Effects of temperature on early development of the sea urchin *Echinometra mathaei* from the intertidal reef of Okinawa Island, Japan

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Abstract: Temperature tolerances of the early development (up to 4-arm pluteus) of *Echinometra mathaei* were investigated at 16-34°C. The critical lower and higher temperatures for embryonic development were 16 and 34°C, respectively. At these two temperatures, 100% of the embryos showed abnormality within 48h after incubation. The lower and higher temperature for development of healthy embryos and larvae were 19°C and 31°C, respectively. The developmental times from the 2-cell to early larval stages showed significant differences among 19 to 31°C. At 19 and 31°C, the embryos reached in healthy 2-arm pluteus stage in 72 and 26h after incubation, respectively. The larval growth performance and relative growth ratios of the different organs of 4-arm larvae showed that they were able to tolerate a wide range of temperature without any abnormality. The present findings will be useful for elucidating the possible mechanisms of larval dispersal, as well as the distribution of this sea urchin in various marine locations worldwide.

Key words: sea urchin, *Echinometra mathaei*, embryo, larvae, temperature

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INTRODUCTION

Echinoderms are sensitive to water temperature, which may affect survival and distribution of adults as well as their gametes and planktonic larvae (Rupp 1973). Several reports have also suggested that the distribution of Echinoderms may be limited by temperature i.e. temperature required for growth or spawning, or hydrographic features may limit larval settlement and juvenile survival (Bougis, 1971; Stephen 1972a, b; McEdward 1985; Bosch et al. 1987; Sewell and Young 1998). Studies on several species of sea urchin living in northern Pacific Ocean have revealed that the optimal temperatures for fertilization (Mita et al. 1984),

early cleavage (Yaroslavtseva and Sergeeva 1992) and normal development to the pluteus stage (Fujisawa 1989; Fujisawa and Shigei 1990) are species-specific and occur within the range of temperatures found in the breeding season. But such types of work have not been done on sub-tropical sea urchin.

The sea urchin fauna have different biogeographical ranges, among which the genus *Echinometra* shows greatest abundance in the world. Particularly Indo-Pacific *Echinometra* has always been a subject of rigorous academic debate with regard to its species composition and taxonomic status due to its wider geographical distributions and spinal color variation. The early taxonomists believed that the Indo-Pacific

Echinometra was composed of a single species, *E. mathaei* (de Blainville 1825), which was followed by Clark and Rowe (1971). After 1984, several workers found morphological (Uehara and Shingaki 1984, 1985), ecological (Tsuchiya and Nishihira 1984, 1985) variation within the species of Indo-pacific *Echinometra*. Recently, Uehara et al. (1986 and 1990) distinguished four types of Okinawan *Echinometra*. Now these types have been referred distinct species (Palumbi et al. 1997, Arakaki et al. 1998). *Echinometra* sp. B is now recognized as *E. mathaei* (de Blainville 1825), while *Echinometra* sp. D belongs to the *E. oblonga* (de Blainville 1825). On the other hand, species name of *Echinometra* sp. A and *E. sp. C* are not yet been given.

These four species of *Echinometra* live sympatrically in the intertidal zone of shallow seas but they show slight microhabitat differences. *Echinometra* sp. A is abundant in constantly submersed habitat which is very clam and situated below the level of MLWS (Mean Low Water Surface). *E. mathaei* inhabits burrows that is affected by wave action and located above the level of MLWS. Whereas *E. sp. C* and *E. oblonga* inhabit burrow above the level of MLWS which is more upper region than *E. mathaei* habitat. Their physiological adaptation is different, due to different microhabitat that is related to temperature (one of the factor). Arakaki and Uehara (1991) showed that the four types (species) of *Echinometra* have different physiological adaptation and reproduction. Still now no published information has been available about the temperature tolerance for early development of these species. Among the four species of the *Echinometra* complex, we choose *E. mathaei* (former type B) as material because this species prefer specific region in their habitat and has a unique distribution in terms of extended zoogeography (Palumbi 1996 and unpublished data). In this paper, we investigate the temperature tolerance capability for early development of *E. mathaei* at various temperatures and to predict the dispersal of larva, and the juveniles and adult distribution in various marine locations worldwide.

