice-versa* when temperature increases. Extreme low and high temperature (16 and 34°C) causes death of embryos after hatching. Similar results were also obtained in other tropical species of sea urchin, *Echinometra mathaei* and *D. setosum* (Rahman *et al.*, 2007; Sarifudin *et al.,* 2016).

To the best of our knowledge, this is the first study to investigate the influence of temperature variations on embryonic and early larval development and morphometric characteristics in the short-spined collector sea urchin, *T. gratilla*. The findings of the present study would not only be helpful to understand the critical limits of temperature but also to find out the appropriate temperature levels for the optimum growth and development of embryo and larvae. This would eventually lead towards the development of breeding and larval rearing techniques for the high valued *T. gratilla* for seed production, stock enhancement and commercial aquaculture.

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