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Effects of Egg Size on Fertilization, Fecundity and Offspring Performance: a Comparative Study Between Two Sibling Species of Tropical Sea Urchins (Genus *Echinometra*)

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Abstract: The effects of egg size on fertilization, fecundity, embryonic and larval development as well as offspring performance were compared between two closely related species of tropical sea urchins, *Echinometra* sp. A (Ea) and Echinometra sp. C (Ec) through laboratory experiments. Ec had significantly larger gametes in terms of egg diameter, egg volume and sperm head length and produced significantly larger but fewer eggs with higher fertilization rate than Ea. Developmental time from fertilization to the formation of echinus rudiment, just prior to the metamorphic competence of Ea were significantly longer than Ec, suggesting that increased allocation to energy reserves in larger Ec eggs reduced the development period as compared with the small Ea eggs. Consequently, smaller eggs of Ea produced smaller larvae than Ec throughout the larval period, due to the small amount of maternal investment as stored nutrients. As Ea and Ec have diverged from their ancestral species, the differences of the above traits related to egg sizes transcended species differences between Ea and Ec.

Key words: *Echinometra* spp. egg size, fertilization-fecundity trade-off, larval and juvenile performance

Introduction

Egg size is correlated with important reproductive and developmental traits including fecundity, larval size, duration of the developmental period, larval habitat, and mode of larval nutrition (Strathmann, 1985; Havenhand, 1995; but see also Roughgarden, 1989). Several reviews have provided further support for these trends in numerous marine taxa (Hermans, 1979; Reaka, 1979; Sastry, 1979; Hadfield and Switzer-Dunlap, 1984; Emlet *et al*., 1987). A central assumption underlying most of the studies is that parental investment per offspring directly determines the fitness of the offspring. A female can not increase offspring number without decreasing the size of individual offspring. Because, juvenile size can influence juvenile survival (Ferguson and Fox, 1984; Henrich *et al*., 1996), the evolution of offspring number and offspring size is presumably mediated by the fecundity advantage of producing small offspring balanced against the survival advantage of large offsprings.

Larvae that develop from larger eggs are thought to utilize increased egg reserves to develop rapidly and to experience a lower mortality relative to larvae from smaller eggs (Strathmann, 1985; Pearse, 1994). Larger eggs are also thought to act as better targets for sperm, thereby enhancing fertilization success but are less numerous (Levitan, 1993, 1998a and b).

Another important component of the trade-off concerns the size-dependence of offspring performance traits likely to affect the fitness (growth, escape from predators, feeding capability). Evolutionary changes in maternal investment per offspring not only affect fertilization success and offspring size per se, but can also affect other offspring traits, such as embryonic and larval development, juvenile and adult performance, which are functionally related to egg size (Emlet and Guldberg, 1997). Such correlations are thought to constrain evolutionary change (Atchley, 1987). An understanding of the developmental and physiological basis of these correlation should become a new focus of study.

On the basis of morphological, ecological, and genetic studies, sympatric *Echinometra mathaei* sensu lato in Okinawa are recognized as four distinct species, designated tentatively as Echinometra species A, B, C and D (Ea, Eb, Ec and Ed) (Uehara *et al*., 1991; Arakaki and Uehara, 1991; Metz and Palumbi, 1996). However, recent studies on morphological characters (Arakaki *et al*., 1998) suggest that Eb and Ed in Okinawa are *Echinometra mathaei* and *Echinometra oblonga*, respectively. The other two Okinawan species will be called E. Sp. Nov. A. (Ea) and E. Sp. Nov. C (Ec), respectively (Palumbi *et al*., 1997, Arakaki *et al*., 1998).

In this study, we presented comparative data describing the effect of egg size on fertilization, fecundity, embryonic and larval development as well as offspring sizes between these two congeners and elucidated the evolutionary consequences in such variations.

Materials and Methods

Collection and maintenance: Mature adults of the sea urchins, Echinometra sp. A (Ea) and Echinometra sp. C (Ec) were collected form Sesoko coast of the Okinawan Island at low tide during their natural breeding season from early July to the end of September, 1999 and from early June to the end of July, 2000 and maintained in aerated acuaria at the University of the Ryukyus, Okinawa, Japan until they were used for experiments.

