EFFECTS OF TEMPERATURE ON FERTILIZATION AND EARLY CLEAVAGE OF SOME TROPICAL ECHINODERMS WITH EMPHASIS ON *ECHINOMETRA MATHAEI* (DE BLAINVILLE)

by

JOHN H. RUPP

A thesis submitted in partial fulfillment
of the requirements for the degree of

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TO THE GRADUATE SCHOOL:

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AN ABSTRACT OF THE THESIS OF John H. Rupp for the Master of Science in Biology presented January 17, 1972.

Title: Effects of Temperature on Fertilization and Early Cleavage of Some Tropical Echinoderms with Emphasis on Echinometra mathaei (de Blainville)

Approved: _____
ROBERT S. JONES, Chairman, Thesis Committee

Select temperatures above normal are shown to reduce success of fertilization and normal early cleavage, in the laboratory, for the echinoderms Acanthaster planci (L.), Culcita novaeguineae Muller and Troschel, Linckia laevigata (L.), Echinometra mathaei (de Blainville), and Diadema savignyi Michelin. The data indicate that cleavage is more sensitive to increased temperature than fertilization. Upper tolerance limits for early cleavage in most of the species is near 34.0°C. The early developmental stages of Acanthaster planci were the most sensitive to elevated temperature, and those of Echinometra mathaei, the least.

Further experiments with E. mathaei showed that unfertilized ova were still viable, dividing normally when fertilized, after two hours of exposure at 36.0°C. The ova were significantly less viable after three hours. Early cleavage stages of E. mathaei were resistant to 36.0°C for exposure times of up to 40 minutes but were inhibited beyond this period.

It is suggested that the ability of E. mathaei to develop normally at 34.0°C (6°C above ambient) and to withstand limited

exposure to 36.0°C may account for the wide distribution of this species in habitats which are often subjected to frequent broad temperature fluctuations, such as reef flats.

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INTRODUCTION

Benthic marine invertebrates, particularly echinoderms, are sensitive to water temperatures above normal ambient (Farmanfarmaian and Giese, 1963; Glynn, 1968). These temperatures may affect survival and distribution of adults as well as their gametes and larval stages which occur in the plankton.

Although the normal annual temperature fluctuation in oceanic surface waters around Guam is less than 3.0°C (26.5-29.0°C), conditions leading to temperature stress on coral reef organisms do exist, both naturally and man-induced. Temperatures in reef flat pools and lagoon areas are often several degrees above normal oceanic and, during periods of low tide and intense solar radiation, may exceed 36.0°C. Other sources of increased water temperature on tropical reefs are steam generating stations which use seawater for condenser cooling and often discharge the effluent directly onto the reef. Discharge water may reach temperatures as high as 35.0 to 36.0°C.

Because echinoderms constitute a large segment of the fringing reef fauna and would likely be affected by major changes in ambient water temperature, a study was designed to determine: if fertilization success (defined as the formation of the fertilization membrane) at select temperatures above normal is significantly reduced from that at normal ambient temperature; if normal cell cleavage at select temperatures above normal is significantly reduced from that at normal ambient temperature; which is more sensitive to increased temperature, fertilization or early cleavage; and how long gametes

and zygotes can be exposed to temperatures above normal, without inhibiting further embryonic development.

The effects of temperature on development of temperate echinoderm species has been studied by numerous workers including Tyler (1936), Farmanfarmaian and Giese (1963), and Andronikov (1967), but there is little information regarding the effects of temperature on the early development of tropical echinoderms. Because so little information is available, this study was conducted in two phases. In the first phase, preliminary information was collected on the effects of temperature on fertilization and early cleavage of tropical echinoderms in general. Species tested in phase one were Acanthaster planci (L.), Culcita novaeguineae Muller and Troschel, and Linckia laevigata (L.) from the class Asteroideae, and Echinometra mathaei (de Blainville) and Diadema savignyi Michelin from the class Echinoideae. Phase one also served to select an experimentally suitable species for more intensive study and to test the experimental apparatus and design.

In phase two, E. mathaei was selected for additional experimentation to answer questions set forth in the objectives.

Phase one was conducted from May to September, 1972, and phase two from September through November, 1972. Experiments were conducted at the University of Guam Marine Laboratory.

MATERIALS AND METHODS

Live specimens of adult asteroids A. planci, C. novaeguineae, and L. laevigata were collected from the fringing reefs of Guam. Adult echinoids D. savignyi and E. mathaei were collected from the intake channels of steam electric generating stations at Tanguisson Point, and Cabras Island, Guam.

Since the spawning periodicity of these animals was unknown, it was often difficult to obtain ripe individuals. Ripe adult males of C. novaeguineae were rarely found between May and September, 1972, and although eggs were easily obtained, sperm were released in sufficient quantity on only two occasions. Ripe adult D. savignyi were found only during late July, 1972.

It was necessary to transport asteroids in individual containers with frequent changes of fresh seawater, because slight increases in water temperature and vibration often caused spawning during transportation and because separate containers prevented individuals which spawned in transit from stimulating spawning in others.

Echinoids were transported in common containers, each carrying three to ten specimens. Handling and transportation did not normally cause spawning of ripe individuals.