MATERIALS AND METHODS

Sample collection and maintenance

Healthy mature adults of *E. mathaei* (brownish color) were collected during summer from Onna point, Okinawa Island, at low tide. After

harvesting, the specimens were transported to the laboratory and maintained in a closed aquarium until use within a following week.

Maturity test

To collect the gametes from the sea urchin, the spines and pedicellaria of the gonopore area were removed using a surgical knife. The dirt from the gonopore area was washed off with filtered seawater. Mouth is cut off by scissor and 0.5% KCl solution is added in to the coelomic cavity to aid gamete release. When gametes were come out through gonopore, maturation of eggs was examined under a compound microscope. A drop of dry sperm was diluted in filter seawater (FSW) and examined the motility.

Experimental protocol

The effect of temperature on the early development of *E. mathaei* was investigated at seven temperature points between $16.34 \pm 0.1^\circ\text{C}$. Water baths (Thermal Robo TR-1A) were equipped with handy cooler (TRL107 NHF). 100-ml flat-bottomed cylindrical bottles were used for embryo and larval culture. The culture bottles were allowed to float in the water bath so that the movement of seawater inside the bottle would prevent coagulation of the embryos.

Early development

Eggs were allowed to inseminate with sperm at ambient temperature ($26\sim 28^\circ\text{C}$) for about 20-25 min (required for the fusion of eggs and sperm nuclei) and transferred to 100-ml culture bottles at a concentration of 300-400 zygotes/bottle. The culture bottles were placed in water baths set at different temperatures: 16, 19, 22, 25, 28, 31 and 34°C . Observations were done in two ways: 1) observations were conducted 4, 8, 12, 24, and 48 h after insemination. At each observation time, the percentage of each embryonic stage was counted by using a compound light microscope at $10\times$ magnifications. 2) Observations were done every 30 min interval. The time was recorded when 50% of embryo reached each progressive stage.

Larval growth performance

Larval growth performance was conducted at 22, 26, 29, 31 and 33°C that are suitable temperature range for development of embryo. Twenty minutes after insemination, the zygotes (4 zygotes/ml) were taken in 100-ml bottles and placed in different preset water baths. The

zygotes were reared without food up to the early larval stage (4-arm pluteus stage). A growth performance experiment was conducted on early larval stages of *E. mathaei* i.e. prism, 2-arm and 4-arm pluteus larvae. Morphometric measurements of prism, 2-arm and 4-arm larvae were done as follows: LL= Larval length, LW = Larval width, BL = Body length, PO = Post-oral arm length and ALA =Anterolateral arm length (Fig. 1). All measurements were made on freshly prepared specimens of larvae (within 1-2 h) following the techniques described previously by McEdward (1984) with slight modifications. Larvae were killed in 6% formalin

in FSW and then concentrated by allowing them to settle on the bottom of a watch glass. A few drops of formalin-seawater containing about 40-50 larvae were placed under an elevated cover slip on a microscope slide and viewed under a compound microscope using a preset objective micrometer.

Statistical analysis

All data were analyzed statistically by one-way ANOVA followed by Tukey/Kramer multiple comparison tests.

Seawater temperature

Data on surface seawater temperature for Okinawa Island were obtained from the Sesoko station (Subtropical Biosphere Research Center, University of the Ryukyus).

RESULTS

Early development

The temperature tolerances for early development of *E. mathaei* were investigated in a single experiment where eggs were inseminated in 35 ‰ filtered seawater at room temperature and reared at 16-34°C.

The 2-cell stage occurred at 5.33, 3.16 and 2.58 h after insemination at 16, 19 and 22°C, respectively (Table 1). Twelve hours after incubation at 16°C, nearly 100% embryos had reached around the 16-cell stage. Embryos that attained the swimming blastula stage showed abnormality after 48 h of incubation (Fig. 2A, 3A). At 19 and 22°C, the embryos reached around the 32-cell stage, and the swimming blastula stage after 12 h where about 70% had reached the gastrula stage and about 86% had reached 2-arm pluteus stage after 48 h of incubation (Fig. 2B, C). Some larvae appeared to show abnormal growth of cells in the blastocoel at 19°C (Fig. 3B).

