ScienceAsia 30 (2004): 75-80

Effects of Egg Size on Fertilization and Embryonic Development of Sibling Tropical Sea Urchins (Genus, *Echinometra*)

Sk. Mustafizur Rahman,* Tsuyoshi Uehara

Graduate School of Science and Engineering, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan.

* Corresponding author, E-mail: mustafizku@hotmail.com

Received 10 Sep 2003
Accepted 17 Nov 2003

ABSTRACT: Egg size has the ability to influence fertilization success and embryonic development in free-spawning marine invertebrates. The effects of egg size on fertilization and embryonic development were compared among three sibling species of *Echinometra* sp. B, *Echinometra* sp. C, and *Echinometra* sp. D. Gamete size of *Echinometra* sp. D is larger than *Echinometra* sp. B and *Echinometra* sp. C. Larger eggs of *Echinometra* sp. D might provide a larger target for sperm and thus, were fertilized at higher rate than smaller *Echinometra* sp. B and *Echinometra* sp. C eggs under moderate and limiting sperm concentrations. Developmental time from fertilization to prism stage (embryonic development) in *Echinometra* sp. B and *Echinometra* sp. C were comparatively longer than *Echinometra* sp. D, suggesting that increased allocation of energy reserve in the cytoplasm in larger *Echinometra* sp. D eggs reduced the developmental period. As *Echinometra* sp. B, *Echinometra* sp. C and *Echinometra* sp. D have diverged from their ancestral species, the differences of the above traits related to egg sizes transcended species differences among them.

Keywords: Sibling species, *Echinometra*, eggs size, fertilization, and embryonic development.

INTRODUCTION

Sibling species, often called cryptic species, are too similar to discriminate in appearance, but they are regarded as independent species in the natural population.[1] The four species of *Echinometra*, one of the most conspicuous organisms found in Okinawa, Japan, are no exception. *Echinometra* species are widely distributed in shallow reef environments across the tropical Pacific and Indian oceans.[2-4] Recent morphological, biochemical, ecological, and reproductive studies have revealed the presence of four sympatric biological species of sea urchin within the *Echinometra mathaei* species complex in Okinawa. These species have been referred as *Echinometra* species A, B, C, and D and now are treated as species rather than type. *Echinometra* sp. B is now recognized *as E. mathaei* [2,5,6], while *Echinometra* sp. D belongs to the *E. oblonga* species complex, which may include at least three species.[7] The existing four species of *Echinometra* in Okinawa were first reported by Uehara[4] based on their morphological characteristics as well as fertilization level, and thereafter, many scientists supported that these species are completely distinct.

Egg size is a central trait in studies of the ecology and evolution of marine invertebrate life histories. Egg size is correlated with important reproductive and developmental traits including fertilization, fecundity, larval size, duration of the developmental period, larval habitat, and mode of larval nutrition in numerous taxa.[10-16] A central assumption underlying most of the above studies is that parental investment per offspring directly determines the performance traits of offspring. Larvae that develop from larger eggs are thought to utilize increased egg reserves to develop rapidly and to experience lower mortality relative to larvae from smaller eggs.[17-19] Larger eggs are also thought to act as better target for sperm, thereby enhancing fertilization success.[10-12] Besides, egg size is an important component of fitness in marine benthic invertebrates through its relationship to fecundity, larval size, development period, and other life history trails.[20-23] While most interpretations of the significance of egg size are in energetic terms, i.e. parental investment as stored nutrients,[21,23-25] it has also a biologically relevant geometric (morphological) interpretation. Egg size may determine the size and shape of an organism independently of growth (increase in biomass), and have important consequences for organismal functions such as feeding, defense, and locomotion.

In this study, we sought to know whether egg size influences fertilization success and embryonic

development among the sibling species, and if so, what are the evolutionary consequences involved in such variations. We addressed this issue by investigating the relationship between egg size and fertilization as well as egg size and embryonic development of three genetically divergent sibling species belonging to genus *Echinometra*. The results of the present study would be an added contribution towards the ongoing debate on the speciation of *Echinometra* species.

Materials and Methods

Adult Collection and Maintenance

Healthy mature adults of *Echinometra* sp. B (recognized *as E. mathaei*), *Echinometra* sp. C and *Echinometra* sp. D (recognized *as E. oblonga*) were collected from the Pacific ocean, Sesoko Island ($26^0$38' N; $127^0$51'E), Okinawa, at low tide during their natural breeding season from May to July, 2001. Immediately after collection, the specimens were transported to the laboratory and maintained in closed aquarium before use, for not more than a week. Algae grown on the inside wall of the aquarium served as food for the urchins.