In the laboratory, individual adult asteroids were kept in containers (14 X 36 cm dia.) of fresh seawater. Spawning was induced by injecting a solution of 10^{-5} M 1-methyladenine (Kanatani, 1969). Three to five ml were injected, depending on the size of the organism.

Ova were collected by pipette from the stream of spawning ova and put in a 400 ml beaker of 28.0°C fresh seawater. Once the ova settled, they were rinsed by decanting and refilling the beaker with fresh seawater. To avoid possible negative effects of overcrowding, ova were concentrated to a density less than one layer thick. Asteroid sperm samples were collected by pipette as they were released from the gonopore. These samples were held in clean glass beakers until experiments began.

Adult echinoids held at 28.0°C were removed from the water and induced to spawn by injecting approximately 1 ml of 0.5 M KCl solution (Tyler, 1949). After injection the animals were rinsed with fresh seawater. Females were inverted over 250 ml beakers of fresh seawater at 28.0°C and the ova collected and concentrated as noted above. Males were inverted over a watch glass and "dry," undiluted sperm (Kobayashi et al, 1972) were collected on the surface. Occasionally, "dry" sperm were pipetted directly from the gonopore.

Four temperature baths set to maintain 28.0 (control), 31.0, 34.0, and 36.0, ± 0.2°C, were made from 30 liter aquaria equipped with 100 watt immersion heaters and thermostatic controls (Fig. 1). The aquaria were filled with fresh tap water. To insure uniform temperature throughout, the water was circulated with two air lifts (flow rate 1.4 liters per minute) driven by an air pump. Plexiglass racks, each holding twelve 40 ml glass vials (experimental chambers), were submerged in the temperature baths (Fig. 1).

In each experiment, the control group (28.0°C) was used to establish normality criteria against which comparisons were made.

Success of fertilization and early cleavage was determined by microscopic examination and calculated as the percent of ova with fertilization membranes or percent of zygotes showing normal cleavage, of the total within a 2 mm microscope field (magnification 10X). Statistical analyses follow procedures outlined by Sokal and Rohlf (1969). Statistical significance was accepted at $P \leq 0.01$.