When seawater temperature were set at 25 and 28°C, the 2-cell stage occurred at 1.43 and 1.23 h where hatching blastula developed in 12.08 and 8.04 h after incubation, respectively (Table 1). The embryos accordingly reached the swimming blastula and 4-arm pluteus stages at 12 h and 48 h after incubation respectively (Fig. 2D, E). All larvae exhibited normal development (Fig. 3C, D).

When the temperature was set at 31°C, embryos attained the hatching blastula stage 8 h after incubation (Table 1). Among the embryos,

Fig. 1. Morphometric measurements of early larval stage of *Echinometra mathaei*. A) prism, B) 2-arm pluteus and C) 4-arm pluteus.

Table 1. Effects of temperature on developmental time of *Echinometra mathaei*: Numbers indicate times (hours) taken for 50% embryos to reach each stage. Each value indicates mean $\pm$ SD (n = 3)

Temperature		Stages						
(°C)	2-cell	4-cell	8-cell	Hatching blastula	Early gastrula	Early prism	2-arm pluteus	4-arm pluteus
16	5.33 $\pm$ 0.06 ^a	7.08 $\pm$ 0.16 ^a	8.40 $\pm$ 0.21 ^a	49.02 $\pm$ 0.24 ^a	*	-	-	-
19	3.16 $\pm$ 0.17 ^b	5.16 $\pm$ 0.17 ^b	5.93 $\pm$ 0.35 ^b	24.02 $\pm$ 0.37 ^b	38.06 $\pm$ 0.55 ^a	52.13 $\pm$ 0.25 ^a	71.99 $\pm$ 0.10 ^a	**
22	2.58 $\pm$ 0.10 ^c	4.33 $\pm$ 0.08 ^c	4.91 $\pm$ 0.15 ^c	12.16 $\pm$ 0.22 ^c	24.16 $\pm$ 0.25 ^b	27.78 $\pm$ 0.20 ^b	48.00 $\pm$ 0.22 ^b	71.88 $\pm$ 0.45 ^a
25	1.43 $\pm$ 0.16 ^d	2.33 $\pm$ 0.06 ^d	2.85 $\pm$ 0.18 ^d	12.08 $\pm$ 0.38 ^c	20.78 $\pm$ 0.20 ^c	26.33 $\pm$ 0.41 ^c	35.43 $\pm$ 0.36 ^c	48.02 $\pm$ 0.24 ^b
28	1.23 $\pm$ 0.11 ^{de}	2.30 $\pm$ 0.08 ^d	2.76 $\pm$ 0.22 ^d	8.04 $\pm$ 0.11 ^d	16.50 $\pm$ 0.16 ^d	22.83 $\pm$ 0.33 ^d	34.20 $\pm$ 0.22 ^d	45.26 $\pm$ 0.50 ^c
31	1.08 $\pm$ 0.07 ^e	2.25 $\pm$ 0.04 ^d	2.56 $\pm$ 0.13 ^d	8.04 $\pm$ 0.11 ^d	12.04 $\pm$ 0.16 ^e	17.06 $\pm$ 0.16 ^e	26.05 $\pm$ 0.52 ^e	41.31 $\pm$ 0.48 ^d
34	1.05 $\pm$ 0.02 ^e	2.16 $\pm$ 0.06 ^d	2.51 $\pm$ 0.03 ^d	8.03 $\pm$ 0.12 ^d	*	-	-	-
ANOVA table	F=1036.37 df=6,28 P <0.0001	F=1681.61 df=6,28 P <0.0001	F=629.56 df=6,28 P <0.0001	F=17217.67 df=6,28 P <0.0001	F=5314.94 df=4,20 P <0.0001	F=11010.97 df=4,20 P <0.0001	F=15891.69 df=4,20 P <0.0001	F=5109.37 df=3,16 P <0.0001

Means in the same column with the same superscript are not significantly different according to the Tukey / Kramer test ($P < 0.0001$). *all larvae showed abnormal development, **Desired larval stage was not observed.

about 53% reached the swimming blastula stage and about 47% reached the gastrula stage at 12 h after incubation, where about 86% of embryo showed 2-arm pluteus larvae at 24 h after incubation (Fig. 2F). Forty-eight hours later, about 60% embryos had reached the 4-arm pluteus stage (Fig. 2F). At 34°C, only about 22% of embryos had attained the swimming blastula stage at 12 h after incubation (not shown in Fig. 2) and all embryos become abnormal at 48 h after incubation (Fig. 3F).

