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Survivorship and vertical distribution of coral embryos and planula larvae in floating rearing ponds

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Abstract: Outdoor floating rearing ponds were used for mass rearing of eggs and embryos of Acroporid corals that were transported from slicks on the sea surface, after simultaneous spawning at Akajima, Okinawa, on May 23, June 13, 2003, and June 1, 2004. The mortality rate of embryos (i.e. until about 70 hrs post-fertilization) was high and varied by slicks, ponds and over time in a single pond. However, the mortality rate lowered among swimming planula larvae. A pond mean of 43% of the stock on Day 3 or 134 inds/l survived until Day 6 when they achieved viability for settlement. The embryos were concentrated on the surface of ponds, but the planulae were distributed more or less evenly throughout the water column. By reducing the density of embryos on the surface and providing shade to avoid direct sunlight, it will be possible to produce in the ponds more planulae able to settle on the substrate.

Key words: Acroporid coral, planula larvae, rearing ponds, mortality, vertical distribution

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INTRODUCTION

Increasingly, coral reefs are facing threats from a number of natural and human causes. A recent report estimates that 20% of the historical extent of coral reefs in the world have been destroyed during the past 40 years. It also predicts that 24% of the world's reefs are under imminent risk of biological collapse through human pressures, and a further 26% are under a longer-term threat of collapse (Wilkinson 2004). To conserve the coral reefs, comprehensive coral reef protection and management legislation is needed to promote research and restoration. However, the today's situation is extremely worrying. One researcher asserts that this unique ecosystem may not be able to show immediate prospects of recovery unless extensive restoration and remediation projects are carried out (Rinkevich 2005).

Transplantation of coral fragments has been accomplished in Japan and other countries at various places as one of the coral reef restoration technique (Okubo and Omori 2001, Rinkevich 2005). Fragment transplants from donor sites to new sites, however, result in high mortality. Also, fragments from a limited number of donor colonies may cause genetic bias in a new coral population at the spot where they are transplanted. Moreover, in many cases the transplantation act itself inflicts additional stress on donor coral populations. The fragment trans-

plants in Japan have been characterized by lack of standard procedures and high labor costs for large-scale transplantation. There are no practices, other than avoidance of physical impacts that can yet be recommended for effective restoration of large areas using fragment transplant techniques.

In light of the current state of transplant knowledge, we have attempted to develop restoration techniques that use sexual reproduction of Acroporid corals, and we have devised methods for obtaining biological material and inducting larval settlement (Hatta et al. 2004, Omori 2005).

In Okinawa, following simultaneous spawning of corals in May and June, large numbers of eggs and embryos aggregate and drift in slicks on the sea surface (Hayashibara et al. 1993). They can be skimmed from the surface and reared using a water tank in the laboratory or an outdoor rearing pond until the planula larvae gain viability to settle. They may then be seeded onto artificial substrates or on natural reef rocks.

The present study seeks an enhanced technique for mass rearing of coral embryos and planulae. Temporal variations of their mortality and vertical distribution in the rearing ponds were studied. We have previously reported partial results (Omori et al. 2004), but we summarize herewith the previous data and some new results of additional experiments for a more conclusive report.

METHODS

The outdoor floating rearing ponds (hereafter referred as the ponds) in Aka Port on Akajima Island, Okinawa Prefecture, are made of nylon-reinforced vinyl fabric, 2.0×2.0×1.0 m - W×L×D. (See another pond for study to vertical distribution). Each contains 3.2 tons of seawater (Fig.1). The ponds were supported by floating frameworks anchoraged in well protected water about 30 m from a public boat ramp. Fresh seawater was provided throughout the perforated showering hose along lip of the pond, by a submerged pumps, yielding approximately 1-ton/h of water. The showering is devised to prevent adhesion of coral embryos and planulae on the vinyl fabric when they are floating on the surface.