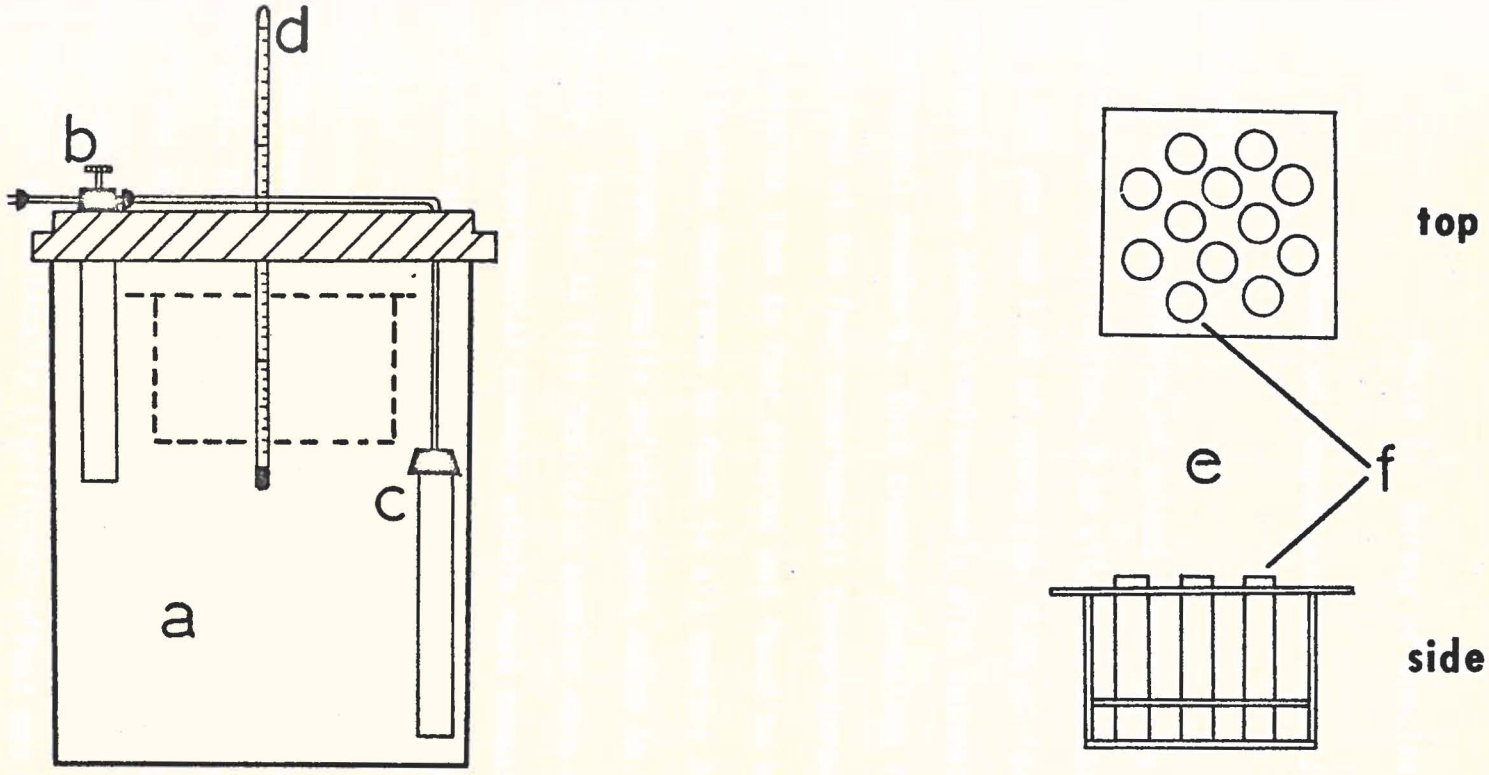

Figure 1. Constant temperature bath. (a) 30 ℓ aquarium, (b) thermostat, (c) 100 watt heater, (d) thermometer, (e) rack for experimental chambers, (f) experimental chambers (40 ml glass vials).

RESULTS

Phase I: Survey of Species

Effects of Temperature on Fertilization

Procedure

To test the effects of select temperatures above 28.0°C (normal ambient) on fertilization, 3 ml aliquots, containing between 1,000 and 2,000 ova and 1 ml of sperm (ca. 0.05 ml "dry" sperm per 50 ml seawater) were added simultaneously with automatic pipettes to the experimental chambers. Each chamber contained fresh seawater maintained at the four experimental temperatures. After approximately five minutes (two to four minutes was found to be sufficient for fertilization membranes to form in all species tested), 10-15 ml of a three percent formalin solution were added to the chambers to fix activity. Three to five replicate samples were aliquoted by pipette from each experimental chamber within each temperature treatment, and percent success of fertilization was determined.

Results

The results of these experiments are summarized in Table 1 and show that, for all five species tested, a temperature treatment of 31.0°C had no apparent effect on the mean percent of fertilization success when compared to the mean at the control temperature (28.0°C). At 34.0°C a statistically significant effect was noted only for A. planci. At 36.0°C the mean percent of fertilization success in both

Table 1. The effects of temperature on fertilization success among select echinoderms expressed as the mean percent of ova showing fertilization membranes. Parentheses enclose two standard errors of the mean. n = 3 except, where asterisk appears n = 1. Means statistically homogeneous ($P \leq .01$) with the control mean are underscored by a horizontal line.

	WATER TEMPERATURE °C			
	28.0 [control]	31.0	34.0	36.0
Acanthaster planci	99.8 (0.4)	90.9 (6.8)	70.2 (10.8)	19.8 (10.1)
Culcita novaeguineae	*95.4 (——)	*93.4 (——)	*89.5 (——)	*70.5 (——)
Linckia laevigata	97.3 (3.6)	97.5 (3.6)	91.0 (9.2)	21.8 (1.2)
Diadema savignyi	*99.8 (——)	*99.4 (——)	*98.2 (——)	*98.6 (——)
Echinometra mathaei	96.4 (2.0)	95.5 (3.4)	95.9 (4.8)	95.4 (1.8)

A. planci and L. laevigata was significantly different from the control. Although the mean percent of fertilization in C. novaeguineae at 36.0°C was 25 percent below the mean at the control temperature, inadequate sample size precluded drawing statistical conclusions. Fertilization success in D. savignyi appeared to be unaffected by temperature treatments, but again, inadequate sample size precluded drawing statistical conclusions. A temperature treatment of 36.0°C showed no statistically significant effect on the mean percent of fertilization success in E. mathaei.

Effects of Temperature on Early Cleavage

Procedure

To test the effects of select temperatures above normal ambient on development to the four cell stage, fertilization was first induced at normal ambient temperature (28.0°C). After fertilization membranes appeared, within five minutes, 3 ml aliquots containing between 1,000 and 2,000 zygotes were added with automatic pipettes to experimental chambers containing fresh seawater maintained at the experimental temperatures. Development at each temperature was monitored microscopically with samples taken from separate monitoring vials. When fifty percent of the zygotes reached the four cell stage, development was fixed by adding a three percent formalin solution. Three to five replicate samples were obtained by the same procedure outlined for the fertilization experiment. Development to the four cell stage as it proceeded at 28.0°C was accepted as the norm for comparative purposes.

Results

For all five species tested, the effects of 31.0°C on normal four cell development were indistinguishable from the control (Table 2). When comparing the results at the control temperature (28.0°C) with 34.0°C, a statistically significant effect was noted in all species except E. mathaei. At 36.0°C, development to the four cell stage was significantly reduced in all species. The occasional embryo reaching the four cell stage at this temperature was generally deformed and ceased to develop further.

Table 2. The effects of temperature on the success of early cleavage in select echinoderms expressed as the percent of zygotes undergoing normal cell cleavage to the four cell stage. Parentheses enclose two standard errors of the mean. n = 3 except, where asterisk appears n = 2. Means statistically homogeneous ($P \leq .01$) with the control mean are underscored by a horizontal line.