Fecundity and gamete sizes: Mature female of various sizes of Ea and Ec were categorized according to their live body weight (g) and were used for study of fecundity. After removing the Aristotle's lantern, the gravid females were placed in glass beakers filled with 1µm filtered sea water (FSW) in such a way that the aboral portion was submerged in FSW. Eggs were collected by pouring 0.5M KCl into the coelomic cavity until approximate shedding of all eggs. The collected eggs were then diluted in 500 ml FSW in glass beakers and a 0.1 ml aliquot of the egg suspensions were placed on the well microscope slides and counted by a differential microscope. For each individual, 3 replicate counts were made. Sperms were collected "dry" and kept in refrigerator at 4-5°C for use in fertilization experiments following the procedure of Uehara *et al*. (1990). Diameter of eggs and length of sperm head were measured (eggs at 400 x in a well slide, sperms at 1000 x on a plain slide) by a differential microscope, using the methods of Amy (1983). Egg volume was calculated according to Emlet and Guldber (1997).

Gamete concentration and fertilization: The methods for fertilization were used from those described by Rahman *et al.* (2000). Briefly, adults were induced to spawn by intracoelmic injection of 0.5 M KCl. Eggs were shed in 1 µm FSW and washed three times with FSW to remove dirties and body fluids. To determine the fertilization rate of eggs, a 0.1 ml aliquot of diluted egg suspension (3,500-4,000 eggs/ml) was placed in a small vial with 0.8 ml of FSW. Fresh "dry" sperms were quickly diluted in a 4, 10-fold dilution (10^{-4} dilution). A 0.1 ml aliquot of this sperm suspension was then placed into the vial, to bring the final volume to 1 ml. Mixing of 0.1 ml aliquot of this sperm suspension was then placed into the vial, to bring the final volume to 1 ml. Mixing of 0.1 ml sperm suspension from this 10^{-4} dilution with 0.9 ml egg suspension in a vial thus, constituted 10^{-5} dilution. Sperms were then allowed to remain with the eggs for 5-10 minutes, then excess sperms were removed by three consecutive washes with FSW.

Twelve replicate conspecific inseminations were performed for each species using gametes, collected from new individuals in each time. The percentage of eggs fertilized was estimated after 1.25-1.5 h of insemination, using a compound microscope. The first 100 eggs encountered were classified as "fertilized" if they had reached the 2-4 cell stage (Rahman *et al.*, 2000, 2001).

Embryonic and larval development: The embryos and larvae were reared in FSW at 27-28° C approximating ambient water temperature (Rahman *et al.*, 2000). Developmental stages of embryos and larvae were observed at time intervals after insemination until they reached the competent (metamorphosis) stage. At each stage, specimens were fixed in 10% formalin for more detailed studies. Observations on both living and fixed specimens, provided information on the times required for embryos to attain specific developmental stages. In each experiment, the times after insemination for 50 % of the embryos to develop to 2-cell, 4-cell, 8-cell, blastula, gastrula, prism, 2-, 4-, 6-, 8-armed pluteus and competent stages were estimated, following Fujisawa (1993).

Morphometrics: Larval growth was examined through random sampling of larvae from each culture bottle. All morphometric measurements (Fig. 1) were made on freshly prepared specimens of 2-, 4-, and 8- armed pluteus larva (within 1-2 h) following the techniques previously described by McEdward (1984) with slight modifications. Larvae were killed in 10% formalin in FSW and were concentrated by settling to the bottom of a vial. A few drops of formalin-sea water containing about 10-20 larvae were put under an elevated coverslip on a microscope slide and finally measured by a compound microscope using a presetting objective micrometer. The skeletons of the larvae were also measured by the same method after dissolving the tissue in distilled water and subsequently treating them with KOH or 10 % haita (soap) solution.

Metamorphosis, juvenile rearing and measurement: After 22-24 days of rearing, the larvae that attained metamorphic competent stage were used for settlement induction (Rahman and Uehara, 2001). Competence was indicated by the presence of large echinus rudiment and a high rate of metamorphosis. Induction of metamorphosis of the larvae for both species was performed on coralline red algal stones. The algal stones were then immersed into 100 ml of FSW in petri dishes (9.0 x 4.0 cm^2). For each species, five replicate petri dishes with 25 larvae were prepared. Five days after metamorphosis, the newly produced juveniles together with attached algal stones were transferred to the aerated aquaria and cultured them up to one month. In all rears, FSW was partially changed every 3 days, and replenished with new sea water to maintain ambient temperature (27-28° C) and salinity (35-36ppt).