Gamete Size

The observation of gamete size was carried out by a differential microscope. Five individuals were examined from each species with 50 eggs and sperms. Gametes were measured (eggs at x 400 in a well slide, sperm at x 1000 on a plain slide) by differential microscope, using the methods of Amy.[26]

Gamete Shedding

Gametes were obtained by injecting sea urchins with 0.05 M KCl. A drop of sperm was diluted in filtered seawater and their motility was observed. If the sperms showed high motility and the eggs contained distinct nucleus, then they were used for fertilization experiment. If the sperms appeared sluggish and the eggs seemed not uniform, the batch was discarded. After injecting KCl, the sperms were collected by pipette in "dry form" and then kept into the refrigerator (3 – 4^0C). The eggs were collected in the same method and washed with sea water at least 2-3 times to remove body fluid and associated dirts.

Gamete Concentration and Fertilization Experiment

Three series of sperm dilutions were prepared from the freshly collected "dry sperm": higher sperm concentration (dilution factor of 5×10^{-5}) where almost all eggs become fertilized (100%); moderate sperm concentration (dilution factor of 4.3×10^{-6}) where slightly lower fertilization rate (about 90%) was observed; and limiting sperm concentration (dilution factor of 1.03×10^{-6}) where about 60% fertilization rate was observed.

In practice, about 7.5 μl of dry sperm was added to 5 ml of sea water resulting in a sperm concentration with a dilution factor of $10^{-3} \times 1.5$ with regard to undiluted dry sperm (A). 100 μl of sperm solution A was diluted with 3 ml of sea water giving rise to a sperm concentration with a dilution factor of $10^{-5} \times 5$ (Higher sperm concentration; B). From sperm solution B, 300 μl was added to 3 ml of sea water to obtain a sperm concentration with a dilution factor of $10^{-6} \times 4.3$ (Moderate sperm concentration; C). Further, 500 μl of sperm solution B was mixed with 3 ml of sea water to obtain a concentration with a dilution factor of $10^{-6} \times 7.2$ (D). Finally, 500 μl of sperm solution D was added to 3 ml of sea water to have a sperm concentration with a dilution factor of $10^{-6} \times 1.03$ (Limiting sperm concentration). All these preparations were made within a minute to ensure the maximum viability of sperm.

For fertilization experiment, same numbers of eggs (1800-2000) were placed in small beaker (20 ml). The eggs were then inseminated with the sperm solutions having different concentrations as mentioned above. The sperms were then allowed to remain with the eggs for a few minutes, and washed 2-3 times with seawater. The fertilized eggs were then re-suspended in sea water for incubation. The percent of egg fertilized was estimated 1.3 hours after the insemination. Fertilization was accomplished at room temperature (26-28^0C). All experiments were conducted with replicates of five individuals from each species.

Developmental Timetable

Developmental stages of embryos were observed at time intervals after insemination until they reached the pluteus stage. At each stage, specimens were fixed in 10% formalin for more detail studies. Observations on both living and preserved specimens provided information on the time intervals required for embryos to attain specific developmental stages. In each experiment, the times after insemination for 50% of the embryos to develop to the 2-cell, 4-cell, 8-cell, blastula, gastrula, prism and pluteus stages were estimated following the methods of Fujisawa.[27]

Data Analysis

Percentages were arcsine transformed to normalize the data and reduce heterogeneity in variances. Homogeneity of variances was analyzed by a Bartlett test;[28] when variances were not significantly heterogeneous and showed no major departure from normality, a one-way ANOVA was done followed by Tukey's multiple comparisons test. For the data that did

Table 1. Chronology of embryonic development. Developmental times (min) are those taken for 50% embryos to arrive at each stage. Developmental times of *Echinometra* sp. D varied significantly (Tukey's test $p < 0.05$) with *Echinometra* sp. C and *Echinometra* sp. B. Five replicate experiments were conducted for each species with gametes from new individuals in each time. All values represent mean ± SD.