	WATER TEMPERATURE °C			
	28.0 [control]	31.0	34.0	36.0
Acanthaster planci	97.5 (2.2)	96.6 (3.0)	35.2 (20.4)	0 (0)
Culcita novaeguineae	96.6 (5.6)	97.6 (4.0)	51.1 (10.2)	0.6 (0.2)
Linckia laevigata	97.9 (2.4)	94.0 (3.8)	30.2 (18.0)	0 (0)
Diadema savignyi	*96.6 (0.1)	*95.4 (3.4)	*26.8 (19.8)	*1.2 (1.2)
Echinometra mathaei	98.3 (0.8)	97.1 (2.2)	92.4 (6.4)	5.4 (8.4)

Phase II: Echinometra mathaei

Despite the preliminary findings that E. mathaei was the most thermally resistant of the species tested, it was still considered the best experimental prospect of the group for carrying out the objectives of the study. It would represent the high end of the thermal resistance scale and might act as a general indicator of effects expected on less resistant species. Additionally, the species was readily available and ripe throughout the study period.

Effects of Temperature on Fertilization and Early Cleavage

The results of the fertilization experiments are presented in Table 3 and confirm the preliminary observations of phase one. Temperature treatments of 31.0, 34.0, and 36.0°C had no significant effect on the mean percent of fertilization success when compared to the mean success at the control temperature (28.0°C).

The results of the cleavage experiments also confirm the results of phase one (Table 3). Temperature treatments of 31.0 and 34.0°C had no significant effect on the mean percent of cleavage success when compared to the mean success at the control temperature (28.0°C). At 36.0°C the mean percent cleavage success was significantly different from the control (28.0°C). Only a small percentage of zygotes were found to undergo cleavage at 36.0°C and these became deformed and died after a few hours.

During two of the experiments, development of the zygotes was followed to the pluteus stage (48 hours) under thermal stress. Normal

Table 3. The effects of temperature on the mean percent success of fertilization (a), and early cleavage (b) of Echinometra mathaei. Parentheses enclose two standard errors of the mean. Means statistically homogeneous ($P \leq .01$) with the control mean are underscored by a horizontal line.

		WATER TEMPERATURE °C			
		28.0 [control]	31.0	34.0	36.0
A.	Percent Fertilized	99.8 (0.2)	99.5 (0.4)	99.5 (0.6)	97.7 (0.4)
		n = 18	n = 18	n = 18	n = 25
B.	Percent Normal Early Cleavage	99.5 (0.6)	99.4 (0.4)	98.9 (0.6)	3.5 (1.0)
		n = 20	n = 20	n = 20	n = 20

development and swimming activity was observed in approximately 100 percent of those at 31.0 and 34.0°C. As noted above, zygotes at 36.0°C did not survive beyond a few hours.

Effects of Exposing Spermatozoa to 36.0°C

Procedure

After establishing that continuous exposure to 36.0°C significantly reduced development to the four cell stage, spermatozoa were tested at this temperature to determine the exposure time required for loss of motility in 100 percent of the cells. Three ml aliquots of spermatozoa, at the same concentration as the fertilization experiments, were added with automatic pipettes to a series of twelve vials of fresh seawater, maintained at 36.0°C. The twelve vials were sampled in rotation every ten minutes until 100 percent of the spermatozoa in a 2 mm microscope field (10X) had lost motility.

Results

At the normal ambient temperature of 28.0°C (control), 100 percent of sperm cells had lost motility after 60 minutes (Fig. 2). At 36.0°C approximately 90 percent of the sperm cells had lost motility after 60 minutes. A few isolated cells remained active after 70 minutes. Eighty minutes was required for loss of motility in 100 percent of the cells. No explanation is readily available for this observation.