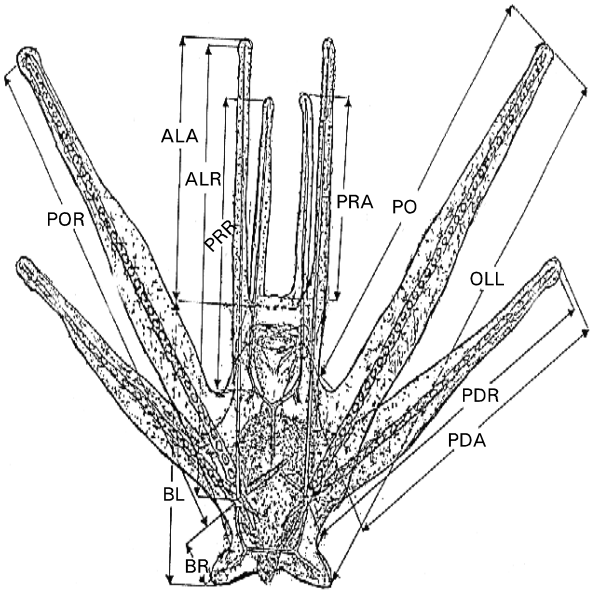

Fig. 1: Outline drawing of eight-armed pluteus stage of *Echinometra* spp., showing measurements compared in Tables 5,6 and 7 respectively. OLL, overall larval length; BL, body length; BR, body rod; POR, postoral rod; POA, postoral arm; ALA, anterolateral arm; ALR, anterolateral rod; PDA, posterodorsal arm; PDR, posterodorsal rod; PRA, preoral arm; PRR, preoral rod. Bar indicates 100µm.

Test diameter of newly metamorphosed juveniles of both Ea and Ec were measured for the first 1 day and at 5 days interval thereafter until 30 days of age. A total of 25 juveniles were randomly measured from each of two species at each time interval. All measurements were made on a compound microscope with presetting micrometer.

Data analysis: Comparison between the two treatment means in this experiment were performed by t-test. Homogeneity of variances were analyzed by "Bartlett's test" (Bartlett, 1937). When variances were not significantly heterogeneous and there were no major departures from normality, Student's t-test was conducted to analyze the differences in means. When variances were significantly heterogeneous or the data were not-normal, an Aspin-Welch's t-test was conducted.

Results

Gamete size: Measurement of Egg diameter, egg volume and sperm head length of Ea and Ec are presented in Table 1. Egg diameter of Ea (mean ± SE: 66.91 ± 0.47 µm, range : 65.0-65.5 µm) was significantly smaller (t-test, $P< 0.01$) than that of Ec (mean ± SE: 71.83 ± 0.33 µm, range: 70.8-75.3 µm). Egg volume and sperm head length also followed the same trend as egg diameter (Table 1).

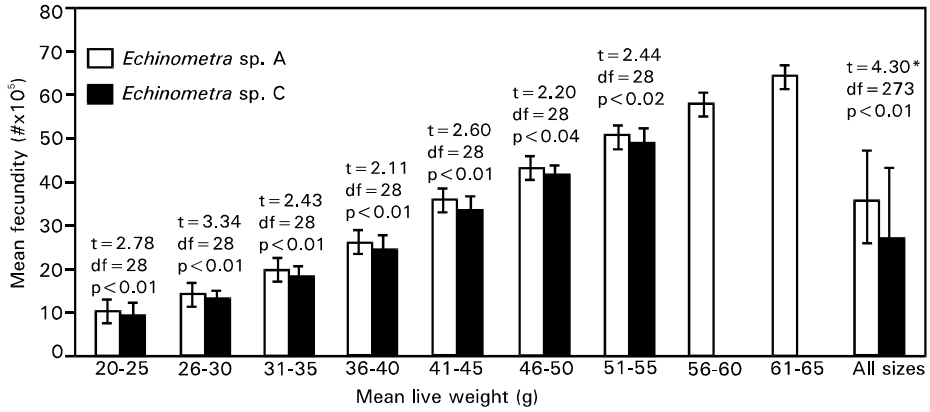

Fig. 2: Fecundity of full matured *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec) under various size groups which were categorized according to live body weight (g). For each size group, eggs from a total of 5 individuals for each species were counted. In case of Ea species, the maximum size was 64.95g, while it was 54.75 g for Ec. All values represent mean± SD in number (# x 10^5)* Due to heterogeneity in variances, an Aspin-Welch's t-test was conducted.

