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Significant drop of fertilization of *Acropora* corals in 1999: An after-effect of heavy coral bleaching?

Abstract—In June l999, after devastating coral bleaching in l998, laboratory fertilization of *Acropora nasuta*, one of the most abundant reef-building corals in Okinawa, Japan, decreased significantly from usual rates (>94%) to an average of 42% at a sperm concentration of 10^5 ml^{-1}. Similar decreases were observed in four other mass-spawning acroporid corals. We also found a decrease in sperm motility in the laboratory. A series of experiments to determine the effects of sperm concentration on fertilization rates revealed that sperm of 10^7 ml^{-1} was needed to obtain a rate >80%. Sperm concentration in surface seawater during mass spawning was highest within 1.0 h of spawning but decreased sharply thereafter. These results suggest that gamete dilution plays an important role in limiting the fertilization of coral eggs in the sea. As successful fertilization appears to have been much lower in 1999, we suspect that production of new coral recruits was also reduced greatly. Current and future sea-temperature increases thus pose a severe potential threat to coral reefs by increasing the frequency of coral bleaching and consequently leading to further declines of coral recruitment and hence, reef corals.

It is well known that devastating coral bleaching occurred in 1997 and l998 worldwide in the Pacific and Indian Oceans, the Persian Gulf, and the Mediterranean, Red, and Caribbean Seas (ISRS 1998*a*,*b*; Wilkinson 1998). Reports from various areas indicate that higher water temperatures affected both hard and soft corals, sea anemones, giant clams, and other invertebrates that depend on photosynthetic symbiotic microalgae (zooxanthellae). Coral reefs in Okinawa, Japan, were hit the hardest in August and September 1998. In Aka Island, Okinawa, where the present study was carried out, the bleaching affected 56–97% of reef corals in September 1998 (Taniguchi et al. 1999). Numerous reports are being published on bleaching in the Indo-West Pacific area, but studies regarding the effects on coral reproduction and recruitment are few (Ward et al. 1998, cited in Hoegh-Guldberg 1999; Hirose and Hidaka in press).

In Aka Island, development of the ovaries of *Acropora* corals commences in autumn. Development of the testes commences ~4 months before the mass spawning, which occurs in Aka Island in early summer on a night around full moon (Hayashibara et al. 1993). In 1999, it occurred between 2135 and 2220 h local time on 2 June, 9 months after the heavy coral bleaching. *Acropora nasuta* and many species experienced the phenomenon. Percentage fertilization of coral gametes of *A. nasuta* and four other acroporid species was determined during the mass-spawning event by cross-fertilization experiments in the laboratory.

Since 1994, techniques developed at the Akajima Marine Science Laboratory to determine fertilization success of single-colony crosses have been standardized to facilitate genetic studies (Hatta et al. 1999). These same techniques were employed for all species in the current study.

We collected mature small-colony fragments and kept them for 1 week at the Aka Port. They were individually transferred into separate bowls just before spawning, then were allowed to spawn their gamete bundles in the dark. The bundles, which contained both eggs and sperm, were allowed to rupture. The floating eggs were collected and washed twice with filtered seawater to remove sperm. Eggs (200–500) were mixed in a 50-ml glass vial with sperm of another colony at a sperm concentration of 10^5 ml^{-1} within 3 h after spawning. They were later washed in filtered seawater. Relative numbers of morulae/gastrulae and uncleaved eggs were counted in replicate samples 6–8 h later to estimate percentage fertilization.

Between 1995 and l997 (no data in 1998), mean percentage fertilization of *A. nasuta* was always >94% at sperm concentrations of l0^5 ml^{-1}. In 1999, however, it dropped to 42% (Table 1). The difference was statistically significant at $P = 0.01$ (Mann–Whitney *U*-test and Student's *t*-test). Similar results were obtained for all other acroporid corals tested, although the number of crosses in some species was too limited for statistical analysis (Fig. 1). The reason for low fertilization of *Acropora gemmifera* in 1997 was not clear, but this species has always shown considerably large variation in fertilization rates.