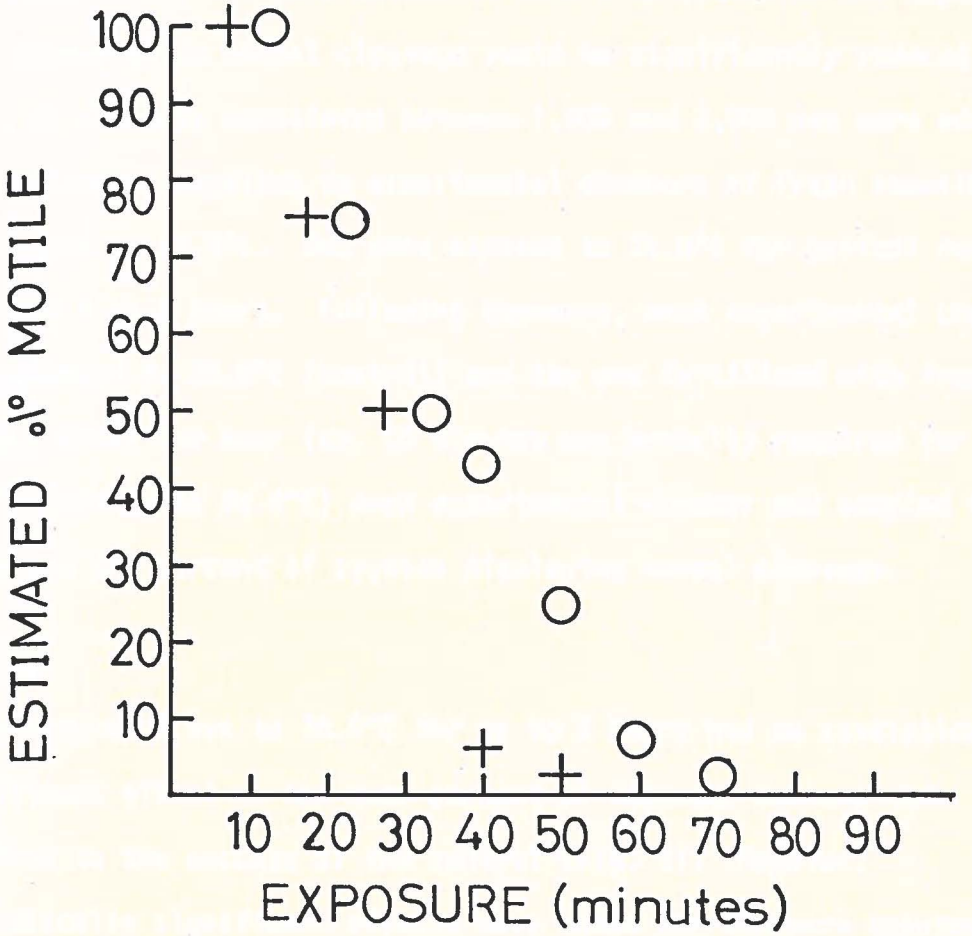

Figure 2. The estimated percent of motile Echinometra mathaei spermatozoa as a function of exposure time to 28.0° C (+) and 36.0° C (o).

Effects of Exposing Unfertilized Ova to 36.0°C

Procedure

Unfertilized ova were tested at 36.0°C to determine the exposure time beyond which normal cleavage would be significantly reduced. Three ml aliquots containing between 1,000 and 2,000 ova were added with automatic pipettes to experimental chambers of fresh seawater maintained at 36.0°C. Ova were exposed to 36.0°C for periods ranging from 1 to 6 hours. Following exposure, each experimental chamber was returned to 28.0°C (control) and the ova fertilized with fresh sperm. After one hour (ca. 55 minutes was normally required for first cleavage at 28.0°C) each experimental chamber was sampled to determine the percent of zygotes displaying normal cleavage.

Results

Exposure of ova to 36.0°C for up to 2 hours had no statistically significant effect on the mean percent of cleavage success when compared to the success of the control (Fig. 3). However, statistically significant effects were noted for exposure treatments of 3 hours and above.

When embryonic development was followed to the pluteus stage, 48 hours after exposure, it was found that approximately 100 percent of those exposed for 1 and 2 hours showed normal development. Those surviving to the pluteus stage after exposure of 3 hours were obviously fewer in number than those exposed for 2 hours and only isolated embryos were observed to reach the pluteus stage after