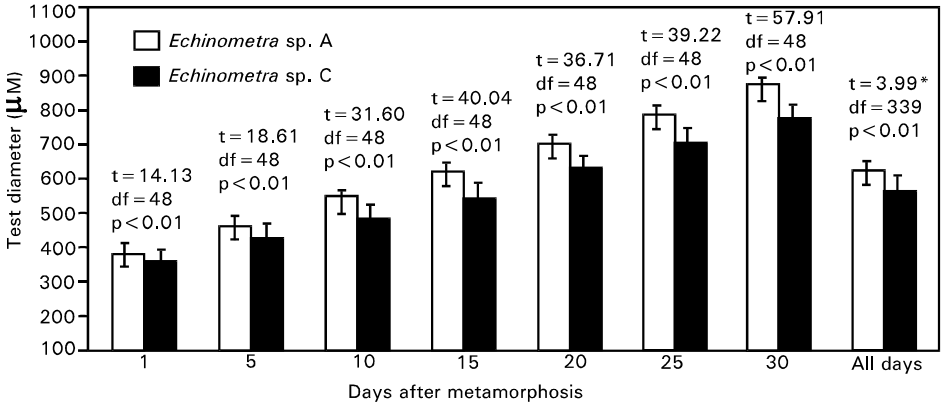

Fig. 3: Test diameter of the juveniles of *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec) after metamorphosis. A total number of 25 juveniles of each species for each of the listed days were measured. All values represent mean± SD in µm.
* Due to heterogeneity in variances, an Aspin-Welch's t-test was conducted.

Fecundity: As both the species had a wide range of body size in the field, they were categorized according to similar sizes in one group in such a way that the live weights of the urchins were within their respective size groups (such as 20-25 g, 26-30 g and so on).

Fecundity was expressed as the total number of eggs laid by fully matured gravid female during peak period of their breeding season. The mean fecundity of Ea and Ec under different size groups as well as all sizes were depicted in Fig. 2. Mean fecundity of each of the size groups of Ea was significantly higher than those of Ec (t-test, $P < 0.01$). Similarly, mean fecundity, pooled from all sizes of Ea (mean ± SD: 35.61 x 10^5 ± 18.51 x 10^5, range: 8.75 x 105-64.31 x 10^5) different significantly (t-test, $P < 0.01$) from that of Ec (mean ± SD: 26.64 x 10^5 ± 13.82 x 10^5, range: 7.75 x 10^5-48.31 x 10^5) (Fig. 2).

Fertilization: Conspecific fertilization rates of Ea and Ec eggs were compared at limited sperm concentration (10^{-5} dilution of 'dry' sperm). At this concentration, the fertilization rate of Ec (mean ± SD: 99.67 ± 0.49 %, range: 99.0 - 100.0%) were significantly higher (t-test, $P < 0.01$) than the percent fertilization of Ec conspecifics (mean ± SD: 90.25 ± 1.36 %, range: 88.0-92.0%) (Table 2).

Embryonic and larval development time table: A list of the embryonic developmental stages and the times required to reach them is given in Table 3. It was apparent that the developmental times sequentially from 2-cell to prism stage of Ec were significantly longer (t-test, $P < 0.01$) than those of Ea. Time tables for the larval development from 2-armed pluteus until competent stage were also assessed by following the similar procedure employed for embryonic stages. It is evident from Table 4 that development of each specific stage of Ea took significantly longer time (t-test, $P < 0.01$) than those of Ec.

Larval sizes: At two-armed pluteus stage of larval development, mean OLL, BL, POA and POR of Ec were significantly larger (t-test, $P < 0.01$) than those of Ea, but the BR did not differ significantly (Table 5). Planktophonic larvae from both species of this experiment started feeding when they attained 4-armed pluteus stage (about 2 days after fertilization). Measurement of all possible morphometric characters (Fig. 1) was also done from Ea and Ec for their subsequent stages of development (4- and 8-armed stages).