Fertilization success for *A. nasuta* and *Acropora tenuis* was increased with increasing sperm concentration, reaching 80% at 10^7 ml^{-1} and 95% at 10^8 ml^{-1} (Fig. 2). These results in 1999 were different from those determined in another year when bleaching did not occur. In 1997, the fertilization rates of *A. nasuta*, *Acropora formosa*, and *Acropora degitifera* were >90%, even at a sperm concentrations of 10^4 ml^{-1} (data not shown). With *Montipora digitata* and three other corals on the central Great Barrier Reef, Australia, maximum percentage fertilization in 1988 and 1989 was reached at sperm concentrations between 10^5 and l0^6 ml^{-1} in all cases (Oliver and Babcock 1992). Thus, at least 100 times higher sperm

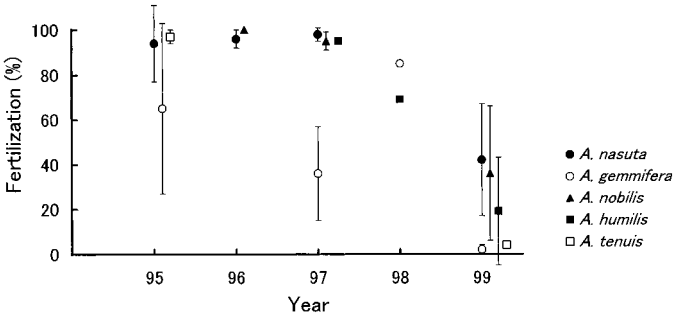

Fig. 1. Change of fertilization of *A. nasuta* and four other acroporids at Aka Island from 1995 to 1999. Values represent mean and standard deviation (n = 6–24). For small number of crosses (n < 5), only mean values are shown. Variability (v) was as follows: *A. nobilis* in 1996 (n = 4) v = 100–100; *A. humilis* in 1997 (n = 4) v = 90–95, 1998 (n = 4) v = 40–98; *A. gemmifera* in 1998 (n = 4) v = 78–91; *A. tenuis* in 1999 (n = 2) v = 0–8.

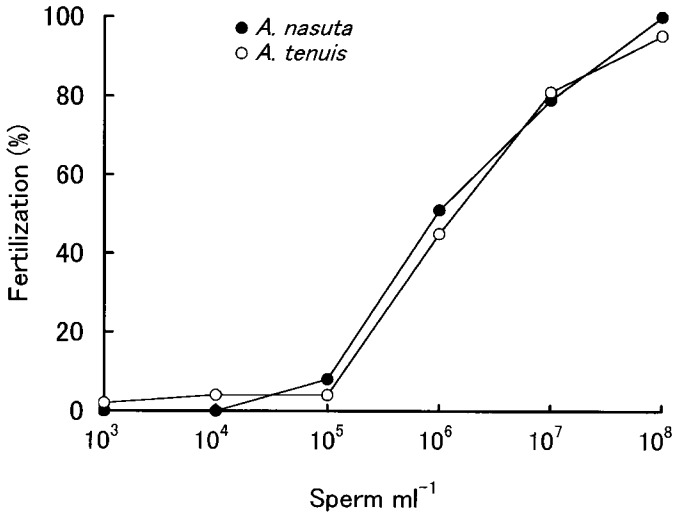

Fig. 2. Effect of sperm concentration on fertilization of *A. nasuta* and *A. tenuis* on 1 and 2 June l999. Eggs (50–300) were mixed in a 50-ml glass vial with a series of sperm concentrations. Individual points for $n = 1$. Spawning of *A. nasuta* on 1 June was artificially induced with hydrogen peroxide.

