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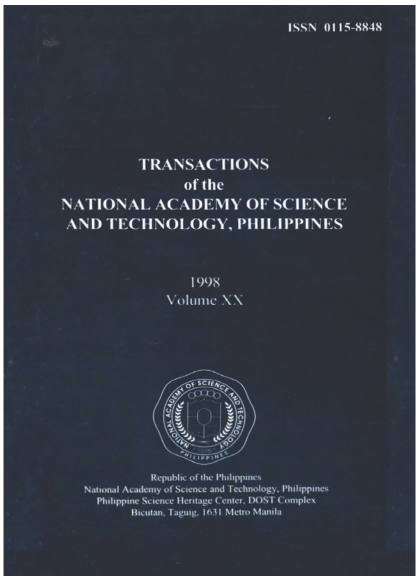

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ABSTRACT

The sea urchin, *Tripnuestes gratilla*, is an important fishery resource. Its roe or gonad is a high value local and export product. Overexploitation has led to the decimation of viable spawning populations. This species has been cultured locally for the first time, through its entire life cycle, at the U.P. Marine Science Institute Bolinao Marine Laboratory. Laboratory experiments to determine optimal rearing and feeding regimes were conducted to improve the production of pre-settlement larvae and juveniles for grow-out culture and reseeding. Preliminary studies on the gonad yield of sea urchins in grow-out culture indicate considerable intra-annual and inter-annual variation in gonad weight to test diameter ratio. Likewise, young adults (6.0-6.4 cm) in high stocking density cages had significant lower gonad yield than those in low stocking density cages. Development and further optimization of mass culture techniques provide an opportunity to utilize mariculture as a resource management tool in the form of sea pen/cage grow-out culture which can enhance the recovery of depleted natural populations and at the same time provide a supplemental source of livelihood for fisherfolk.

Key words: sea urchin, *Tripnuestes gratilla*, mariculture, grow-out culture

INTRODUCTION

Tripnuestes gratilla is widely distributed in the Indo-Pacific (Shokita et al. 1991) and is the most commercially important sea urchin in the Philippines. Its roe or gonad is a high value local and export product. In Bolinao, Pangasinan, Northwestern Philippines, for example, the commercial fishery of this species from 1987 to 1992 generated multimillion peso earnings per annum which provided substantial income for most coastal families in the area (McManus and Kesner 1995), However, overexploitation precipitated the collapse of the sea urchin fishery in 1993 (Juinio-Meñez et al. 1997). Even after five years (starting in January 1993 until the present) of moratorium on commercial harvesting in Bolinao, no significant juvenile recruitment has been observed. This may be due to the small size of the remaining local and adjacent spawning stocks. Anectodal accounts of traders on the shortage of sea urchin products in the local and international market in the last few years indicate that the natural stocks of this species in other parts of the country are overexploited. Given this grave situation, a viable management strategy has to be developed for the recovery of the natural stocks of the sea urchin. The strategy proposed involves the mass production of *T. gratilla* in the laboratory to provide seedstock for family-/village-scale grow-out culture which will serve as mini-reproductive reserves and provide supplemental livelihood for the fisherfolk (Juinio-Meñez et al. 1997).

Implementation considerations for grow-out culture include regular availability of seedstock, suitable culture areas, favorable markets, and interest of local fishers (Juinio-Meñez et al. 1997). Thus, lack of seedstock for grow-out culture poses a major impediment. The first and only successful local culture of *Tripnuestes gratilla* through its entire life cycle at the U.P. Marine Science Institute Bolinao Marine Laboratory provides an opportunity to pursue grow-out culture as a resource management strategy. This paper focuses on the development of mariculture protocols to enhance the production of larvae and seedstock and the preliminary assessment of the effects of seasonality and stocking density on gonad yield of sea urchins in grow-out culture.

MATERIALS AND METHODS

Larval Survivorship and Development Rate

The major aspects in the culture of *T. gratilla* are shown in Fig. 1. To scale up production of seedstock, induction of artificial spawning (using 0.5 M KCl), and fertilization were conducted every two months in 1996 and 1997. During each spawning period, two to three different batches of larvae (i.e., from three different parents) were reared in three 50-L pails for the mass culture set-up and in nine 3-L culture jars for the experimental scale. In both set-ups, stocking density of larvae was about 1 larva mL^{-1}. Larvae were kept in suspension in the water column with the use of motorized stirrers running continuously for the entire duration of the larval period. Larvae were fed daily according to the standard mixed feeding protocol in Table 1.

For nine batches spawned in 1996, monitoring of larval development and survivorship in the mass culture set-up was conducted every two days from the time embryos were placed in the rearing culture chambers until pre-settlement stage (i.e., ~29-47 days after spawning). Sampling of larvae involved the collection of three 2-L samples of the rearing medium from each pail in the mass culture set-up. The larvae in each 2-L beaker were estimated by counting larvae in three 50-mL subsamples. Larvae were examined and counted under a stereoscope. The