The development times of 2, 4 and 8-cell stages showed significant differences among fifteen combinations of temperature, whereas there were no significant differences among six combinations of temperature (Tukey/Kramer, $P < 0.0001$). The developmental time of 2-cell decreased with increasing temperature (Fig. 4), and within the temperature range (25 to 28°C), the times were almost similar. When the temperature was set higher than 28°C, the development time of 2 cell stages decreased. The hatching time was decreased sharply from 16 to 22°C, but was nearly constant at higher temperatures (25–34°C). From the early gastrula to 4-arm pluteus stages, the developmental time differed significantly (Table 1) among the all temperatures (Tukey/Kramer, $P < 0.0001$).

Larval growth performance

As shown in Table 2, the prism and 2-arm pluteus larvae were found alive at 22–33°C. The maximum length and width of prism larvae were 107 and 115 μ m at 29°C whereas the minimum length and width were 90 and 87 μ m at 22°C. The growth rate of prism larva increased staidly up to 29°C, where it decreased at 31°C (Table 2). The length and width of prism larvae differed significantly among six temperature combinations. No significant differences were noted among four temperature combinations (Tukey/Kramer, $P < 0.0001$).

In 2-arm pluteus larvae, the maximum values of larval length (LL), body length (BL) and post-oral arm length (PO) were 140, 111 and 45 μ m, whereas the minimum values were 104, 92 and 33 μ m at 29 and 33°C, respectively. The growth rate of different organs increased with increase the temperature (22–29°C) whereas growth declination occurred at 31°C (Table 2). The LL showed significant differences among seven temperature combinations (Tukey/ Kramer, $P < 0.0001$). On the other hand, BL exhibited significant differences among six temperature combinations (Tukey/ Kramer, $P < 0.0001$) and PO exhibited significant differences among five temperature combinations (Tukey/Kramer, $P < 0.0001$).

The maximum values of LL, BL, PO and

Fig. 2. Effects of different temperature (16-31°C) on early development of *Echinometra mathaei*. Bar shows mean $\pm$ SD of three replicate culture bottles. Insemination was done at room temperature (26-28°C) and were transferred to different temperature 20-25 min after insemination. Developmental stages; 1, fertilized eggs; 2, 2-cell; 4, 4-cell; 8, 8-cell; 16, equal or more than 16-cell; 32, equal more than 32-cell; B, swimming blastula; G, gastrula; Pr, prism; 2P, 2-arm pluteus; 4P, 4-arm pluteus. Asterisk on the bar indicates abnormality of respective stage.

Fig. 2. Continued.

Fig. 3. Photomicrographs showing early development of *Echinometra mathaei* at various temperatures. Photomicrographs were taken 48 h after insemination. A, B, C, D, E and F indicates culture temperature 16, 19, 25, 28, 31 and 34°C respectively. A; all hatching blastula larvae show abnormality, B; larvae show gastrulation, C, D and E; normal 4-arm pluteus larvae and F; all larvae cease their development. Scale bar indicates 100 µm.

ALA for 4-arm pluteus larvae were observed at 31°C (Table 2). The growth of different organs increased steadily from 22–31°C and no larvae were found alive at 33°C. All parameters differed significantly among five temperature combinations (Tukey/Kramer, $P < 0.0001$), whereas there were no significant differences in 22 and 26°C combination. The ALA showed significant differences among all temperature combinations (Tukey/Kramer, $P < 0.0001$).