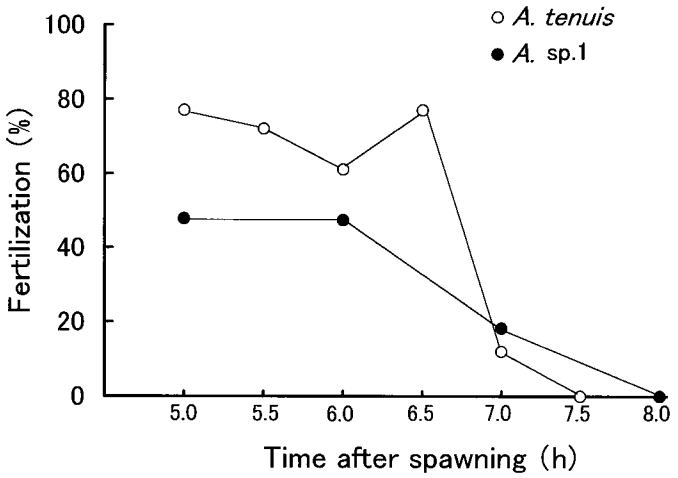

Fig. 3. Gamete age effects for fertilization of *A. tenuis* and *Acropora* sp. 1 at a sperm concentration of ~10^7 ml^{-1}. Sperm and eggs were separated at the time of spawning and later recombined with gametes from other colonies at varying time. They were of the same age at the time of mixing. Individual points for $n = 1$. Experiment with *A. tenuis* was done on 2 June 1999 and *Acropora* sp. 1 on 5 August 1999.

concentrations were needed in 1999 to obtain fertilization levels similar to those of previous years and in other studies.

A noteworthy finding in 1999 was that some sperm of *A. nasuta* did not move vigorously in the laboratory (data not shown). Low sperm motility was also found in 1999 for spawn of *M. digitata* and *Pocillopora verrucosa* from the Okinawa main island (Hirose and Hidaka in press). Hirose and Hidaka observed apparent testes shrinkage and decrease in number of eggs in both species also. Although egg size did not change, fertilization of *P. verrucosa* dropped sharply from 80 to 90% in 1998 to <5% in 1999. Decreased sperm motility suggests limited energy reserves in each sperm cell.

In the laboratory, we found that maximum fertilization of *A. tenuis* and *Acropora* sp. 1 as identified by Hayashibara (1995) was obtained at all concentrations tested, when eggs were fertilized within 30–50 min of spawning (data not shown). To determine if the viability of gametes is a function of time after release, eggs and sperm were held separately, after which we mixed eggs with sperm (l0^7 ml^{-1}) at intervals up to 8 h. The gametes maintained reasonable viability for up to 6–6.5-h postreleases, but fertilization dropped off rapidly to <20% by 7 h later (Fig. 3). A similar result has been obtained for *Acropora millepora* at sperm concentrations effective during a normal year (Willis et al. 1997). The present study suggests that competence of eggs did not change after the bleaching, but rather, that the number of mobile sperm at a given concentration was reduced significantly.

In the field, gamete dilution occurs rapidly. During the mass spawning event on 2 June l999, we sampled water by skimming the sea surface at time intervals from a boat on the reef flat where coverage of corals was ~30%. Weather conditions at the time of spawning were calm. Synchronous gamete release occurred around the floodtide (2137 h local). Because the sea was glassy, large numbers of gamete bundles released from coral polyps were visible near the surface around the boat. During the first 1.0 h after spawning, sperm concentrations were about 10^6 ml^{-1}, but they dropped quite rapidly over the next few hours, and sperm concentration was virtually zero 6 h later (Fig. 4). This decrease may have been caused by diffusion and advection at the surface as well as by sinking of the sperm through the water column. In any case, for hermaphroditic species that broadcast spawn into the water column, high concentration of sperm is necessary, and we cannot expect successful fertilization 1.0 h after spawning.

We found few conspicuous slicks of eggs and larvae at the surface of our water-sampling site the next morning. Percentage fertilization of various coral species in five water samples from the slicks was 65–78% at 0950 h. In the laboratory, some of the uncleaved eggs had begun to disinte-

Table 1. Change in fertilization of *Acropora nasuta* at Akajima Island during the period from 1995 to 1999. No data for 1998. Statistical analysis was made with Mann–Whitney *U*-test and Student's *t*-test.