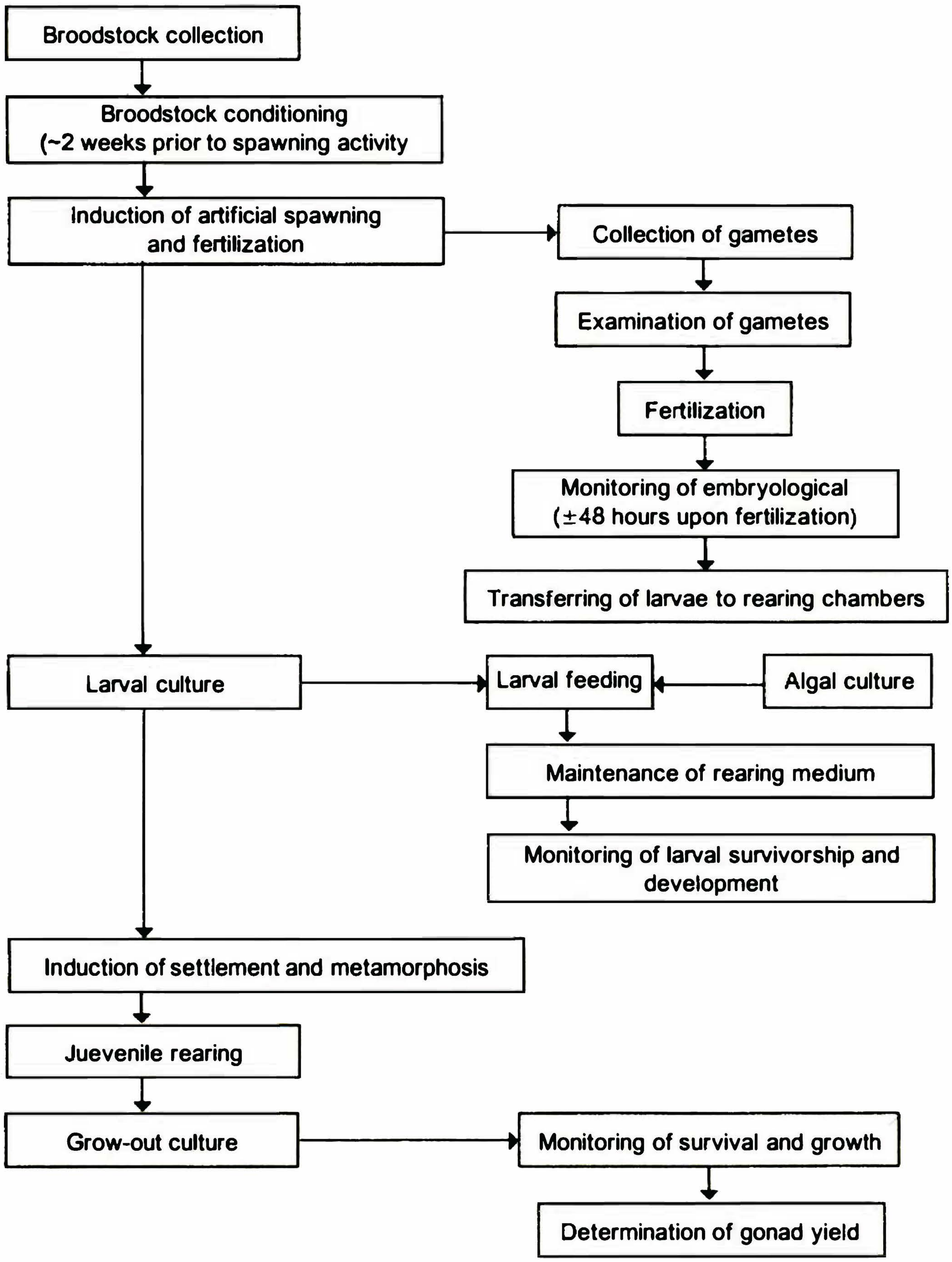

Figure 1. Major aspects in the mass culture of *Tripnuestes gratilla.*

Table 1. Mixed feeding diet protocols for *Tripnuestes gratilla* larvae.

Development stage	Type of food		Number of cells mL^{-1}	Ratio	
	Standard	Experimental		Standard	Experimental
2-arm	*Ig*	*Ig* & *Cg*	20,000	1	1:1
4-arm	*Ig*	*Ig* & *Cg*	20,000	1	1:1
4- to 6-arm	*Ig* & *Cg*	*Ig* & *Cg*	40,000	1:1	1:1
6- to 8-arm	*Ig* & *Cg*	*Ig* & *Cg*	40,000	1:3	1:1
8-arm	*Cg*	*Ig* & *Cg*	40,000	1	1:1
rudiment	*Cg*	*Ig* & *Cg*	40,000	1	1:1

proportions of larvae in various stages of development (i.e., 2-arm, 4-arm, 6-arm, 8-arm, rudiment) and larval density were recorded. Comparisons of larval survivorship and development rate between the mass culture pails and the experimental culture jars were conducted for three batches of larvae. For the latter, the number and stage development of larvae in three 50-mL subsamples were used to estimate survivorship and development rate following the same protocol as that described for the mass culture pails.

Feeding Regime

A study on various feeding regimes was conducted to determine what feeding regime would enhance larval growth rate and survivorship of cultured *T. gratilla* larvae. Four feeding regimes were either mixed or pure diets of *Isochrysis galbana* (*Ig*), and *Chaetoceros gracilis* (*Cg*). Different proportions of each alga were given at specific developmental stages. The feeding protocol for the mixed diets: standard and experimental are shown in Table 1. In the two pure diet treatments, larvae were fed the same amount (cells mL^{-1} of either *Ig* or *Cg* during the entire larval period. Three replicate batches of larvae from artificial spawning and fertilization on September 10, 1996, were used in the experiment. Stocking density was estimated at 1 larva mL^{-1} (2.5-L volume in each experimental culture jar). The larvae were fed daily with the prescribed protocol for each feeding treatment. Monitoring of the larvae was done every two days, using three 50-mL subsamples of larvae from each of the twelve replicate jars. The larvae were counted and examined under a stereoscope. The number of surviving larvae in the different developmental stages was noted. Observations on the occurrence of abnormal larvae and mortalities were likewise noted. Differences in the mean percent survivorship of *T. gratilla* larvae grown under four diet treatments per sampling time were determined using one-way Analysis of Variance (ANOVA, $p<0.05$). If significant differences were found, a Turkey's Honesty Significance Difference Test was used to determine which means were significantly different from each other.

Rearing Medium

The effect of different types of larval rearing medium, i.e., boiled filtered seawater (BFSW) and UV sterilized filtered seawater (UVFSW) on larval developmental and survivorship was also conducted to improve larval survivorship and streamline culture maintenance activities. For this experiment, two batches of larvae were used. Each batch was reared in replicate 3-L jars with BFSW and UVFSW. Monitoring of the number of surviving larvae in a specific developmental stage was conducted as in the feeding regime experiment. The differences in the mean percent survivorship of *T. gratilla* larvae reared in BFSW and UVFSW per sampling time were determined using Student's t-test ($p<0.05$).

Grow-out Culture

Newly-settled juveniles were provided with benthic diatoms (*Navicula ramossisima*) for food. This was supplemented with seagrass when the juveniles were approximately 0.5 mm test diameter (TD), about one month after induction of larval settlement and metamorphosis in the laboratory. Juveniles in the outdoor hatchery were maintained in a flow-through system with unfiltered seawater and aeration was provided for 24 hours. When juveniles reached about 1.0 cm TD, *Sargassum* was given as food. Fresh *Sargassum* was placed in the rearing tanks thrice a week to ensure *ad libitum* supply of food for the juveniles. Food debris and wastes were siphoned out and the seawater was replaced every two days to maintain good water quality. At 1.5-2.5 cm TD, sea urchins were transferred to sea cages on the reef flat. Sea cages were made of PVC pipes and polyethylene netting, raised with bamboo posts about 0.5 m from the ground of the seagrass area in Lucero, Bolinao, Pangasinan. About 200-300 sea urchins were stocked in 1 m x 1 m cages. *Sargassum* was replenished every 10 days to ensure *ad libitum* supply of food for the sea urchins. The sea cages were regularly cleaned. Waste materials were removed from the cages and the polyethylene netting was brushed to minimize overgrowth that would impede water flow in the cages.

Gonad Yield

After 6-7 months of rearing, sea urchins grown in the sea cages were periodically sampled in 1996 and 1997 to determine test diameter and weight of gonad. In 1997, total body weight was also determined. Only reproductively mature sea urchins (i.e., 5.0 to 10.0 cm TD) were shucked during each of the sampling period. In 1996, bi-monthly sample collection was conducted for the months of January, March, April, June, and September. During the months of May, July, October, and November, only one sampling activity was conducted for each month due to the limited number of reproductively mature sea urchins in the cages. No sampling was done for the month of August. Random samples of 45-339 individuals were taken from different cages per month. The average monthly gonad weight to test diameter ratio was determined.