Table 1: Diameter and volume of eggs and sperm head length of *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec). Six individuals were examined from each species with 100 eggs and 100 sperms from each species. All values represent mean ± SE with ranges in parentheses.

Species	Egg diameter (µm)	Egg volume (10^5.µm^3)	Sperm head length (µm)
Echinometra sp. A	66.91± 0.47 (65.00-67.50)	1.57± 0.02 (1.53-1.57)	3.93± 0.13 (3.83-4.11)
Echinometra sp. C	71.83± 0.33 (68.75-73.50)	1.94± 0.02 (1.90-1.94)	6.37± 0.23 (5.96-7.44)
Student's t-test	t = 21.09 df = 10 P< 0.01	t = 21.40 df = 10 P< 0.01	t = 21.28 df = 10 P< 0.01

Table 2: Percent fertilization of *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec) at limited sperm concentration (10^{-5} dilution of "dry" sperm). Each item under the "Number" column indicates number of conspecific crosses. In all crosses gametes (sperms and eggs) from new individuals were used. Fertilization rate was calculated 1.25-1.5 h after gamete mixing by counting 100 eggs that had reached 2-4 cells stage. All values represent mean ± SD with ranges in parentheses.

Species	Number	Fertilization (%)	Aspin-Welch's t-test
Echinometra sp. A	12	90.25± 1.36 (88.0-92.0)	t = 22.60* df = 13 P< 0.01
Echinometra sp. C	12	99.67± 0.49 (99.0-100.0)	

* Due to heterogeneity in variances, an Aspin-Welch's t-test was conducted.

Table 3: Developmental time of *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec)., required for the embryos to reach the stages listed at a laboratory temperature of 27-28° C. Developmental times (min) are those taken for 50 % embryos to arrive at each stage. Six replicate experiments were conducted for each species with gametes from new individual each time. All values represent mean ± SD in min with ranges in parentheses.

Developmental stages	*Echinometra* sp. A (Min)	*Echinometra* sp. C (Min)	Student's t-test
2-cell	77.67± 1.86 (75.00-80.00)	72.16± 1.16 (71.0-74.0)	t = 6.13 df = 10 P< 0.01
4-cell	133.17± 1.17 (132.0-135.00)	124.33± 121 (123.00-126.00)	t= 12.85 df = 10 P< 0.01
8-cell	160.33± 1.63 (158.00-162.00)	151.83± 1.60 (150.00-154.00)	t = 9.97 df = 10 P< 0.01
Blastula	435.33± 1.37 (433.00-437.00)	423.83± 1.47 (442.00-426.00)	t = 14.03 df = 10 P< 0.01
Gastrula	742.67± 1.36 (741.00-745.00)	732.00± 1.90 (730.00-735.00)	t = 11.17 df = 10 P< 0.01
Prism	986.33± 1.86 (984.00-989.00)	971.17± 1.94 (968.00-973.00)	t = 13.81 df = 10 P< 0.01

Table 4: Developmental time of *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec)., required for the larvae to reach the stages listed at a laboratory temperature of 27-28° C. Developmental times (min) are those taken for 50 % embryos to arrive at each stage. Six replicate experiment were conducted for each species with gametes from new individual each time. All values represent mean ± SD in min with ranges in parentheses.

Developmental stages	*Echinometra* sp. A (Min)	*Echinometra* sp. C (Min)	Student's t-test
2-arm pluteus	1,901.0± 7.57 (1,885.0-1,920.0)	1,833.8± 9.5 (1,820.0-1,845.0)	t = 8.23 df = 10 P< 0.01
4-arm pluteus	2, 755.5± 14.1 (2,735.0-2,775.0)	2,709.3± 14.1 (2,692.0-2730.0)	t = 5.67 df = 10 P< 0.01
6-arm pluteus	8,271.3± 13.9 (8,290.0-8,250.0)	8,207.9± 9.1 (8,195.0-220.0)	t = 9.38 df = 10 P< 0.01
8-arm pluteus	21,612.8± 10.9 (21,612.0-21,640.0)	21,549.5± 11.6 (21,535.0-21,565.0)	t = 6.48 df = 10 P< 0.01
Competent	31,648.8± 12.7 (31,620.0-31,680.0)	31,550.8± 13.9 (31,535.0-31,570.0)	t = 9.01 df = 10 P< 0.01

Table 5: Comparison of five morphometric characters of the larvae in *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec) at two-armed pluteus stage. A total of 25 larvae were measured for each species. All values represent mean ± SD in µm with ranges in parentheses. See Fig. 1 for measurements.