Experiments were carried out during the periods from May 23 to 29, and June 13 to 17, 2003, and from June 1 to 9, 2004, when slicks appeared on the sea surface at Aka Port or neighboring waters after simultaneous spawning at night

Fig. 1. Floating rearing pond

(around 22:00hr). On May 23, 2003, the slicks were found 9h after spawning [Exp. A (May 2003) in Fig. 2], whereas they were found immediately after spawning on June 13, 2003 [Exp. B (June 2003), C (June 2003) and on June 1, 2004 [Exp. D (June 2004)]. The slicks were skimmed and introduced within hours to the ponds without measuring the initial densities.

Temporal variation of densities (number of individuals/l) of eggs, embryos and planulae was determined at 7 ponds. At a single pond only, the pond water was sampled at 9 evenly spaced positions by inserting an acrylic tube-sampler from the surface to bottom [Exp. A (May 2003) and B (June 2003)]. The tube, 1.3 m long and 3 cm in diameter, collected about 650 ml of the water-column at one manipulation (Yokokawa 2004). At the other 6 ponds, 5 to 10 replicate samples (50 ml each) were collected from the surface immediately after the pond water was stirred well to remove bias and stratification of the distribution [Exp. C (June 2003) and D (June 2004)]. The number of eggs, embryos, and planulae in each sample was counted using a dissecting microscope.

Vertical distribution of embryos and planulae in daytime was measured for 7 days at a single pond that had the same dimension as other ponds, but was 2.0 m deep. The slicks were brought to the pond soon after the spawning on May 31, 2004. Samples of water from 7 discrete layers between the surface and 1.8 m deep were sampled from four positions on the surface using the bottle sampler of 130 ml each (Yokokawa 2004).

The water temperature and salinity of the ponds were measured daily with a portable TS meter (model ACT20-D, Alec Electronics Inc.) during the rearing experiments. The surface

water temperature in the outdoor ponds in 2003 and 2004 varied between 24.5 and 29.0℃, salinity varied from 31.19 to 34.04 psu. The water in the ponds was well mixed. Variation of the temperature between the surface and bottom was less than 0.5℃. Variation of the surface temperature between inside and outside of the pond was always less than 0.3℃.

RESULTS

Eggs and embryos collected were exclusively scleractinian corals from the genera *Acropora* and *Montipora*. Larvae of *Acropora* could be distinguished from *Montipora* by the absence of zooxanthellae in the body, and *Acropora* always comprised more than 90% of embryos in the slicks in 2003 and 2004.

The temporal variation of the mean density of embryos and planulae during the four rearing experiments is shown in Fig.2. Results of Exp. A (May 2003) and B (June 2003) shown are at the same pond but from different slicks of different months. The average density of embryos in Exp. B (June 2003) lowered suddenly from 1198 inds/l to 78 inds/l during the first 16 h post-fertilization. High mortality among those embryos was seen to relate to their high density in certain places at the surface. The difference of densities among 9 samples at 16 h post-fertilization was ~8.8 times (S.D. 43.6 inds/l).

The starting times of the experiments and initial densities of the populations were not standardized. However, the results apparently indicated that mortality at the embryo stage was significantly higher than that at the planula stage (Fig. 2). In Exp. D (June 2004), the mean density of embryos over the first 48 h varied significantly among 6 ponds. In 4 out of 6 ponds the original density at 16 h post-fertilization had increased at 40 h. The mortality rate in the embryos between 14 h and 67 h post-fertilization was 29.6%/day, whereas, that between the 67 h and 135 h post-fertilization was 20.3%/day. That is to say, the mean number of planulae at a pond, about 15% of the original stock or 429×10^3 inds (134 inds/l), survived on Day 6 (135h post-fertilization).

In this calculation a considerable number of unfertilized eggs and small, abnormal embryos with separated blastomeres usually was included in the count until Day 3. Normal eggs of *Acropora* are larger than 510 μm (Wallace 1985, Ohya and Iwao, 1998), whereas the small embryos we observed at the present study were always less than 300 μm.