Stages	*Echinometra* sp. D(min)	*Echinometra* sp. C(min)	*Echinometra* sp. B(min)
2 - Cell	67.23 ± 1.36 [b]	70.25 ± 1.86 [a]	71.84 ± 1.17 [a*]
4 - Cell	116.11 ± 1.07 [b]	126.21 ± 1.90 [a]	128.45 ± 1.16 [a]
8 - Cell	139.19 ± 1.33 [b]	154.37 ± 1.36 [a]	152.15 ± 1.86 [a]
Blastula	386.60 ± 1.56 [b]	421.91 ± 1.47 [a]	427.61 ± 1.41 [a]
Gastrula	654.31 ± 1.73 [b]	719.46 ± 1.53 [a]	731.73 ± 2.13 [a]
Prism	878.23 ± 1.94 [b]	986.50 ± 0.93 [a]	1001.89 ± 1.83[a]

* Mean values in the same row having the same superscript are not significantly different (Tukey's test $p > 0.05$).

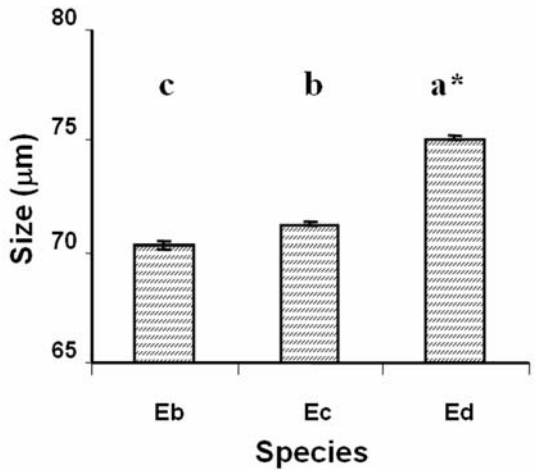

Fig 1. Mean values of egg diameter (mm) of *Echinometra* spp. Five individuals were examined from each species with 50 eggs. Error bar indicates standard error. There were significant differences (Tukey's test $p < 0.05$) in egg size of all *Echinometra* species.
Eb – *Echinometra* sp. B
Ec – *Echinometra* sp. C
Ed – *Echinometra* sp. D
* Same superscripts are not significantly different (Tukey's test $p > 0.05$).

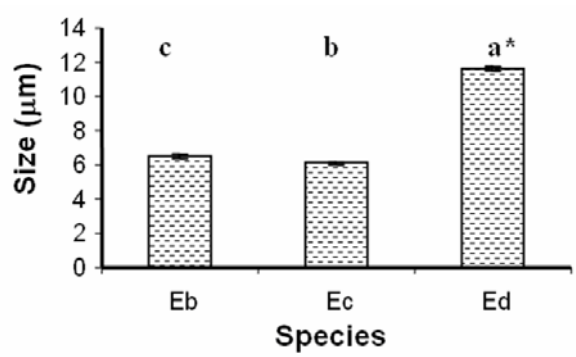

Fig 2. Mean values of sperm head size (mm) of *Echinometra* spp. Five individuals were examined from each species with 50 sperms. Error bar indicates standard error. There were significant differences (Tukey's test $p < 0.05$) in egg size of all *Echinometra* species.
Eb – *Echinometra* sp. B
Ec – *Echinometra* sp. C
Ed – *Echinometra* sp. D
* Same superscripts are not significantly different (Tukey's test $p > 0.05$).

not meet the normality, assumption of parametric analysis were analyzed using non-parametric statistics. This was done by transforming values to ranks and then applying one-way ANOVA followed by Tukey's multiple comparison tests. The level for statistical significance was set at 0.05. Untransformed values are presented in the tables and figures.

Results

Gamete Size

There were significant differences (Tukey's Test, $p < 0.05$) in egg size of all three species (Fig 1). The mean egg diameters were 70.28 μm, 71.25 μm, and 75.08 μm for *Echinometra* sp. B, *Echinometra* sp. C and *Echinometra* sp. D, respectively. Sperm head also followed the same trend as with egg size. As shown in Fig 2, it was found that the mean sperm head size of *Echinometra* sp. D (11.64 μm) was significantly larger (Tukey's Test, $p < 0.05$) than that of *Echinometra* sp. C (6.07 μm) and *Echinometra* sp. B (6.49 μm).

Fertilization

Fertilization experiment demonstrated a direct relationship between the probability of fertilization and egg diameter for the three species of *Echinometra* (Fig 3). Cross-fertilization of three sibling species of *Echinometra* B, C and D were conducted using various sperm concentrations. Fertilization success of crosses was highly dependent on sperm concentrations, i.e., the higher the sperm concentration gave rise to, the higher fertilization rate (Fig 3).