Morphometric characters	*Echinometra* sp. A (µm)	*Echinometra* sp. C (µm)	Student's t-test
OLL (Overall larval length)	211.6± 6.72 (200.3-225.5)	229.7± 5.9 (220.9-240.4)	t = 10.12 df = 48 P< 0.01
BL (Body length)	124.5± 4.0 (118.6-132.5)	137.9± 4.6 (128.9-146.0)	t = 10.9 df = 48 P< 0.01
BR (Body rod)	80.3± 2.3 (75.6-83.5)	81.2± 1.9 (77.6-85.7)	t = 1.57 df = 48 P> 0.01
POA (Postoral arm)	103.8± 4.2 (96.8-110.6)	118.9± 5.0 (109.7-127.9)	t = 12.02 df = 48 P< 0.01
POR (Postoral rod)	121.9± 3.2 (115.6-128.6)	136.1± 4.5 (127.7-143.9)	t = 12.93 df = 48 P< 0.01

Table 6: Comparison of seven morphometric characters of the larvae in *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec) at four-armed pluteus stage. A total of 25 larvae were measured for each species. All values represent mean ± SD in µm with ranges in parentheses. See Fig. 1 for measurements.

Morphometric characters	*Echinometra* sp. A (µm)	*Echinometra* sp. C (µm)	Student's t-test
OLL (Overall larval length)	298.0± 9.4 (281.4-318.7)	337.2± 7.2 (325.9-350.7)	t = 14.17* df = 40 P< 0.01
BL (Body length)	144.1± 4.8 (136.6-155.7)	162.8± 5.6 (155.5-172.5)	t = 12.71 df = 48 P< 0.01
BR (Body rod)	81.3± 2.0 (77.7-83.9)	84.9± 1.7 (81.3-87.7)	t = 6.99 df = 48 P> 0.01
POA (Postoral arm)	169.5± 5.3 (158.5-175.8)	202.3± 9.0 (190.7-215.6)	t = 15.67 df = 48 P< 0.01
POR (Postoral rod)	203.2± 8.4 (185.2-215.4)	239.8± 7.1 (228.7-250.9)	t = 16.6 df = 48 P< 0.01
ALA (Anterolateral arm)	42.1± 3.4 (35.6-47.6)	55.1± 5.0 (47.8-60.2)	t = 10.79 df = 48 P< 0.01
ALR (Anterolateral rod)	107.5± 6.7 (92.6-115.7)	229.9± 6.0 (120.0-140.9)	t = 10.79 df = 48 P< 0.01

* Due to heterogeneity in variances, an Aspin-Welch's t-test was conducted.

It could be seen from Table 6 and Table 7 that all morphometrics differed significantly (t-test, P< 0.01) between the two species. Larvae of Ea always showed smaller sizes compared with that produced by Ec.

Juvenile sizes: All the pelagic larvae attained competent stage (bottom dwelling mode of life) within 22-24 days post-fertilization. They were then allowed to metamorphose on coralline red algal stones. Complete metamorphosis from feeding larva to feeding juveniles took place in about 1 day. This included the complete development of internal organs as well as the formation of the adult mouth, anus, tube-feet and spines (Rahman and Uehara, 2001). Five days after metamorphosis, all the tiny juveniles of Ea and Ec were cultured in the similar rearing conditions. Test diameters from 1 day old juveniles until 30 days of age at every 5 days interval were measured. In each time interval, it was observed that test diameters were significantly larger (t-test, P< 0.01) in Ea than Ec, though Ea have significantly smaller eggs than Ec (Fig. 3). Similarly, mean test diameter, pooled from all ages (1-30 d) of Ea (mean ± SD: 625.95± 162.73 µm, range: 396-885 µm) was significantly larger (t-test, P< 0.01) than that of Ec (mean ± SD: 561.30 ± 139.34 µm, range: 348-785 µm) (Fig. 3).

Discussion

Our laboratory experiment has revealed five clear results: 1) Ea produced the larger number of eggs of the smaller size than Ec; 2) large eggs of Ec are fertilized at a greater rate than smaller Ea eggs; 3) small eggs of Ea yield larvae that are small in size; 4) small eggs result in slower development through the embryonic and larval stages; 5) effects of egg sizes are restricted to larval stages (i.e., juvenile sizes are independent of egg size).