Date of measurement	No. of crosses used	Fertilization (%)			Statistical comparison with 1999	
		Mean	SD	Range	*U*-test	*t*-test
16 June 1995	24	94.2	18.6	7–100	<0.01	<0.01
6 June 1996	12	95.8	4.3	84–99	<0.01	<0.01
26 & 27 May 1997	14	98.4	2.6	90–100	<0.01	<0.01
1 & 2 June 1999	12	41.7	24.7	4–64	—	—

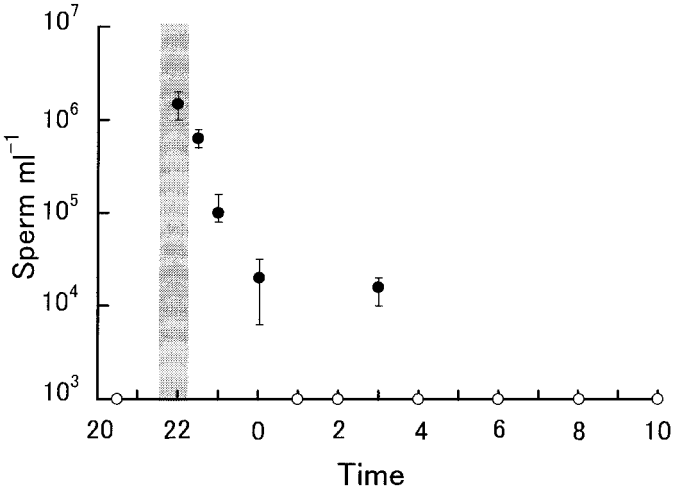

Fig. 4. Concentrations of sperm in water samples around Aka Island on a night of mass spawning, 2 June 1999. Three replicate samples of surface water were taken from an anchored boat on the coral reef flat. Mean values and variability are shown. Time of mass spawning (2135–2220 h local time: shadowed area in the figure) was determined by field observation. Sperm concentrations at 2030, 0100, 0200, and after 0400 h (open circles) were below our limits of detection ($<10^3$ ml^{-1}).

grate by this time; therefore, actual fertilization rates in the field must be lower than these figures. Together, the results in the field (Fig. 4) and fertilization in laboratory experiments (Fig. 2) indicate that successful coral fertilization was probably much lower in 1999 than in normal years. We cannot be sure if lowered reproduction of the corals was caused by the temporary loss of zooxanthellae during the bleaching 9 months previously. Regardless, sperm limitation and dilution may severely constrain the rate of successful crossing, and thus determine reproductive success, so that environmental stress that lower gamete numbers and viability can have major effects on fertilization rates of those gametes that are released. The sharp drop in fertilization rates after the 1998 bleaching episode may thus be followed by a substantial reduction in coral recruitment.

Various models have shown that seawater temperatures in tropical regions are increasing under a moderate greenhouse warming scenario and that future El Niño Southern Oscillation (ENSO) events are likely to result in higher and higher sea temperature maxima (Timmermann et al. 1999). Hoegh-Guldberg (1999) suggested that bleaching episodes as severe as the 1998 event are likely to become commonplace within 20 yr. Assuming that the sea temperatures at which corals bleach in the Okinawa region are somewhere between 29.5 and 30.0°C, based on past records, and that corals and their zooxanthellae are unable to acclimatize to rapid and continued warming of sea temperature, coral bleaching episodes will increase in frequency. Decreased recruitment may occur everywhere after such bleaching and will retard the ability of corals to recover from the bleaching damage. In some areas where coral coverage is below the threshold level for production of sufficient sperm, reproductive failure may follow bleaching. Consequently, bleaching could lead to decline of reef corals both as a direct cause of coral loss and later, by causing recruitment failure.

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