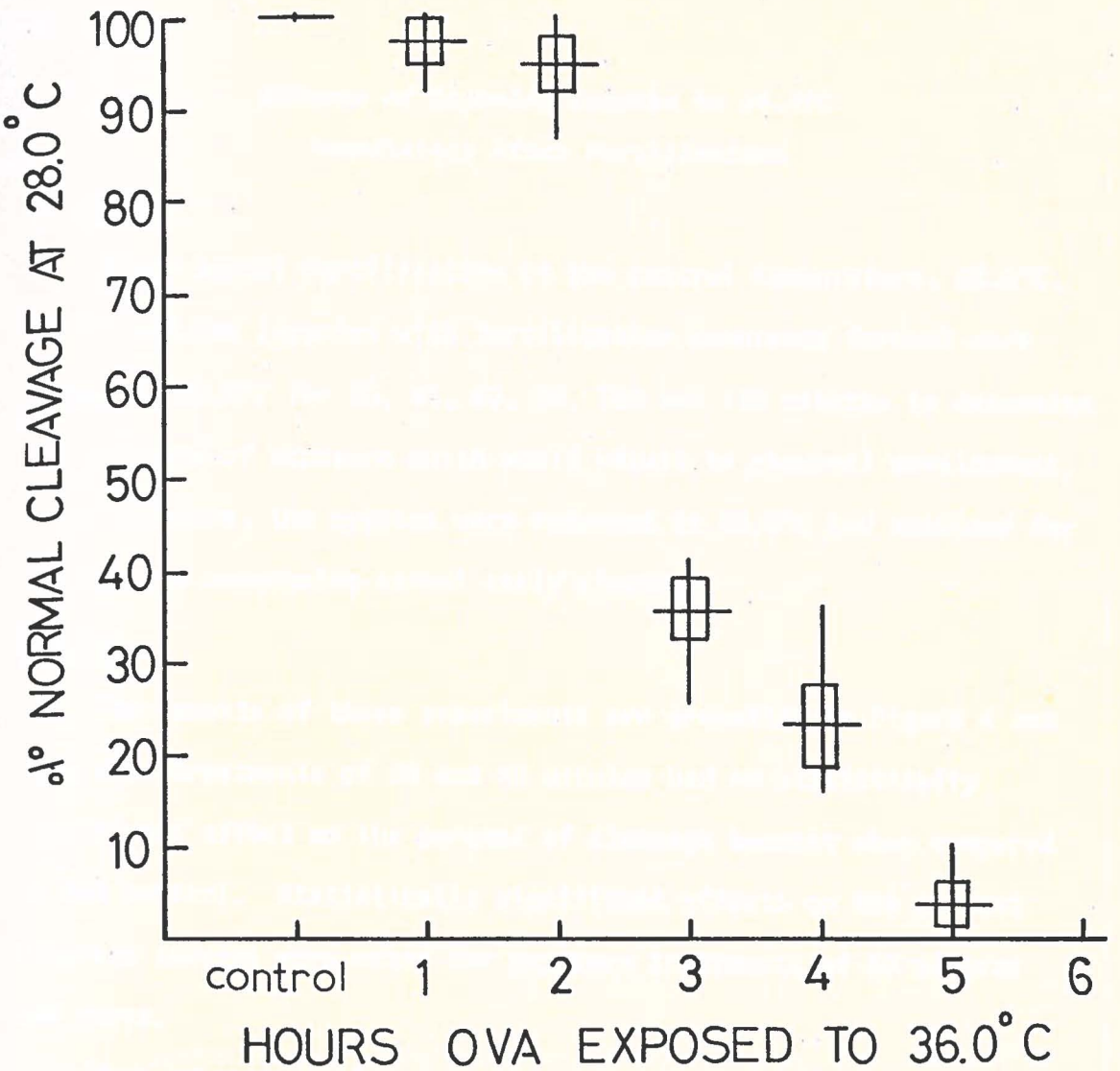

Figure 3. Percent of Echinometra mathaei zygotes undergoing normal early cleavage at 28.0° C, after exposure to 36.0° C for varying times before fertilization. Vertical line represents range; white bar, two standard errors; and the horizontal line, the mean. n = 9

exposure of 4 and 5 hours. None of the embryos survived exposure of 6 hours.

Effects of Exposing Zygotes to 36.0°C Immediately After Fertilization

Procedure

After normal fertilization at the control temperature, 28.0°C, fertilized ova (zygotes with fertilization membranes formed) were tested at 36.0°C for 20, 40, 60, 80, 100 and 120 minutes to determine the duration of exposure which would result in abnormal development. After exposure, the zygotes were returned to 28.0°C and examined for the percent undergoing normal early cleavage.

Results

The results of these experiments are presented in Figure 4 and show that treatments of 20 and 40 minutes had no statistically significant effect on the percent of cleavage success when compared to the control. Statistically significant effects on the percent cleavage success were noted for exposure treatments of 60 minutes and above.

When embryos developed to the pluteus stage (48 hours after exposure) it was found that approximately 100 percent of those exposed for 20, 40 and 60 minutes developed normally. However, few zygotes survived to the pluteus stage after exposure of 80 and 100 minutes, and none survived exposure of 120 minutes.