In 1997, sampling was conducted three times a month (i.e., at 10-day intervals). On an average, about 30 reproductively mature individuals were sampled for each interval in a month. No sampling was conducted for the months of April and May due to the unavailability of reproductively mature individuals. For the month of June, only one sampling (i.e., with 32 individuals) was conducted.

Effect of Stocking Density

The effect of stocking density on the gonad yield of individuals from six batches (i.e., from two different spawning dates) of sea urchins reared in sea cages in Lucero for about 6-7 months was determined. The cages were grouped into two categories, low density (mean = 212.38 ; range = 137-162 individuals m^{-3}). Twenty-eight individuals were shucked from each of the three replicate cages per density on August 1997. Gonad index was determined for each individual from the ratio of gonad weight to body weight expressed in percentage and was plotted against size (test diameter). Differences in the gonad index of *T. gratilla* grown between high and low stocking densities were determined using the Student's t-test ($p<0.05$).

RESULTS AND DISCUSSION

Larval Survivorship and Development

There was a high variability in larval survivorship and development rate among nine different batches of larvae that were monitored in the mass culture set-ups in 1996. Percent survivorship of the larvae (n= ~ 50,000 larvae per 50-L pail) up to pre-settlement ranged from 12.3 to 70.4%, while the total duration of the planktonic larval period ranged from 29 to 47 days. Since rearing conditions for these batches were the same, differences may be attributed to genetic variability.

Comparison of average larval survivorship of three batches of larvae reared in the mass culture set-up and the experimental culture set-up is shown in Fig. 2. Batches reared in the experimental culture set-up had higher average percentage survivorship than their mass culture counterparts. Average survivorship at pre-settlement for the three batches in the experimental jars was 47.0% ± 13.0, while average survivorship for the mass culture pails was 25.0% ± 6.0. Mass mortalities also occurred more often in the mass culture set-up. These larvae were discarded due to physical abnormalities (e.g., small in size, with deformities in arm). Differences can be attributed to the different rearing conditions in the mass culture pails and experimental jars. The latter were kept indoors which had relatively more constant temperatures (25°C-28°C) compared to the mass culture set-up temperatures (26°C-31°C). Moreover, maintenance of water quality was better in the experimental culture set-up.

The rearing medium in the experimental jars was boiled filtered sea water (BFSW) whereas UV filtered sea water (UVFSW) was used in the mass culture set-up. Notably, the experiment on rearing medium showed that percent survivorship

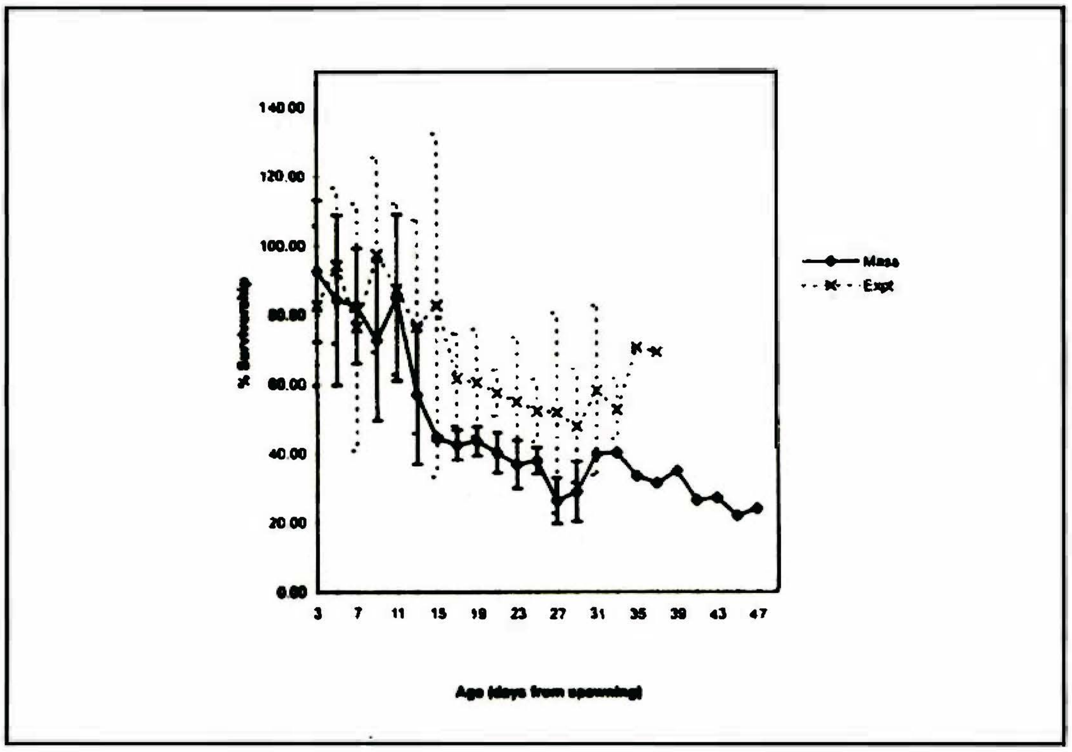

Figure 2. Average percent survivorship during the entire larval period of three spawning batches reared in mass culture pails (50-L) and their corresponding larvae reared in experimental jars (3-L).

of larvae in UVFSW is generally higher compared to that of the larvae in BFSW. However, mean percentage survivorship of *T. gratilla* in the UVFSW was significantly higher only on the 19^{th}, 21^{st}, and 25^{th} day (Table 2, Student's t-test, $p<0.05$). This indicates that either BFSW or UVFSW is suitable for larval rearing. However, UVFSW is more practical for the mass production of *T. gratilla* since boiling large volumes of water is very tedious and time consuming. The UVFSW used to rear the mass culture larvae in 1996 was stored in holding tanks for a couple of days prior to use. Likewise, high bacterial activity due to the accumulation of wastes (e.g., excess food, excreta) significantly decreased water quality in the mass culture set-ups. Thus, the differences in survivorship in the mass and experimental culture experiments cannot be attributed to the type of rearing medium per se, but rather on the maintenance of water quality.

In general, the highest rate of larval mortalities was observed from day 12 (i.e., 4-arm stage) to around day 28 (i.e., early 8-arm stage). From day 14, larvae are expected to be in the 8-arm stage. During the fifteenth day until the pre-settlement stage, rearing chambers are cleaned thoroughly by siphoning water out and collecting the larvae in a sieve (with a mesh size of 120 μ). Abnormal and small larvae are siphoned out of the rearing chambers. Survivorship is based on only competent/fully-developed larvae. This also accounts for the sharp decline in the survivorship of the larvae from day 15 (Fig. 2).