In this experiment, high percentage of fertilization was achieved at high sperm concentration ($10^{-5} \times 5$; Fig 3i). But fertilization success dropped slightly at moderate

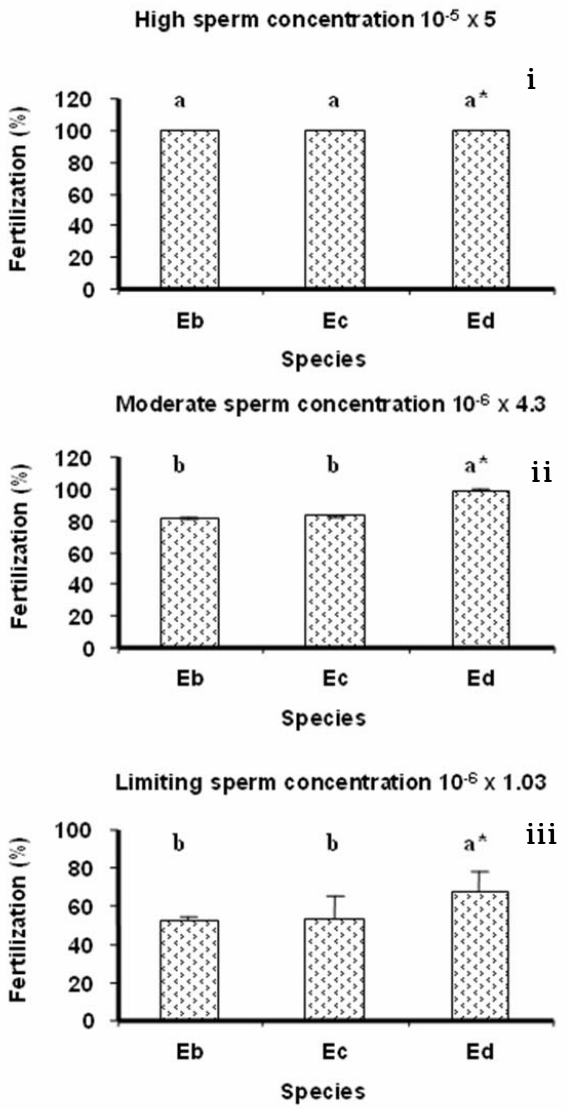

Fig 3. Percent fertilization of *Echinometra* sp. B, C, and D. Fertilization rate was calculated 1.3 h after gamete mixing when 2-4 cell stage occur. Fertilization success of *Echinometra* sp. D significantly differed (Tukey's test $p < 0.05$) from that of *Echinometra* sp. B and *Echinometra* sp. C at both moderate and limiting sperm concentrations. Each mean value represent 5 replicate crosses.
Eb – *Echinometra* sp. B
Ec – *Echinometra* sp. C
Ed – *Echinometra* sp. D
* Same superscript are not significantly different (Tukey's test $p > 0.05$).

sperm concentration (10^{-6} x 4.3; Fig 3ii). At this concentration, fertilization success of *Echinometra* sp. B (81%) and *Echinometra* sp. C (82.90%) did not differ significantly (Tukey's test $p > 0.05$), whereas the fertilization rate of *Echinometra* sp. D (99.2%) differed significantly (Tukey's test $p < 0.05$) when compared with the other two species. On the other hand, at limiting sperm concentration (10^{-6} x 1.03), fertilization rate declined sharply (Fig 3iii). Fertilization rate of *Echinometra* sp. B, *Echinometra* sp. C, and *Echinometra* sp. D was 52.15%, 53.73% and 67.44%, respectively. Fertilization rate of *Echinometra* sp. D differed significantly from that of *Echinometra* sp. B and *Echinometra* sp. C. (Tukey's test $p < 0.05$), but there was no difference between *Echinometra* sp. B and *Echinometra* sp. C (Tukey's test $p > 0.05$).

Developmental Timetable

A list of the embryonic developmental stages and the times required to reach them is given in Table 1. It was apparent that the developmental times sequentially from 2-cell to prism stage of *Echinometra* sp. D differed significantly (Tukey's test $p < 0.05$) from *Echinometra* sp. B and *Echinometra* sp. C, while the difference between *Echinometra* sp. B and *Echinometra* sp. C was not statistically significant (Tukey's test $p > 0.05$).