Effects of Exposing Zygotes to 36.0°C at First Cleavage

Procedure

Zygotes at the two cell stage were tested at 36.0°C to determine

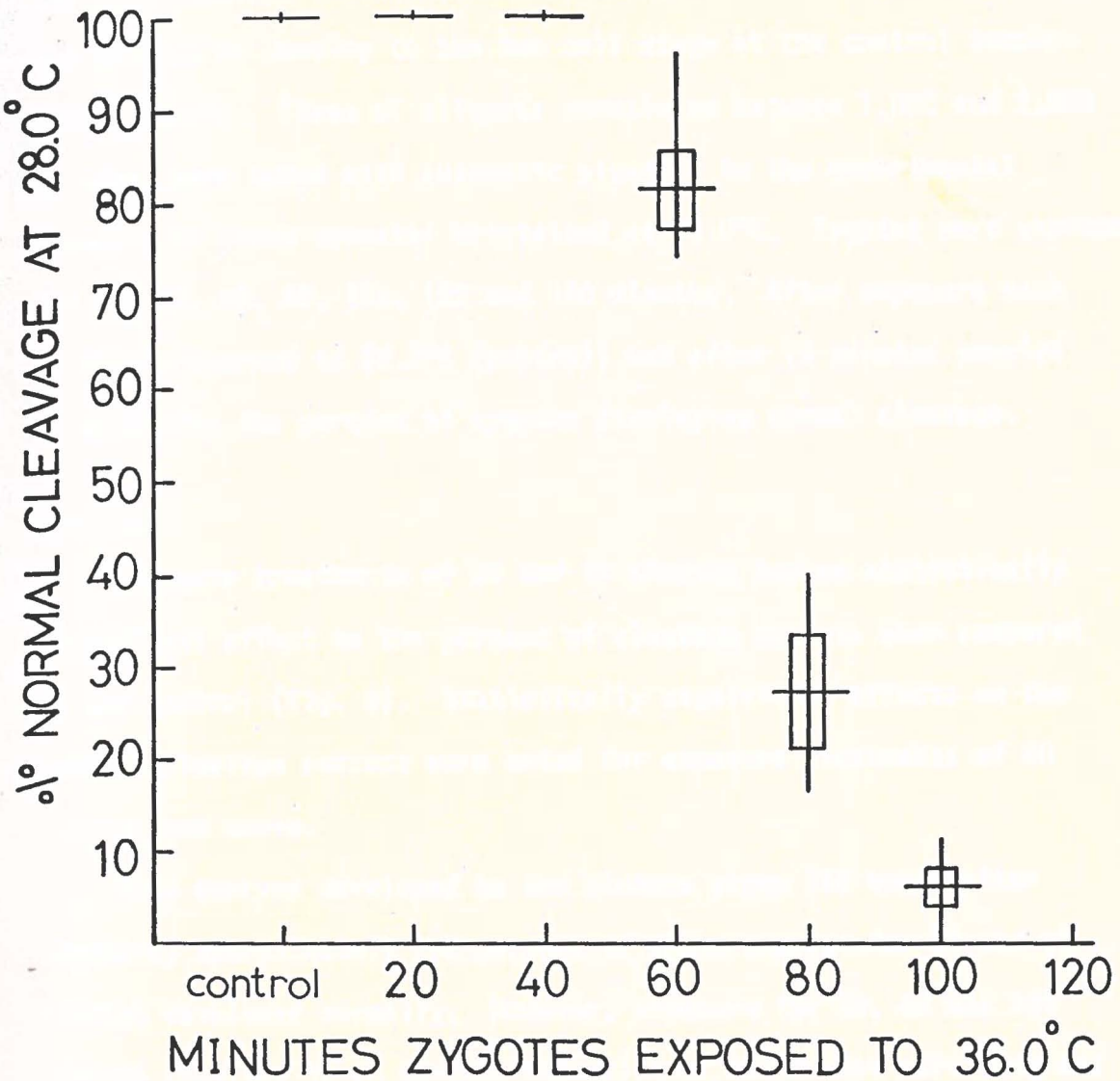

Figure 4. Percent of Echinometra mathaei zygotes undergoing normal early cleavage at 28.0° C, after exposure to 36.0° C for varying times immediately after fertilization. Vertical line represents range; white bar, two standard errors; and the horizontal line, the mean. n = 9

the time required to inhibit normal development. Ova were fertilized and allowed to develop to the two cell stage at the control temperature, 28.0°C. Three ml aliquots containing between 1,000 and 2,000 zygotes, were added with automatic pipettes to the experimental chambers of fresh seawater maintained at 36.0°C. Zygotes were exposed for 20, 40, 60, 80, 100, 120 and 140 minutes. After exposure each vial was returned to 28.0°C (control) and after 15 minutes sampled to determine the percent of zygotes displaying normal cleavage.

Results

Exposure treatments of 20 and 40 minutes had no statistically significant effect on the percent of cleavage success when compared to the control (Fig. 5). Statistically significant effects on the percent cleavage success were noted for exposure treatments of 60 minutes and above.

When embryos developed to the pluteus stage (48 hours after exposure) approximately 100 percent of those exposed for 20 and 40 minutes developed normally. However, exposure for 60, 80 and 100 minutes resulted in an obvious reduction of the number surviving and none survived exposure of 120 and 140 minutes.