The production of pre-settlement larvae and juvenile seedstock was higher in 1997 than in 1996 (Fig. 3). Average survivorship to pre-settlement increased from about 22.0 % to 56.0 %. About 22,932 early juveniles (i.e., test diameter >

1.0 cm) were produced from 20 batches during the second year. This is significantly higher compared to only about 3,804 early juveniles from 22 batches during the first year. Maintenance of water quality improved significantly by using freshly UV-sterilized filtered water every time larval rearing water was routinely changed. Thus, contamination by larval wastes was minimized. Likewise, mass larval settlement (in 450-L tanks rather than in 10-gal aquaria) which was initiated during the last quarter of the first year, became a regular routine in the mass culture protocol.

The average survivorship of cultured *Tripnuestes gratilla* larvae in this study is comparable to that reported for *Strongylocentrotus intermedius* where survivorship is about 53.0 % from initial stocking of 1.5 larvae mL^{-1} (Hagen 1996). However, high mortalities still occurred during the settlement (Fig. 3). Even in 1997, average survivorship up to newly settled stage was only about 3.0 % of the initial number of larvae reared. Mortalities after settlement up to the early juvenile stage (size 1.0-2.5 cm TD) are generally minimal. It is evident that studies that will enhance settlement and metamorphosis are critical in improving the production of juveniles. Initial experiments on the induction of settlement and metamorphosis show that water conditioned with *Sargassum* significantly increases metamorphosis of larvae from settlement (manuscript, in preparation). Further studies are needed to overcome this bottleneck in the mass culture protocol.

The average duration of the different larval stages of three batches of *T. gratilla* reared in the experimental and mass culture set-ups are summarized as shown in Figs. 4a and 4b, respectively. The pre-pluteus stage took about 2 days for both the experimental and mass culture set-ups. The 2-arm, 4-arm, and 6-arm stages lasted an average of 4, 14, and 12 days, in the experimental culture, compared

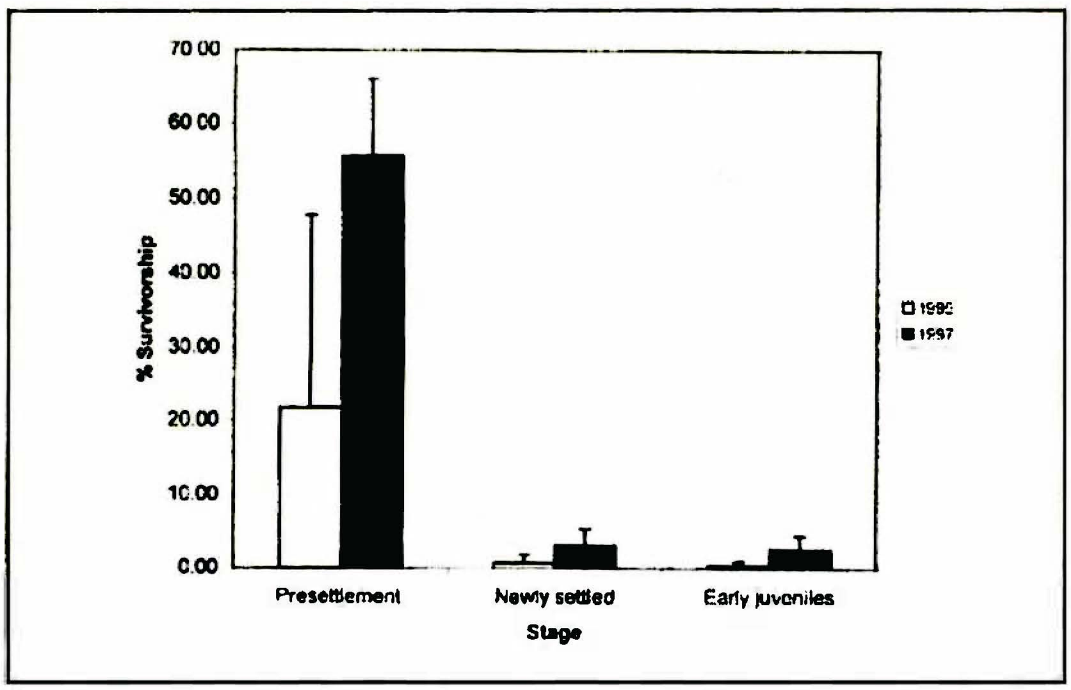

Figure 3. Percent survivorship of mass cultured *Tripnuestes gratilla* larvae at presettlement, newly settled, and early juvenile stages in 1996 (n-22 batches) and 1997 (n=20 batches).

Table 2. Larval survivorship by age (in days) in two rearing media: Boiled filtered seawater vs UV filtered seawater. Two-tailed significance: 95% CI (Student's t-test); NS = not significant.

Treatment	Mean % Survivorship																			
BFSW	81.0	83.0	86.3	54.7	75.0	76.3	68.7	79.3	76.0	69.3	74.0	46.7	60.3	50.3	32.7	33.0	37.7	41.0	49.7	49.0
UVFSW	101.0	96.0	85.7	59.0	94.7	90.3	87.3	88.0	95.3	88.3	86.7	84.3	82.0	64.0	49.7	42.0	55.3	42.7	44.0	59.3
Age (days)	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	35	37	39	41	45
	NS	NS	NS	NS	NS	NS	NS	NS	*0.014*	*0.004*	NS	*0.010*	NS	NS	NS	NS	NS	NS	NS	NS

Table 3. Mean larval counts (n=3, 50-mL test tube per replicate; 3 replicates per feeding regime) of developing *T. gratilla* larvae grown under 4 feeding regimes. (Means with different letters vary significantly from each other based on one-way ANOVA, using Tukey's HSD test, $p < 0.05$)

Treatments	Age (from spawning)																			
	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41
Pure *Ig*	a 57.00	a 56.67	a 59.67	a 55.00	a 53.00	a 59.33	a 67.33	ab 41.67	a 50.44	ab 32.44	b 15.89	b 8.67	b 8.89	b 3.78	b 3.33	b 3.00	-	-	-	-
Pure *Cg*	a 44.67	a 60.00	a 70.00	a 64.00	a 63.00	a 57.67	a 64.00	b 27.56	b 17.56	b 9.78	b 6.56	b 4.56	b 2.89	b 4.11	b 6.44	b 3.11	b 5.11	b 3.00	b 3.11	b 0.00
Standard mixed *Ig-Cg*	a 31.33	a 49.33	a 62.33	a 50.00	a 49.67	a 48.67	a 53.67	a 58.56	a 50.44	a 47.89	a 46.78	a 43.11	a 38.89	a 35.22	a 42.56	a 39.78	a 38.33	a 36.89	a 34.56	a 31.33
Experimental mixed *Ig-Cg*	a 44.33	a 50.67	a 62.33	a 65.67	a 64.00	a 65.67	a 89.33	a 62.00	a 62.44	a 47.11	a 57.00	a 47.44	a 40.44	a 41.78	a 41.67	a 43.33	a 46.00	a 42.56	a 35.22	a 32.89