Discussion

Egg Size and Fertilization

Fertilization success is very sensitive to changes for all the three species of *Echinometra*. The influences of egg size on fertilization were seen most clearly in the comparison among the closely related but genetically divergent *Echinometra* sp. B, *Echinometra* sp. C, and *Echinometra* sp. D. Our result demonstrated that larger egg of *Echinometra* sp. D had a significantly higher fertilization rate than that of *Echinometra* sp. B and *Echinometra* sp. C at both moderate ($10^{-6} \times 4.3$) and limiting ($10^{-6} \times 1.03$) sperm concentrations used. Sperm limitation can lead to important variation in the rates at which eggs of different sizes are fertilized. The free-spawning of gametes into the environment is a common mechanism of reproduction for many diverse taxa.[29] After release, the sperms can quickly become diluted to a concentration where fertilization becomes unlikely.[30-32] If there are situations where a large proportion of eggs is not fertilized, sperm limitation could be an important constraint restricting reproductive success.[33] Working with three temperate congeneric sea urchins, *Strongylocentrotus droebachiensis*, *S. purpuratus*, and *S. franciscanus*, Levitan[12] demonstrated that larger eggs were fertilized at a greater rate because they provide a higher target for sperm. He further concluded that conditions of sperm limitation could select for larger eggs and that variations in such conditions could contribute to observe patterns of interspecific variation in egg size. Recognizing this advantage of large egg size makes an important contribution to evaluating the fitness consequences of how resources are divided among gametes. When life

history models incorporate effects of egg size on developmental and larval mortality,[20,23] the fertilization advantage could shift optimal egg size in a way that depends on sperm limitation.[12] However, incorporating fertilization success into life-history theory will be particularly important if gamete provisioning directly influences gamete fertilization. If so, then egg size will influence not only offspring number and survival but also the probability of fertilization.

Egg Size and Embryonic Development

The experiment revealed that embryonic stages (2-cell to prism stage) were slower for embryo from the smaller *Echinometra* sp. C and *Echinometra* sp. B eggs than the larger *Echinometra* sp. D eggs. There was an important correlation between small egg size in *Echinometra* sp. C and *Echinometra* sp. B in comparison to *Echinometra* sp. D, and increased developmental time. Similar results were also obtained in other marine invertebrates.[16,26,34,35] Interspecific comparisons[16,36,37] and experimental manipulation of egg size[16,38] both indicated longer periods of development for larvae from smaller eggs. All of these experiments with feeding larvae demonstrated that increased allocation was necessary to provide materials and energy reserves to eggs resulting in reduced larval development period. These findings are consistent with interpretations of variation in eggs size reflecting the rates of larval mortality.[9,18,39] Thus, it is plausible that the dependence of developmental rates on egg size transcends species differences among *Echinometra* sp. B, *Echinometra* sp. C, and *Echinometra* sp. D.

The general conclusion is that for these *Echinometra* spp., egg size (parental investment of stored nutrients per offspring) is traded off against developmental time. The larger egg should result in shorter developmental time after fertilization, and the larger eggs may act as better target for sperm, thereby enhancing fertilization success. Other gamete attributes that are likely to influence fertilization success include receptiveness of the egg surface to sperm, sperm morphology, sperm velocity, sperm longevity, sperm age, 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[11,12,15,30,40-45] and gamete recognition molecules.[46-48] Previously, our laboratory reported that larger eggs resulted in larger larval size,[49,50] which, together with the results of the present study, clearly demonstrates that egg size has a direct effect on fertilization, larval size and developmental time.

Sequences from the COI gene region of mitochondrial DNA indicate that congeneric *Echinometra* spp. is of recent origin, having diverged within the middle Pleistocene (less than 3 million years old).[3] 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 three sibling species. The relationship between the adaptive evolution of life histories and the evolution of ontogeny of these three species clearly deserves further attention.

Acknowledgements

The study was supported by MONBUSHO, Ministry of Education, Japan. We thank Dr. Nazmul Ahsan, Associate Professor, Khulna University, Bangladesh for constructive comments and valuable suggestions on this manuscript.