The effect of egg size on fecundity and fertilization are the most clearly seen in comparison between these two closely related and genetically divergent Ea and Ec. The results demonstrated that Ec produced significantly larger but fewer eggs with higher fertilization compared with significantly smaller but numerous eggs produced by Ea. Working with three congeneric sea urchins, *Stongylocentrotus droebachiensis, S. purpuratus* and *S. franciscanus,* Levitan (1993) observed that larger eggs were fertilized at a greater

Table 7: Comparison of eleven morphometric characters of the larvae in *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec) at eight-armed pluteus stage. A total of 25 larvae were measured for each species. All values represent mean ± SD in µm with ranges in parentheses. See Fig. 1 for measurements.

Morphometric characters	*Echinometra* sp. A (µm)	*Echinometra* sp. C (µm)	Student's t-test
OLL (Overall larval length)	1026.4± 9.3 (1010.7-1040.5)	1078.5± 7.4 (1065.5-1090.7)	t = 21.05 df = 48 P< 0.01
BL (Body length)	558.3± 8.7 (545.4-570.9)	584.3± 8.7 (568.7-592.3)	t = 10.6 df = 48 P< 0.01
BR (Body rod)	112.7± 4.8 (104.7-120.3)	128.2± 3.7 (120.9-133.9)	t = 12.72 df = 48 P> 0.01
POA (Postoral arm)	619.5± 8.7 (604.7-632.4)	669.2± 9.7 (640.3-673.4)	t = 23.62 df = 48 P< 0.01
POR (Postoral rod)	770.7± 8.9 (756.7-785.7)	827.1± 7.8 (815.9-840.6)	t = 23.80 df = 48 P< 0.01
ALA (Anterolateral arm)	420.7± 8.5 (406.9-433.3)	462.9± 8.3 (450.7-475.7)	t = 17.81 df = 48 P< 0.01
ALR (Anterolateral rod)	776.7± 7.0 (768.2-788.7)	832.0± 10.1 (818.5-850.7)	t = 22.50 df = 48 P< 0.01
PDA (Posterodorsla arm)	517.7± 8.5 (504.3-530.4)	551.5± 9.0 (535.8-565.7)	t = 13.62 df = 48 P< 0.01
PDR (Posterodorsla rod)	712.2± 12.4 (690.6-727.4)	767.1± 8.0 (755.8-781.2)	t = 18.60* df = 41 P< 0.01
PRA (Preoral arm)	312.5± 8.2 (298.2-325.7)	354.3± 7.4 (340.1± 364.2)	t = 18.93 df = 48 P< 0.01
PRR (Preoral rod)	475.0± 6.0 (465.2-485.7)	530.6± 7.2 (520.3-542.1)	t = 29.15 df = 48 P< 0.01

* Due to heterogeneity in variances, an Aspin-Welch's t-test was conducted.

rate because they provide a higher target for sperm. Other gamete attributes are most likely to influence fertilization success including receptiveness of the egg surface to sperm, sperm velocity, sperm longevity, viscosity and dispersability of gametes, proportion of sperm egg collisions, egg buoyancy, the size and presence of egg jelly coat or other structures that can capture sperms, the presence of sperm chemoattactants (Denny and Shibata, 1989; Levitan, 1993, 1998a, b; Thomas, 1994; Podolsky and Strathmann, 1996 and Levitan, 1998b) and most recently gamete recognition molecules (Vacquier *et al.*, 1995; Metz and Palumbi, 1996; Palumbi, 1998).

The experiment revealed that embryonic stages (2-cell to prism stage) and larval stages (2-arm pluteus to competent stage) were slower for larvae from smaller Ea eggs than the comparatively larger Ec eggs. An important correlate of small egg size in A in comparison to Ec is the increased developmental time to metamorphic competence. Similar results were also obtained in other marine invertebrates (Amy, 1983; Sinervo and McEdward, 1988). Interspecific comparisons (Sinervo and McEdward, 1988; Havenhand, 1993; Kohn and Perron, 1994) and experimental manipulation of egg size (Sinervo and McEdward, 1988; McEdward, 1996) both indicate longer periods of development for larvae from smaller eggs. All of these experiments with feeding larvae demonstrate that increased allocation has been considered necessary to provide materials and energy reserves to eggs can reduce the larval development period and is consistent with interpretations of variation in egg size reflecting rates of larval mortality (Strathmann, 1985; Emlet *et al.*, 1987).