to 4, 11, and 10 days, respectively for the mass culture set-up. The duration of the 8-arm stage was longest and most variable in both culture set-ups (i.e., 11-37 days for experimental culture and 11-47 days for mass culture). Development of rudiment was observed starting day 17 in the experimental culture and day 15 in the mass culture. This lasted for about 19 days in both culture set-ups. Overall, larval development in the mass culture was comparable to the experimental. However, the 8-arm stage in the mass culture was protracted (Fig. 4b). This indicates that attainment of competence to settle took longer in the mass culture set-up. The average total larval duration (i.e., prior to settlement) for these three batches of larvae was also more variable, being 35.0 ± 10.4 days, compared to 33.7 ± 5.0 days in the experimental culture set-up. Notably, the total larval duration of *T. gratilla* in this study is longer than that reported for the same species in Okinawa, Japan which is only 20-30 days (Shokita et al. 1991).

Effect of Feeding Regime

Larval survivorship from days 3-15, from the four feeding treatments did not significantly differ from each other (one way ANOVA $p<0.05$, Table 3). However, from days 17-41, larval survivorship under standard and experimental mixed feeding regimes were generally significantly higher than those in the pure *Ig* and pure *Cg* treatments. In contrast to larvae in the two mixed feeding regimes, larval survivorship significantly declined from day 17 to 41 both in pure *Ig* and pure *Cg* treatments (Figs. 5a-c). Larvae given pure diets of *Ig* and *Cg* (Fig.5c) did not survive up to 41 days. Two replicate jars from each pure diet treatment were discarded on day 20 because of low survivorship. Remaining larvae in the pure *Ig* treatment were discarded on day 34, whereas few surviving larvae in the pure *Cg* treatment were ready to settle on day 40. In addition, more abnormalities were

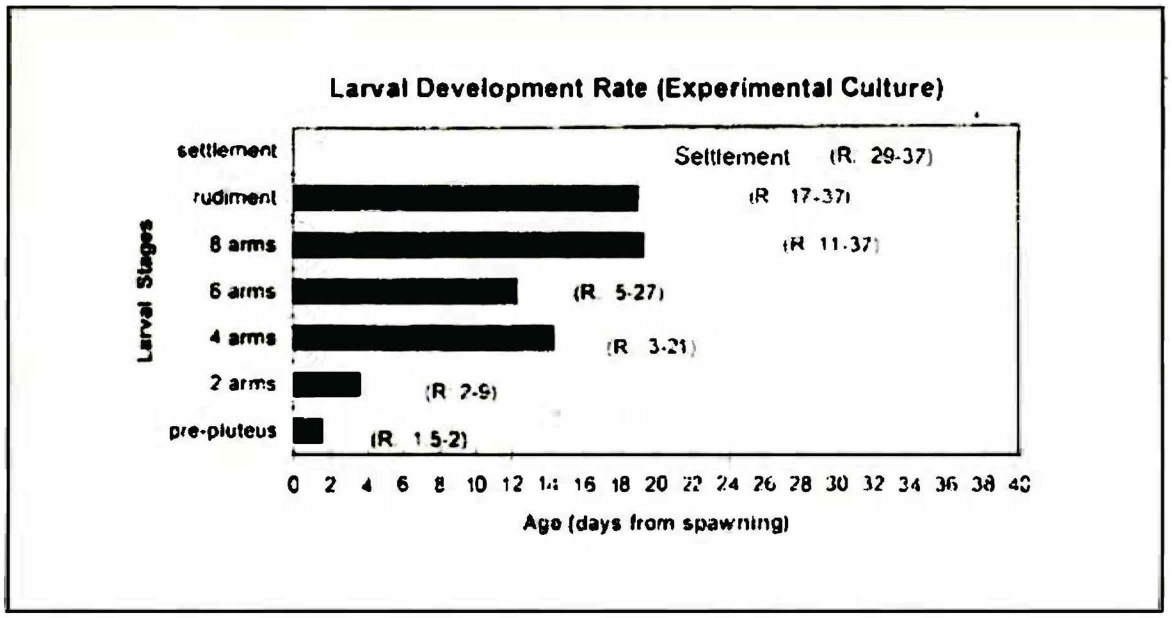

Figure 4a. Average duration of different larval stages of *T. gratilla* at the Bolinao Marine Laboratory based on three batches of larvae reared in experimental culture set-up. (R-range of duration of each larval stage).

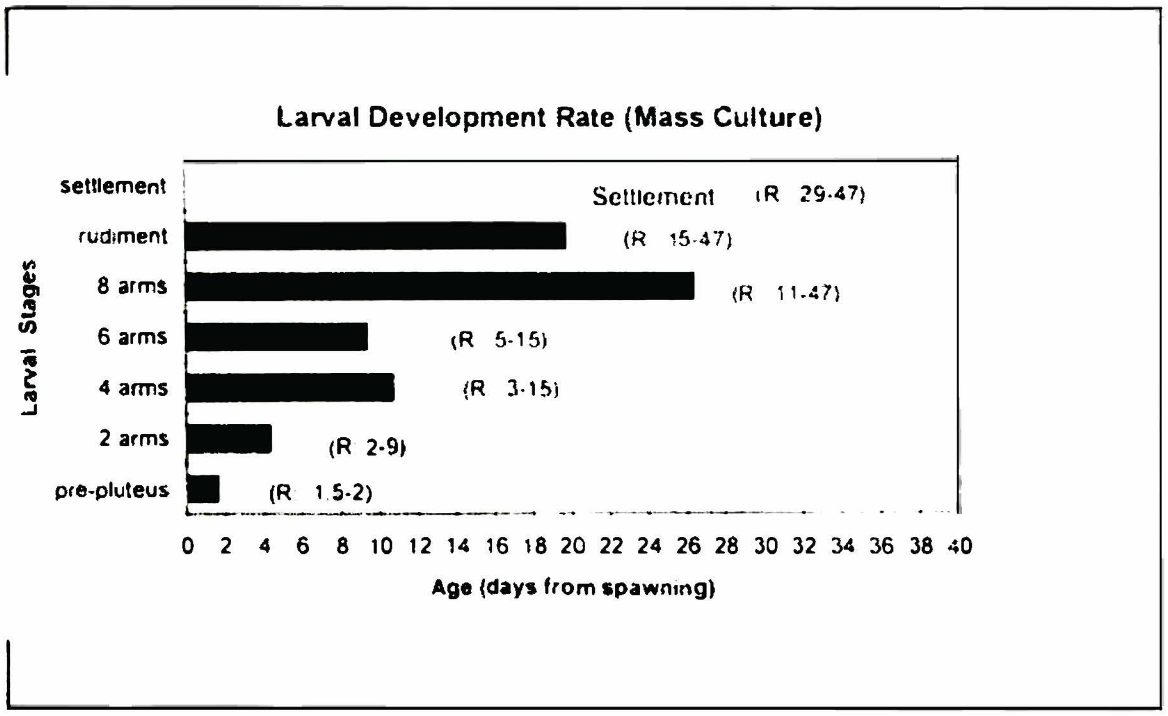

Figure 4b. Average duration of different larval stages of *T. gratilla* at the Bolinao Marine Laboratory based on three batches of larvae reared in mass culture set-up. (R-range of duration of each larval stage).

observed in larvae reared under the pure diet regimes.