Until 62 h post-fertilization, embryos were concentrated near the surface (>90%) (Fig.3). After developing into swimming larvae (about 70 h post-fertilization), they began to disperse, and were distributed more or less evenly throughout the water column. On Day 4, when some larvae were viable for settlement, 15% of them were found near the bottom. A tendency for all larvae to move toward bottom was not evident, however, at any time during the rearing period.

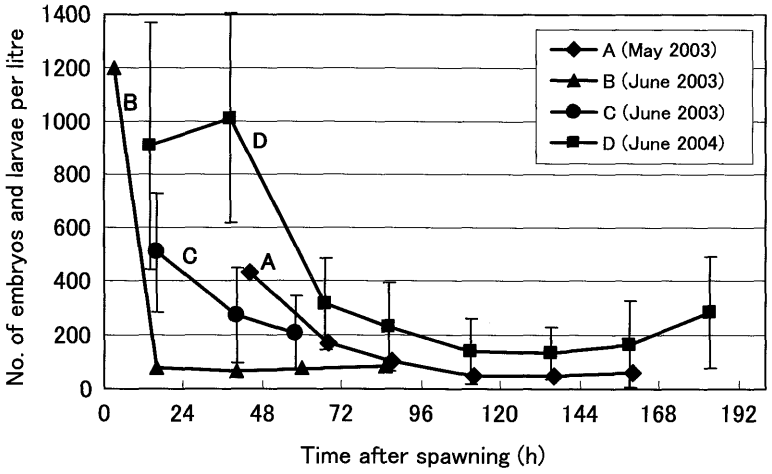

Fig. 2. Mean density of coral embryos and planulae over time in 7 ponds from May 23 to 29 [Exp. A (May 2003)], June 13 to 17 [Exp. B (June 2003), Exp. C (June 2003)], and June 1 to 9, 2004 [Exp. D (June 2004)]. Error bars show ±S.D.

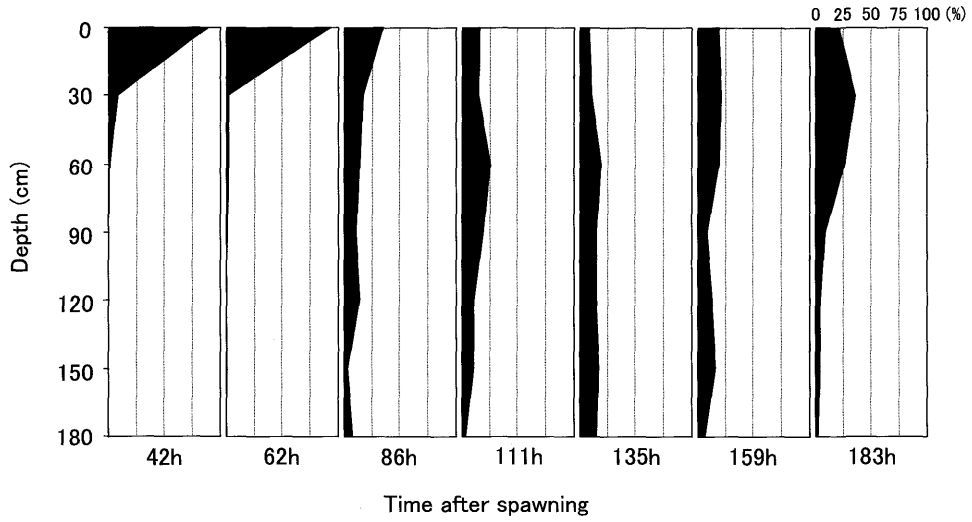

Fig. 3. Vertical distribution of coral embryos and planulae in the pond during daytime.