The relative growth ratio between the different organs of early 4-arm pluteus larvae at different temperatures is shown in Fig. 5. The

PO/BL ratio showed a linear relationship with increasing temperature (Fig. 5A). It exhibited significant differences among three temperature combinations (Tukey/Kramer, $P < 0.0001$). The highest and lowest ratio values were observed at 31 and 22°C, respectively. In figure 5B, the maximum and minimum ALA/BL ratio values were found at 26 and 22°C where the ratio values showed significant differences among three temperature combinations (Tukey/Kramer, $P < 0.0001$). The highest and lowest values of ALA/PO ratio being observed at 26 and 22°C (Fig. 5C) and significant differences were found

Fig. 4. Embryonic developmental times of *Echinometra mathaei* at various temperatures (16–34 °C). Time was counted after the embryos were transfer in each respective temperature. Times required for fertilized eggs to reach 2-cell stage (A) and hatching blastula (B). Each point indicates the mean of five replicates.

in three temperature combinations (Tukey/Kramer, $P < 0.0001$).

DISCUSSION

Several workers had been studied on *E. mathaei* of Indo-Pacific region. They treated all species belong to the genus *Echinometra* is one species, *Echinometra mathaei*. Although the species showed inter species morphological variation but no body tried to preciously identify. After 1984, on the basis of morphology (Uehara et al., 1986) and genetics (Palumbi and Metz, 1991), four distinct species of sea urchin belonging to the genus *Echinometra* are most

abundant in coral reef areas of Okinawa Island. Although they exist simpatrically in their natural habitat, their microhabitats (Nishihira et al. 1991) and the physiological adaptation (Arakaki and Uehara 1991) are different. *E. mathaei* is one of species name among the four species, which is widely distributed in the indo-pacific region from central Japan in the north, to southwest Australia in the south, and from Clarion Island off Mexico in the east, to the Gulf of Suez in the west (Palumbi 1996; Pearse and Phillips 1968; Uehara unpublished data). This is the first time we detail investigate the early development of this species in various temperature that are usually occurring in its habitat in the world. Here we will rename all previous works on *E. mathaei* as “former *E. mathaei*” for the convenience of our discussion. Pearse (1969) reported that the reproduction of former *E. mathaei* occurs throughout the year in most areas of its distribution, but is restricted to summer off central Japan (Onoda 1936) and within the Gulf of Suez (Pearse 1968). The spawning season of *E. mathaei* in Okinawa is late July to late September. Spawning period of this species varies with the former *E. mathaei* in the rest of the world (Table 3) because it is not clear which species (among four species) is used. The peak gonadal index of *E. mathaei* occurs July and decreases in the fall (Arakaki and Uehara 1991). From this viewpoint, gametogenesis probably start from May in Okinawa, when the seawater temperature begins to increase, and spawning occurs when the seawater temperature is around 25–28°C (Fig. 6).

Environmental factors such as temperature directly determine the embryonic development of sea urchins. Figure 2 indicates that the temperature tolerance for embryogenesis is around 16–34°C. Rupp (1973) detained a similar result for the tropical sea urchin former *E. mathaei* (de Blainville) and showed that the upper temperature tolerance limit for early development of this species is between 34 and 36°C. Farmanfarmanian and Giese (1963) also investigated the temperature sensitivity for early development of the urchin *Strongylocentrotus purpuratus*, which is distributed in temperate regions. They reported that at 25°C, 8°C above the ambient range, the fertilization membrane developed but division of the fertilized eggs was abnormal. Our present finding reveal that embryos appear abnormal both critically low and high temperature (Fig. 3). At low temperature (16°C) larvae stop

Table 2. Growth performance of early larval stages of *Echinometra mathaei* at different temperatures (22–33°C). A total of 30 larvae were measured at each experimental temperature. Abbreviations: LL, larval length; LW, larval width; BL, body length; PO, postoral arm length; ALA, anterolateral arm length.