Slower development was also observed in the pure diet treatments compared to the mixed diets. Specifically, majority of the larvae in the pure diet treatments were still at the 4-arm stage at day 11 in contrast to the standard mixed diet treatment wherein most larvae were at the 6-arm stage. Notably, upon introduction of a mixed diet at the 4-arm stage, in the standard mixed diet regime, development rate increased (Fig.5a). Results from this experiment indicate that mixed diets enhance larval growth and survivorship relative to pure diets of cultured *T. gratilla* larvae, specifically from the time when majority of the larvae are in the 8-arm stage (about 17 days) up to the pre-settlement stage. Invertebrate larvae often grow and develop better on a mixture of microalgal species than they do on any species individually (Hinegardner 1969; Pechenick 1987; Strathmann 1987; Basch 1996). Presumably each species contributes some necessary micronutrient that is lacking in the others. The diatom *Chaetoceros gracilis* was reported to contain high levels of the biochemically important fatty acid, arachidonic acid, while *Isochrysis galbana* was reported to contain a high proportion of the fatty acid, 18:5ω3 (Napolito et al. 1990). Microalgal lipid composition has been suggested as critical for promoting optimal growth and metamorphosis in bivalve larvae. This seems to be also true for other invertebrate larvae like sea urchins.

Gonad Yield

Average monthly gonad weight to test diameter ratios for 1996 and 1997 are shown in Fig. 6. The total numbers of sea urchins sampled were 1,436 and 1,388

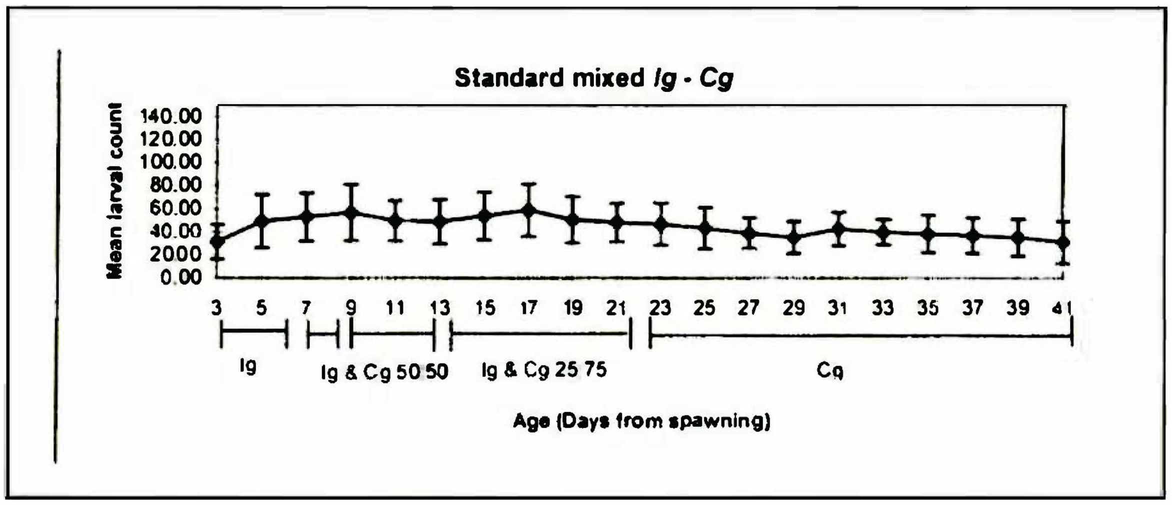

Figure 5a. Mean larval count (n=3 50-mL test tube per replicate; 3 replicates per feeding regime) of *T. gratilla* fed in the standard feeding regime used for culture of sea urchin in Bolinao Marine Laboratory.

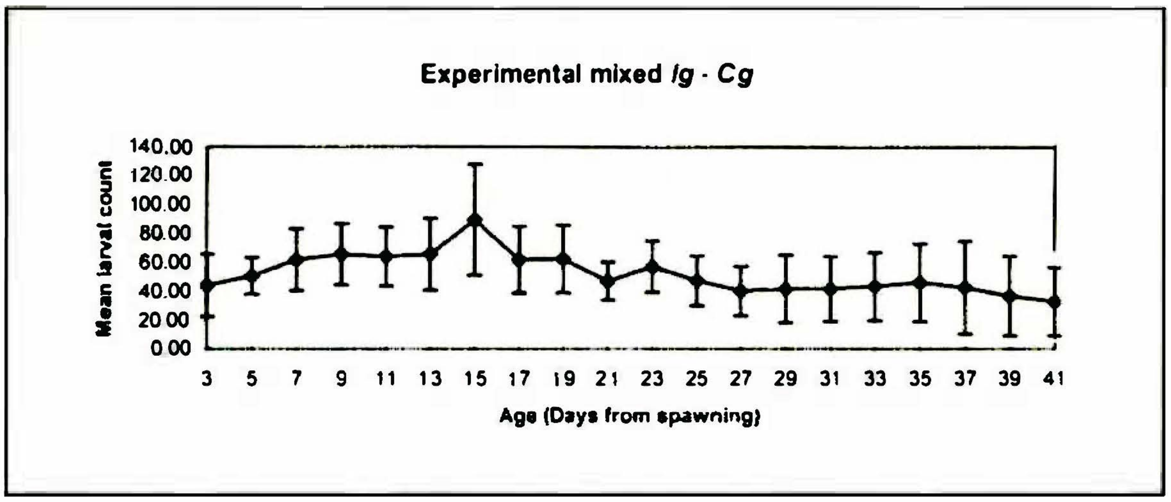

Figure 5b. Mean larval count (n=3 50-mL test tube per replicate; 3 replicates per feeding regime) of *T. gratilla* fed with mixed diet of *I. galbana* and *C. gracilis.*