DISCUSSION

The rearing in the ponds resulted in fairly high mortality of embryos in 3 days post-fertilization. The mortality was lower on Day 4 and afterward when they became planulae. The average density of planulae on Day 6 (134 inds/l) in the ponds [Exp. E (June 2004)] was very close to that obtained by Heyward et al. (2002) who reared embryos and planulae from the slick, using a self-contained floating pond of 1.5 tons water capacity in the sea. They produced a pond mean of 5% of the original stock found on Day 2 or 133 inds/l surviving to Day 6.

Generally, the unfertilized eggs dissolve within 18-36h after spawning (Iwao, unpublished data). Pollutants leached from dead eggs and embryos on the surface layer could be highly harmful to physiologically sensitive embryos. Given that the initial densities of eggs and embryos on the surface water in Exp. B (June 2003) and Exp. D (June 2004) were 958×10^3 inds/m^2 and 726×10^3 inds/m^2, respectively, it is considered that their excess concentration and exposure to ultraviolet radiation at the surface might cause high mortality. Differences in mortality of embryos and planula larvae with and without ambient UV radiation, particularly UV-B (280-320 nm), were not investigated for the present eggs and embryos. But, harmful effect of UV-B on survivorship of eggs and planulae has been pointed out for 3 species of broadcast-spawning reef corals (Wellington and Fitt 2003). They also found that survivorship of larvae originating from shallower parent colonies was higher compared to those from deeper parent colonies due to higher UV-tolerance.

The planulae began to swim using cilia, and dispersed to the whole range of pond depth on Day 4 (86 h post-fertilization in Fig. 3), indicating viability of movement to settle. Their vertical distribution is, however, likely to be dependant largely on their specific gravity, that is almost equivalent to the surrounding water.

Our outdoor experiments indicated that it was possible to produce planulae able to metamorphose with a pond mean of 429×10^3 inds or 134 inds/l. If the initial densities of eggs and embryos in the pond were lowered and exposure to UV-B radiation was reduced with shade, it might be possible to increase survivorship and produce many more planulae able to settle on substrates. In this connection, using a large water tank under shade, Shimomura (2004) has successfully produced 250×10^3 inds (238 inds/l) of planulae by rearing lower densities (100×10^3 inds/m^2) of eggs and embryos of *Acropora* spp. that had been transported from the slick.

Rearing of coral eggs and embryos in transported slicks using floating ponds is simple and yields good genetic variability of the larvae. With manipulation of the slick, it will be possible to rear some tens of millions of coral larvae. However, appearance of the slicks near stationery rearing ponds is not always guaranteed. Thus, it is risky to count only on slicks to provide a continuous supply of eggs and embryos for rearing. We must consider alternative methods of collecting eggs and embryos.

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大型飼育水槽の中でのミドリイシ属サンゴの胚および幼生の生残率と鉛直分布の変化

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要旨：サンゴ礁修復のためのサンゴ幼生の大量飼育を目的として、野外の浮体に取り付けた大型ビニール水槽（水量3.2t、以下水槽）を用いて、一斉産卵のあとに形成されたスリックから取り込んだミドリイシ属サンゴの胚と幼生を育てた。2003年の実験では、初期密度300-1200個/ｌのすべての水槽で受精後３日目までに胚数が大きく減少した。2004年の実験でも、同様に受精後３日目までの胚期に大きな減耗が認められた。この間の日間死亡速度は平均で29.6%であった。しかし、プラヌラ幼生になってからの減少は小さく、日間死亡速度は20.3%となり、初期密度の15%にあたる134個体/ｌ、１槽あたりおよそ43万個体の幼生を着生能力を獲得するまで飼育できた。胚の90%以上が海面付近に集中していたのに対し、それ以降のプラヌラ幼生は、海面から下層までほぼ万遍なく分布していたことから、海面での高密度状態と紫外線、殊にUV-Bへの暴露、および未受精卵などの分解による水質の悪化が胚期減耗の原因であると思われた。胚密度と水質を管理調整し、水槽に覆いをつけて直射日光をさえぎることにより、野外で着生能力をもつ幼生をもっと大量に育てることができるだろう。