The present study demonstrated that egg size has a significant effect on larval sizes throughout the period of larval development of A and Ec. This is due to the fact that the eggs of A are smaller than those of Ec. Smaller eggs produce smaller larvae. This result may be general to many echinoid species (Emlet *et al.*, 1987; McEdward, 1996; Emlet and Guldberg, 1997 etc). Other experimental studies provide support for a general relationship between egg size and form in Echinoderm larvae (McEdward, 1996; Sinervo and McEdward, 1988). However, Sinervo and McEdward (1988) demonstrated that the differences that are related to egg size do not persist throughout the larval development. Beyond the six-armed pluteus stage, larval size are the same among experimental treatments in the two species of *S. droebachiensis* and *S. purpuratus.* Their results differ from ours in that larger eggs of Ec produces significantly bigger larvae than A with smaller eggs throughout the larval period. The amount of material (maternal investment) the mother provides to each egg determine on how large and elaborate a larvae can develop from an egg. Larvae that develop from smaller eggs must be smaller due to shortage of stored nutrients in terms of energetic contents for larval development and growth (Sinervo and McEdward, 1988).

Experimental manipulate of egg size has been shown to affect the offspring size and growth performance. Egg size has also been experimentally manipulated in sea urchins to explore the consequences for larval developmental rates. McEdward (1996) carried out two and four fold reductions of egg size in species with feeding larvae of *Strongylocentrotus droebachiensis.* Reduction in egg size slow down the rate of development through

the early feeding larval period and increased overall length of the larval period.

Traditionally large eggs of marine invertebrates have been considered necessary to provide materials and energy reserves that permit rapid larval development (Strathmann, 1985). Some echinoderm and opisthobranch taxa with large eggs also produce significantly larger juveniles than those with smaller eggs (Hadfield and Miller, 1987). However, comparative studies on echinoderm that showed little or no effect of egg biomass in the newly metamorphosed juveniles (McClintock and Pearse, 1986). Considering the above findings, the influence of egg size on juvenile size is assessed with 1-30 days old urchins from both A and Ec. Our results demonstrate that juveniles from smaller A eggs are significantly larger than those from bigger Ec eggs, which is completely opposite to the larval performances. This may be due to the fact that juvenile size is not correlated with egg size, because regulation is completed during the larval period (Sinervo and McEdward, 1988). This further suggests that larval sizes are more likely controlled by cytoplasm as stored nutrients, while juvenile sizes are controlled genetically (Sinervo and McEdward, 1988).

The general conclusion is that for these *Echinometra* spp., egg size (parental investment of stored nutrients per offspring) is traded off against developmental time, that is the larger the egg, the shorter the development time and larger eggs may act as better target for sperm thereby enhancing the fertilization success. Larger Ec eggs yield larger larvae compared with those of the smaller A eggs during the whole period of larval development but do not persist in juvenile stages, instead they rapidly undergo metamorphosis, and grow rapidly into larger larvae than A with smaller eggs. Our results clearly demonstrate that egg size has a direct effect on fecundity, fertilization, larval size and developmental time. Even the substantial differences in the above traits that separate A and Ec seem to be explained by differences in egg size in concordance with the study on the two congeneric sea urchins, *S. droebachiensis* and *S. purpuratus* (Sinervo and McEdward, 1988).

Sequences from the COI gene region of mt DNA indicate that congeneric *Echinomera* spp. are of recent origin, having diverged within the middle Pleistocene (less than 3 million years old) (Palumbi, 1996). Moreover, the molecular phylogenetic tree constructed on the basis of the Nei's genetic distances revealed the close affinities between A and Ec (Matsuoka and Hatanaka, 1991). Due to their evolutionary diversification from the ancestral species, natural selection on a life history trait such as egg size is likely to influence a number of functionally important traits in these two sibling species. The relationship between the adaptive evolution of life histories and the evolution of ontogeny of these two species clearly deserves further attention. The identification of developmental couplings is essential to our understanding of the evolution of suites of life-history traits.

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