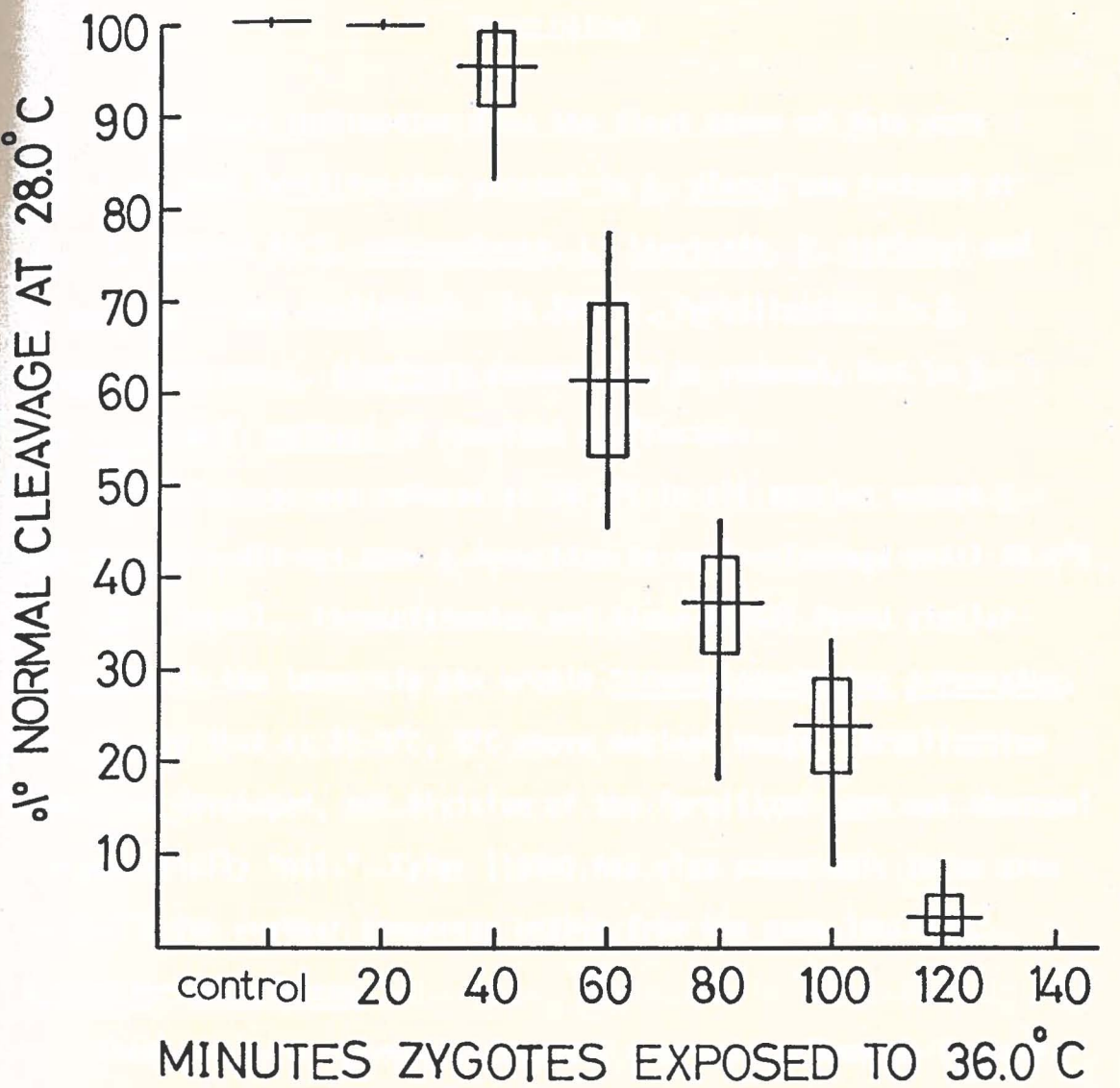

Figure 5. Percent of Echinometra mathaei zygotes undergoing normal early cleavage at 28.0° C, after exposure to 36.0° C for varying times at first cleavage stage. Vertical line represents range; white bar, two standard errors; and the horizontal line, the mean. n = 9

CONCLUSIONS

Preliminary information from the first phase of this work indicates that fertilization success in A. planci was reduced at 34.0°C, whereas in C. novaeguineae, L. laevigata, D. savignyi and E. mathaei it was unaffected. At 36.0°C, fertilization in C. novaeguineae and L. laevigata appeared to be reduced, but in D. savignyi and E. mathaei it remained unaffected.

Cell cleavage was reduced at 34.0°C in all species except E. mathaei which did not show a reduction in early cleavage until 36.0°C, 8°C above normal. Farmanfarmaian and Giese (1963) found similar results with the temperate sea urchin Strongylocentrotus purpuratus. They showed that at 25.0°C, 8°C above ambient range, fertilization membranes developed, but division of the fertilized eggs was abnormal and essentially "nil." Tyler (1936) has also shown this to be true at 25.0°C for another temperate urchin from the same locality, Dendraster excentricus.

These preliminary results suggest that early cleavage is more sensitive to increased temperature than fertilization for each species tested except A. planci in which both processes appear to be inhibited at 34.0°C.

Although development is inhibited in E. mathaei by continuous exposure at 36.0°C, it was found that both gametes and zygotes of this species are resistant to this temperature for short periods. Unfertilized ova are able to withstand exposure to 36.0°C for up to

two hours, but when exposed for three hours and then fertilized at normal temperature, the percent of zygotes undergoing normal cleavage is significantly reduced. Ova with fertilization membranes and zygotes at first cleavage were unaffected by exposures of 40 minutes or less, but showed a significant reduction in normal cleavage after one hour. The difference in duration of thermal resistance between unfertilized ova and zygotes suggests that activation of the ova by fertilization might render them somewhat less thermally resistant.

In any case, the ability of the early developmental stages of E. mathaei to withstand extreme temperature elevation for at least short exposure periods would presumably have survival value. For example, resistance for short periods might protect ova and zygotes from elevated temperatures brought about by a mid-day low tide, until relieved by the cooling water of the next flood tide. This, of course, would not be the case for reef environments adjacent to power plants. The continuous operation of these facilities would ensure a virtually constant thermal stress on the organisms and would effectively mask the flushing action of the tide cycle.

In summary, these data point to an upper thermal tolerance limit between 34.0°C and 36.0°C for early embryonic development of these species. The exact temperature affecting fertilization and cleavage may vary somewhat in a species specific manner and needs further investigation.

The critical temperatures are probably correlated with the environmental temperature conditions to which each species is

exposed. For example, fertilization and cleavage in A. planci, which usually inhabits deeper water with little diurnal temperature variation, appears to be significantly inhibited at 34.0°C, whereas fertilization and cleavage in E. mathaei, which often inhabits shallower water with broad temperature variations, is unaffected at 34.0°C.

The fact that E. mathaei appears to be a thermally resistant species in terms of its early embryonic development may therefore be a factor in its broad distribution on the coral reef.

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