Larval stage	Temperature (°C)	Mean (μm) ±SD			
		LL	LW		
Prism	22	90 ± 3.76 ^a	87 ± 7.62 ^a		
	26	103 ± 6.07 ^b	103 ± 6.21 ^b		
	29	107 ± 7.86 ^c	115 ± 7.24 ^c		
	31	98 ± 9.07 ^b	106 ± 8.61 ^b		
	33	98 ± 10.40 ^b	105 ± 7.59 ^b		
ANOVA table	<i>F</i>	19.74	56.63		
	<i>df</i>	4,150	4,150		
	<i>P</i>	<0.0001	<0.0001		
		LL	BL	PO	
2-arm pluteus	22	132 ± 11.57 ^a	90 ± 13.52 ^a	42 ± 9.99 ^a	
	26	139 ± 11.52 ^a	111 ± 12.88 ^b	45 ± 8.65 ^a	
	29	140 ± 15.32 ^a	111 ± 8.77 ^b	45 ± 5.81 ^a	
	31	116 ± 11.05 ^b	96 ± 13.74 ^a	38 ± 7.39 ^{ab}	
	33	104 ± 11.40 ^c	92 ± 13.73 ^a	33 ± 9.99 ^b	
ANOVA table	<i>F</i>	51.40	20.33	11.15	
	<i>df</i>	4,150	4,150	4,150	
	<i>P</i>	<0.0001	<0.0001	<0.0001	
		LL	BL	PO	ALA
4-arm pluteus	22	137 ± 17.22 ^a	100 ± 18.82 ^a	44 ± 9.65 ^a	10 ± 5.08 ^a
	26	138 ± 18.19 ^a	111 ± 18.74 ^a	52 ± 10.64 ^a	22 ± 4.08 ^b
	29	165 ± 23.18 ^b	114 ± 9.17 ^b	69 ± 16.62 ^b	26 ± 5.92 ^c
	31	204 ± 33.43 ^c	128 ± 8.54 ^c	92 ± 26.01 ^c	32 ± 5.51 ^d
	33	*	*	*	*
ANOVA table	<i>F</i>	72.34	29.27	48.96	71.57
	<i>df</i>	3,120	3,120	3,120	3,87
	<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001

Mean values in the same row with the same superscript are not significantly different (Turkey / Kramer's test $P < 0.0001$). *No larvae survived.

to continue further development while embryo reared at the temperature 34°C are arrested or in some case dead at prism or 2-arm pluteus stage. However, embryos with other five-tested temperature (19, 22, 25, 28 and 31°C) show normal development and the development time varies with increasing temperature, which is line with the findings of others (Matsumoto et al. 1988; Fujisawa 1993). The factors determining the temperature tolerance of sea urchin embryo are still unclear. Bělehrádek (1930) proposed that in general biological processes including development protoplasmic viscosity is

the main factor determining the effect of temperature, whereby different time periods are required for completion of egg cleavage patterns.

Development times up to the blastula stage only differed significantly among low temperatures (Table 1), as the embryos of this species are genetically and environmentally tolerate higher temperature (seawater above 25°C). The organogenesis (gastrula to 4-arm pluteus) of this species may occur within a certain temperature range (19 to 31°C). Rupp (1973) reported that the normal larval development of

Fig. 5. Ratio between the sizes of different organs of 4-arm pluteus larvae of *Echinometra mathaei* at various temperatures. **A** Ratio between post oral arm length (PO) and body length (BL) **B** Ratio between anterolateral arm length (ALA) and body length **C** Ratio between ALA and PO. Means and standard deviation are calculated based on the measurements of 30 larvae. Means with different superscripts are significantly different (Tukey/Kramer, $P < 0.0001$).

tropical sea urchins only occurs below 34°C. The synchronized development of larvae is another point to consider in the effect of temperature on the mode of development. Figure 2 clearly shows that embryo of *E. mathaei* develops synchronously from 19 to 28°C and asynchronously development occurs at 31°C. Most of the larvae are morphologically normal at this tested temperature (31°C), which indicating a wide range of temperature tolerance for this species. Fujisawa (1989) has stated that the embryo's temperature tolerance characteristic determines the distribution of sea urchins.

The average annual seawater temperature of Okinawa ranges between 22 to 30°C (Fig. 6), coincident with the average temperature ranges in different regions of the world where this species and other *Echinometra* species are exists (Table 3). Comparing the development time between *E. mathaei* and *E. sp. A*, a little variation are observed between two species (unpublished data). Table 1 shows that developmental speed of the studied species also varies with temperature fluctuation and lethal when the temperature are extreme low or high. Our experiment reveals that lowering the temperature decrease the embryo/larval developmental speed and vice-versa when the temperature is increased. Extreme low or high temperature (16 and 34 °C) causes embryo death after hatching (Table 1). Within the range of temperature along with reef current or strong current/wind cause by typhoon, a common natural calamity of this Island allows the embryos/larvae to the highly dispersed area. Developmental speed of this species is directly related to the total length of pelagic life, which influences the distance of larval dispersal as well as the annual recruitment pattern. Thorson (1961), Mileikovsky (1971) and Scheltema (1977) reported that the length of pelagic larval life is an important factor in recruitment of young population or the distribution of a species.