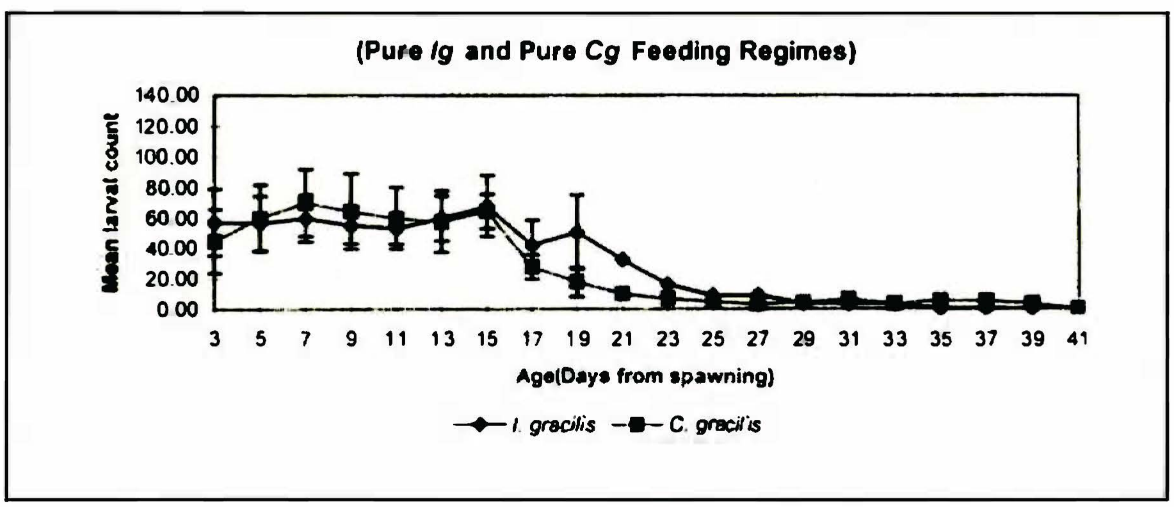

Figure 5c. Mean larval count (n=3 50-mL test tube per replicate; 3 replicates per feeding regime) of *T. gratilla* fed with pure diet of *I. galbana* and pure diet of *C. gracilis.*

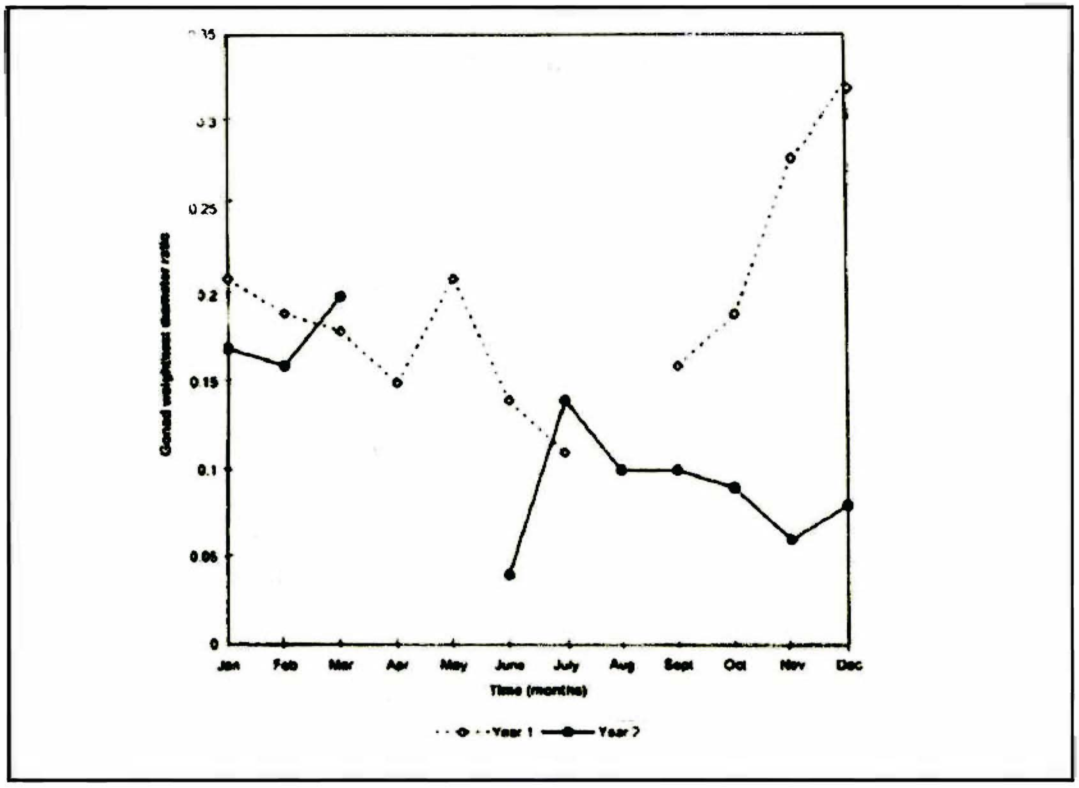

Figure 6. Mean monthly gonad test diameter ratio of reproductively mature sea urchins from Lucero, Bolinao, Pangasinan.

for year 1 and year 2, respectively. Although some months were not sampled, data show considerable inter-annual variation in the gonad weight and test diameter. In 1996, the highest gonad-test diameter ratio was observed during the onset of colder months, October to December. The lowest average ratio was observed during the month of July (i.e., during the peak of the rainy season). In 1997 gonad weight to test diameter ratio was higher in January to February and was lowest during the rainy season in June. Notably, gonad weight to test diameter ratio was considerably lower during October to December in contrast to the very high ratios in 1996 during the same period.

Average gonad indices (gonad weight to body weight ratio) of different size groups of sea urchins ranged from 4.0 to 13.4 in the low stocking density cages and 1.4 to 14.3 in the high stocking density cages. Results showed significantly lower (Student's t-test, $p<0.05$) gonad index of *T. gratilla* for sea urchins with sizes ranging from 6.0-6.4 cm test diameter in the high stocking compared to low stocking cages. However, for larger individuals, with sizes ranges 6.5-6.9 cm and 7.0-7.4 cm test diameters (Fig.7), no significant difference was found in relation to stocking density. This suggests that the smaller sized (e.g., 6.0-6.4 cm TD) urchins are the ones most affected by increased stocking density of *T. gratilla.* This size-dependent effect indicates that smaller individuals may be outcompeted in feeding by larger individuals resulting in decreased gonad yield particularly when stocking densities are high. In this case, gonad yield may be optimized by rearing individuals of similar sizes and determination of optimal stocking densities with respect to size. Density has been reported to influence the size of the gonads in the echinoid *Evechus chloroticus* (6.0 ± 2.2 cm TD), being generally larger at