References

1. Walker TJ (1964) Cryptic species among sound-producing esiteran Orthoptera (Gryllidae and Tettigoniidea) *Quart Rev Biol* **39**, 345-355.
2. Arakaki *et al* (1998) Comparative studies of the genus *Echinometra* from Okinawa and Mauritius *Zool Sci* **15**, 159-168.
3. Palumbi SR (1996) What can molecular genetics contribute to marine biogeography? An urchin's tale *J Exp Mar Biol Ecol* 203, 75-92.
4. Uehara T (1990) Speciation of *Echinometra mathaei*. *Iden* **44**, 47-53 (in Japanese).
5. de Blainville HM (1825) Dictionnaire des sciences naturelles, dans lequel un traite methodiquement des differences tetres de la *nature* **15**, 93-120.
6. Uehara T and Shingaki M (1985) Taxonomic studies in the four types of sea urchin, *Echinometra mathaei* from Okinawa *Zool Sci* **2**, 1109.
7. Arakaki Y and Uehara T (1999) Morphological comparison of black *Echinometra* individuals among those in the Indo-west Pacific *Zool Sci* **16**, 551-558.
8. Emlet RB and Guldberg OH (1997) Effects of egg size on post larval performance: experimental evidence from sea urchin *Evolution* **52(2)**, 141-152.
9. Emlet *et al* (1987) Echinoderm larval ecology viewed from the egg. In: *Echinoderm studies*, 2nd vol. 2 (Edited by Jangoux M and Lawrence JM). pp. 55-136. Balkema Press, Rotterdam, The Netherlands.
10. Levitan DR (1998a) Does Bateman's principle apply to broadcast-spawning organisms? Egg traits influence in situ fertilization rates among congeneric sea urchins *Evolution* **52**, 1043-1056.
11. Levitan DR (1998b) Sperm limitation, gamete competition, and sexual selection in external0fertilizers In: *Sperm Competition and Sexual Selection*, (Edited by Birkhead TR and Moller AP). pp 175-217. Academic Press, San Diego.
12. Levitan DR (1993) The importance of sperm limitation to the evolution of egg size in marine invertebrates *Am Nat* **141**, 517-536.
13. McEdward LR (1986a) Comparative morphometrics of echinoderm larvae. I. Some relationships between egg size and initial larval form in echi-noids *J Exp Mar Biol Ecol* **96**, 251-265.
14. McEdward LR (1986b) Comparative morphometrics of