Another physical factor that cannot be ignored is ocean circulation, as it is responsible for the horizontal distribution, recolonization, transport and dispersal of free-floating larval stages of many marine organisms. Several oceanic currents such as the North Equatorial Current, Kuroshio Current and Ryukyu current system (RCS) exist around the Okinawa Islands and are highly likely to transport embryos and larvae short or greater distances, either locally or between islands or to more remote regions of the world. Tyler and Young (1998) have suggested

Table 3. Annual seawater temperature and spawning period of *Echinometra mathaei* (de Blainville 1825) in various regions of the world.

Location	Temperature range	Spawning period	Reference
Rottneest Island, Western Australia	18-23°C	Year round	Pearse and Phillips, 1968
Wadi El Dom, Egypt	18-28°C	July-September	Pearse, 1969
Al-Ghardaqa, Egypt	17-29°C	July-September	Pearse, 1969
Shirahama, Japan	14-28°C	July-August	Onoda, 1936
Shirahama, Japan	-	July-September	Tohara and Okada, 1968
Equatorial Pacific	-	Year round	Pearse, 1968
Okinawa, Japan	20-29°C	Late July-Late September	Unpublished data*

*Only for *E. mathaei* out of the four Okinawan *Echinometra* spp.

Fig. 6. Surface seawater temperature of Okinawa Island from 2000 to 2004 (source: annual temperature data from Sesoko Staion, Subtropical Biosphere Research Center, University of the Ryukyus). Each point indicates mean $\pm$ SD.

that embryo's pressure tolerance is correlated with the depth distribution of three sea urchins, *Genus Echinus*. Though in this study, we did not examine the pressure tolerance of the embryo or larvae, it has been predicted that vertical distribution of *E. mathaei* is limited by pressure tolerance or other factors, as it is globally distributed in shallow water.

The larva of *E. mathaei* is planktotrophic, and temperature has effects on the mode of larval development, such as growth, metabolic activity. Our results also indicated that the size, shape and relative growth of larvae differed according to temperature. Table 2 and Fig 5 clearly indicate that the larvae show different growth performance with temperature in a certain temperature range but morphologically normal within this range. At various temperatures, biochemical metabolic activity of larva may responsible for the larval growth. Normal growth of larva and its different organs in various temperatures also proves that larva of this species is well adapted in widely temperature fluctuating environment. Pechenik (1980) suggested that the eventual end to larval life may be "programmed" into the development of at least some species. According to this hypothesis, slowly developing larvae should be able to delay metamorphosis for a longer period than larvae that develop more quickly. Bayne (1965) has also reported that temperature influences the potential duration of the delay period for larvae of the mussel, *Mytilus edulis*. Under laboratory conditions, competent larvae of *E. mathaei* show arrest of metamorphosis in the absence of suitable natural substrates (Pers. comm. with Rahman). Although not yet been observed but it has been predicted that during this period larvae utilize either stored body energy or continuously ingest food from the environment and extend their pelagic life. The southern coast of mainland Japan is the northern distribution limit of the genus, *Echinometra* where only *Echinometra* sp. A is found among the four species. The temperature tolerance is not only factor for distribution of these species. Habitat preference is likely to be involved. All over the oceanic world, habitat characters and area of *E. mathaei* is more common than other three *Echinometra* species.

On the basis of its wide range of temperature tolerance, mode of embryonic and larval development and habitat preference, *E. mathaei* is capable of wide dispersal and broad distribution, not only near shallow-water equatorial

regions but also in areas far from the equator. The present study is the first to investigate the early development of this species at various temperatures. It is anticipated that the data obtained will help to reveal further unknown interesting biological consequences with regard to the mode of development, juvenile recruitment pattern, population dynamics and distribution strategy of this species in the diverse oceanic environment.

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