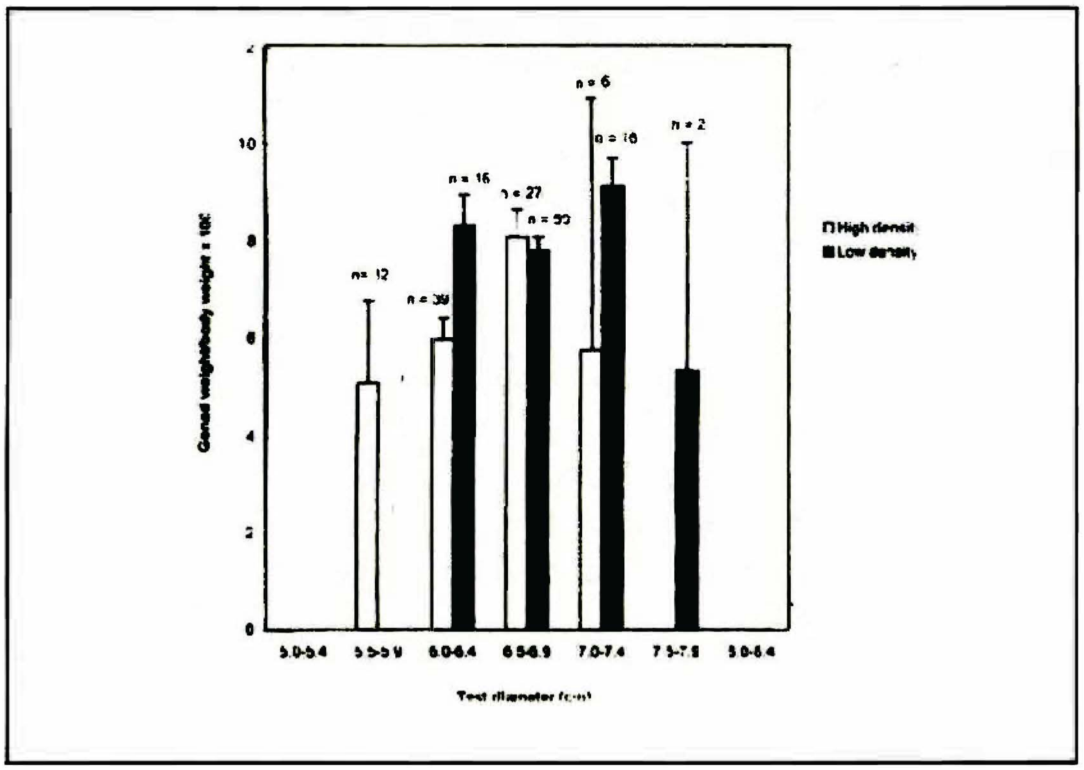

Figure 7. Average gonad index of different size classes of sea urchins reared in cages with different stocking densities. (High density = 291.50 m^{-3}, low density = 212.38 m^{-3}).

lower stocking density (Andrew 1986). In addition, consumption rates of individuals at high densities were lesser than those in the low density cages. It was suggested that crowding may have deprived some individuals access to algae or may have had a depressing effect on the feeding behavior of the sea urchins.

The correlation of gonad indices with water temperature reported for cultured *T. gratilla* in Okinawa, Japan is opposite from that found in this study. High gonad indices (i.e., percent gonad weight to body weight ratio) coincided with the months when water temperature was highest (~26-27 °C, October 1978 and August 1979) and low gonad indices during cold months (~21 °C, January of 1978 and 1979) (Shokita et al. 1991). The average water temperature in Bolinao, Pangasinan during the colder months (i.e., October-February) when gonad indices (gonad weight to test diameter ratios) were relatively higher, is ~ 24-25 °C. This is close to the water temperature in Okinawa when gonad indices were also higher. These clearly show that there is an optimal water temperature range for gonad production. In addition, the lower gonad indices during the rainy season in Bolinao indicate that salinity changes brought about by the monsoon rains have a significant influence of the gonad yield of tropical sea urchins. Seasonality in gonad yield of different sea urchin species has likewise been reported (e.g., *Paracentrotus lividus* – Blin 1997; *Anthocidaris crassispina* – Chiu 1988; *Tripnuestes esculentus* – Lewis 1958). A gonad weight to body weight ratio of 10.0 is considered the benchmark for a very good gonad yield (i.e., *P. lividus*, Blin 1997). The gonad weight to body weight ratio of cultured *T. gratilla* in Japan ranges from 12.0-14.0 depending on the season and sex. In this study, the average gonad weight to body weight ratio in

August 1997 (i.e., based on the stocking density experiment) ranged from 5.0-9.0. A more systematic sampling of cultured sea urchins at different times of the year and under different rearing conditions, to better elucidate the effects and interaction of stocking density and size, is needed to determine ways by which reproductive output and harvest of sea urchin roe may be optimized.

CONCLUSION

Significant improvements on the mass culture potentials for the sea urchin *Tripnuestes gratilla* larvae have been accomplished. Mass production of pre-settlement larvae (i.e., 30,000 per batch compared to 5,000 per batch in the experimental culture set-up) were realized for a number of larval batches. Likewise, spawning and larval rearing were conducted on a regular basis to optimize output of the pilot sea urchin hatchery at the UPMSI Bolinao Marine Laboratory. Despite the lower percent survivorship in the mass culture set-up, production of pre-settlement larvae was increased to tens of thousands compared to the less than ten thousand per experimental jar. Even with higher survival rates, this level of production will entail the maintenance of dozens of culture jars which will be very tedious and therefore not cost-effective. Improved maintenance of water quality in the mass culture rearing set-up and the installation of a UV filtration system, to provide regular supply of fresh UV filtered seawater, more than doubled the production of pre-settlement larvae in 1997.

With the mass production of pre-settlement larvae, a significant increase in the production of juvenile seedstock was realized in part by scaling up settlement protocols in the outdoor hatchery. Improving survivorship of newly settled sea urchins is presently the major bottleneck in the mass production of juveniles. Further studies on the induction of settlement and metamorphosis of pre-settlement larvae (manuscript in preparation) indicate an enhanced metamorphosis of settled larvae into juveniles.

The potential of *T. gratilla* grow-out culture as a resource management tool is high. Cultured sea urchins have high growth rates and attain reproductive maturity at 6.0 cm TD only 7-8 months after artificial fertilization (Juinio-Meñez et al. 1997). The wide seagrass reef flat and *Sargassum* beds around Bolinao, Pangasinan provide suitable grow-out sites for sea urchins. Grow-out culture of sea urchins in pens utilizing either the wild or hatchery-reared seedstock can contribute to the enhancement of the recovery of the natural sea urchin populations by: (1) increasing the probability of successful fertilization by aggregating the adults (e.g., Levitan 1991; Levitan et al. 1992) and (2) enhancing local recruitment by the presence of adult conspecifics which facilitates larval settlement (e.g., Tegner and Dayton 1977). Moreover, grow-out culture of sea urchins can easily be undertaken by fishers as a natural resource enhancement tool and a source of supplemental income or food (Juinio-Meñez et al. 1995).

Pilot testing of the grow-out culture technology together with the development of protocols to improve gonad yield will be valuable in ensuring the realization of the potential ecological (i.e., increased reproductive output) and economic benefits (i.e., optimum harvest of roe) of sea urchin grow-out culture.

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