echinoderm larvae. II Larval size, shape, growth, and the scaling of feeding and metabolism in echinoplutei *J Exp Mar Biol Ecol* **96**, 267-286.
15. Podolsky RD and Strathmann RR (1996) Evolution of eggs size in free-spawners: consequences of the fertilization-fecundity trade-off *Am Nat* **148**, 160-173.
16. Sinervo B and McEdward LR (1988) Developmental consequences of an evolutionary change in egg size: An experimental test *Evolution* **42(5)**, 885-899.
17. Pearse JS (1994) Cold-water echinoderms break "Thorson's rule". In: *Reproduction, larval biology, and recruitment in the deep sea benthos* (Edited by Young CM and Eckelbarger KJ). pp 26-43. Columbia Univ. Press, New York.
18. Strathmann RR (1985) Feeding and non-feeding larval development and life-history evolution in marine invertebrates *Annu Rev Ecol syst* **16**, 339-361.
19. Thorson G (1950) Reproduction and larval ecology of marine bottom invertebrates *Biol Rev* **25**, 1-45.
20. Christiansen FB and Fenchel TM (1979) Evolution of marine invertebrate reproduction patterns *Theoritical Population Biology* **16**, 267-282.
21. Crisp DJ (1976) Settlement responses in marine organisms. In: *Adaptation to environment: essays on the physiology of marine animals* (Edited by Newell RC). pp 83-124. Butterworths, London.
22. Strathmann RR (1974) Introduction to function and adaptation in echinoderm larvae. *Thalassia Jugoslavica* **10**, 321-339.
23. Vance RR (1973) More on reproductive strategies in marine benthic invertebrates *Am Nat* **107**, 353-361.
24. Strathmann RR and Vedder K (1977) Size and organic content of eggs of echinoderms and other invertebrates as related to developmental strategies and egg eating. *Marine Biology* **39**, 305-309.
25. Turner RL and Lawrence JM (1979) Volume and composition of echinoderm eggs: implications for the use of egg size in life history models. In: *Reproductive ecology of marine invertebrates* (Edited by Stancyk SE). pp 25-40. Univ. of South Carolina Press, Columbia.
26. Amy RL (1983) Gamete sizes and developmental timetables of five tropical sea urchins. *Bull Mar Sci* **33(1)**, 173-176.
27. Fujisawa H (1989) Differences in temperature dependence of early development of sea urchins with different growing seasons *Biol Bull* **176**, 96-102.
28. Bartlett MS (1937) Some examples of statistical methods of research in agriculture and applied biology *Suppl J R Stat Soc* **4**, 137-170.
29. Giese AC and Kanatani H (1987) Maturation and spawning. In: *Reproduction of marine invertebrates, General aspects: seeking unity in diversity*, Vol. 9. pp 252-329. (Edited by Giese AC, Pearse JS, and Pearse VB). Boxwood Press, Pacific Grove, California, USA.
30. Denny MW and Shibata MF (1989) Consequences of surf-zone turbulence for settlement and external fertilization *Am Nat* **134**, 859-889.
31. Levitan DR (1991) Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate *Biol Bull* **181**, 261-268.
32. Pennington JT (1985) The ecology of fertilization off echinoid eggs: the consequences of sperm dilution of echinoid eggs, adult aggregation and synchronous spawning *Biol Bull* **169**, 417-430.
33. Mortensen T (1938) Combinations to the study of the development of larval form of echinoderms. IV. *Det Kongelige Danske Videnskabernes Selskabs Skrifter Naturvidenskabeling Mathematisk Afdeling* ***9*** *raekke* 7,1-59.
34. Marcus NH (1979) Developmental aberrations associated with twinning in laboratory- reared sea urchins *Develop Biol* **70**, 274-277.
35. Strathmann RR (1977) Egg size, larval development, and juvenile size in benthic marine invertebrates *Am Nat* **111**, 373-376.
36. Havenhand JN (1993) Egg to juvenile period, generation time, and the evolution of larval type in marine invertebrates *Mar Ecol Prog Ser* **97**, 247-260.
37. Kohn JA and Perron FE (1994) Life history and biogeography: patterns in *Conus*. Oxford University Press, Oxford.
38. McEdward LR (1996) Experimental manipulation of parental investment in echinoid echinoderms *Am Zool* **36**, 169-179.
39. Herrera *et al* (1996) Diversity of energetic strategies among echinoid larvae and the transition from feeding to nonfeeding development *Oceanol Acta* **19**, 313-321.
40. Eckelbarger *et al* (1989) Modified sperm in echinoderms from the bathyal and abyssal zones of the deep sea. In: *Reproduction, Genetics and Distribution of Marine Organisms* (Edited by Ryland JS and Tyler PA, Olsen and Olsen). pp 67-74. Fredensborg.
41. Franzén A (1987) Spermatogenesis. In: *Reproduction in Marine Invertebrates*, Vol. 9, (Edited by Giese AC, Pearse JS and Pearse VB). pp 1-47. The Boxwood Press, Pacific Grove, California.
42. Levitan DR (1996a) Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism *Nature* **382**, 153-155.
43. Levitan DR (1996b) Predicting optimal and unique egg sizes in free-spawning marine invertebrates *Am Nat* **148**, 174-188.
44. Thomas FIM (1994) Physical properties of gametes in three sea urchin species *J Exp Mar Biol Ecol* 194, 263-284.
45. Vogel *et al* (1982) Fertilization kinetics of sea urchin eggs *Math Biosci* **58**, 189-216.
46. Metz EC and Palumbi SR (1996) Positive selection and sequence rear-rangements generate extensive polymorphism in the gamete recogni-tion protein bindin *Mol Biol Evol* **13**, 397-406.
47. Palumbi SR (1998) Species formation and the evolution of gamete recognition loci. In: *Endless Forms: Species and Speciation* (Edited by Howard DJ and Berlocher SH). pp. 271-278. Oxford Univ. Press, New York.
48. Vacquier *et al* (1995) What have we learned about sea urchin sperm bindin? *Dev Growth Diff* **37**, 1-10.
49. Aslan *et al* (1999) Relationship between egg size and larval size of two closely related species of sea urchins genus *Echinometra* and the egg size effects to the larval size of their reciprocal hybrids *Biol Mag Okinawa* **37**, 67-73.
50. Rahman *et al* (2001) Hybrids of two closely related tropical sea urchins (genus *Echinometra*): Evidence against postzygotic isolating mechanisms. *Biol Bull* **200**, 97-106.