Zoological Studies **48**(4): 442-447 (2009)

Zoological Studies

Sexual Reproduction in Transplanted Coral Fragments of *Acropora nasuta*

Nami Okubo[1,*], Hiroki Taniguchi[2], and Makoto Omori[2]

[1]*Japan Society for the Promotion of Science/ Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University, Shirahama, Nishimuro, Wakayama, 649-2211 Japan*

[2]*Akajima Marine Science Laboratory, 179 Aka, Zamami-son, Shimajiri-gun, Okinawa, 901-3311 Japan*

(Accepted November 13, 2008)

Nami Okubo, Hiroki Taniguchi, and Makoto Omori (2009) Sexual reproduction in transplanted coral fragments of *Acropora nasuta*. *Zoological Studies* **48**(4): 442-447. The survival rate and sexual reproduction were examined after fragmentation and transplantation of the reef-building coral *Acropora nasuta*. Fragments of 2 different lengths, of approximately 5 and 10 cm, were transplanted onto a reef substrate in July 2001 (n = 85 at 5 cm and 71 at 10 cm) and Feb. 2002 (n = 66 at 5 cm and 66 at 10 cm), corresponding to the early and late vitellogenic stages of oocyte development, respectively. Oocyte development, fecundity, and spawning, were monitored over a 3 yr period. Oocyte development was influenced by both fragment size and season of transplantation. In smaller fragments, oocytes were resorbed, while development continued in larger fragments, suggesting that smaller fragments could not afford to invest in sexual reproduction and converted resources from oocytes into growth/survival. Oocytes of July-transplanted fragments (in the early vitellogenic stage) were resorbed, while oocytes of Feb.-transplanted fragments (in the late vitellogenic stage) continued to develop. This may have occurred because of the large amount of energy needed for further oocyte development in July-transplanted fragments until spawning in June of the following year. Transplanted fragments spawned in the 1st year; none, except for 1 fragment, spawned in the 2nd year; and no gametes were produced in the 3rd year, indicating that fragments reallocated energy resources, and that infertility occurred for a certain period of time. http://zoolstud.sinica.edu.tw/Journals/48.4/442.pdf

Key words: Fragmentation, Conservation, Fragment size, Season, Sexual reproduction.

Studies on survivorship and the reproductive ability of transplanted coral fragments are important for coral reef restoration (Okubo et al. 2005, Forsman et al. 2006). It is especially important to determine the ideal collection time and minimum fragment size that are necessary for successful propagation. This is because the maximum survival rate with the possibility of spawning needs to be established in order to develop successful restoration techniques. For example, aquariums try to establish coral breeding facilities and nurseries using sexually reproducing corals. The smallest fecund fragments should be collected to avoid stress to the donor corals and their environments (e.g., Petersen et al. 2005), and mature transplants may enhance sexual recruitment. Larvae from transplants can be introduced to sites where very little natural larval recruitment is expected due to damage to existing field colonies (Cowen et al. 2000, Mathews 2007). Furthermore, this study can also contribute to knowledge of various aspects of coral biology, including size-dependent survivorship of fragmented corals and diverse reproductive modes of colonies. The coral genus *Acropora* is widespread throughout the tropical Indian, Pacific, and west Atlantic Oceans, and typical colony shapes include arborescent, corymbose, and tabular forms (Wallace 1999). However, *Acropora* species are currently so rare that they are the first corals on the ESA threatened species list (http://

*To whom correspondence and reprint requests should be addressed. E-mail:namiokubotitech@yahoo.co.jp

ecos.fws.gov/tess_public/SpeciesReport.do).

Although several reports have stated that naturally or artificially occurring fragments reduce fecundity or stop gonad development, those studies were performed only once or just a few times after fragmentation (e.g., Smith and Hughes 1999, Zakai et al. 2000). Survivorship and growth of transplanted fragments have been surveyed and discussed (e.g., Rodgers and Cox 2003, Yap 2004), but the spawning of fragments has never previously been reported. Fecundity may differ depending on the developmental stage of oocytes at fragmentation (e.g., Schreck et al. 2001), and the reduction in colony size due to fragmentation may affect reproductive output (Sebens 1982). Connell (1973) postulated that the occurrence of sexual reproduction in a colony is determined by the size of the colony or age of the polyps comprising the colony. Few data are available, especially on the corymbose form, on physiological responses of fragments after transplantation, and fragment growth and physiological response are thought to be species-specific. Furthermore, Cumming (2002) described injury, including fragmentation, as a highly significant predictor of colony decline (shrinkage or death) for corymbose acroporids, but not for arborscent acroporids. In the present study, we report long-term observations on the survivorship, oocyte development, and spawning versus fragment size and timing of fragmentation/transplantation in the corymbose coral *Acropora nasuta*.

MATERIALS AND METHODS

This study was conducted at Majanohama Beach, Akajima I., Okinawa, Japan (26°3'52"N, 127°5'30"E). Oocytes of *A. nasuta* are usually observed in the mesoglea in Oct., and spawning generally takes place in May or June of the following year (Hayashibara et al. 1993). In total, 288 fragments of *A. nasuta* were randomly trimmed from 42 large donor colonies (each ca. 15 cm in height and ca. 120 cm^2 in plane area on 13 July 2001 and 20 Feb. 2002. Small colonies of *A. nasuta* (5-8 cm in diameter) at the experimental site were immature and did not have oocytes (Hayashibara et al. 1993). Fragments were taken from the tip (marginal part) of each colony, as described previously (Okubo et al. 2007), and each fragment was weighed underwater using a handmade balance and grouped into 2 size classes: small (ca. 5 cm from the tip, 7.6 ± 0.7 g) and large (ca. 10 cm, 20.8 ± 0.5 g). We drove 8 cm long concrete nails into a coral pavement substrate, leaving 5 cm protruding from the surface, in an area with similar light and temperature conditions to the donor colonies, and at a depth of 2-3 m. A 20 cm space was maintained between the nails at a distance of about 50 m from the donor colonies. Each fragment was vertically fastened along the nail using 1 polyethylene cable tie (Okubo et al. 2005). After transplantation, the condition and survival rate of the fragments were monitored every 2-4 mo for 2.5 yr, until Jan. 2004 while scuba diving. Fragments in which all polyps had died were counted as dead fragments. Oocyte development was studied in separate fragments from those in which the survival rate was measured. Three fragments removed from the transplants (2 sizes) and donor colonies were collected and studied every 2 mo post-transplantation during the 1st year (2002), in Apr. of the 2nd year (2003), and in Jan. of the 3rd year (2004).

Oocyte development in the 3 fragments from the donors and 3 fragments from each of the 2 transplant size classes was examined after fragmentation. In each fragment, the condition of 9 polyps with oocytes was examined as described by Okubo et al. (2007). Samples were fixed and decalcified in Bouin's fluid. As the volume and number of oocytes in a polyp vary according to the position of the polyp (e.g., Chornesky and Peters 1987), polyps from each fragment size with a similar position relative to the donors (i.e., at 3-4 cm from the tip) were respectively examined. We examined the condition of all polyps in the fragments from Aug. 2002 to June 2004 in the 2nd and 3rd gametogenesis. Three factors were quantified: (a) the average number of oocytes per polyp, (b) the average volume of each oocyte, and (c) the fecundity (i.e., the average volume of oocytes per polyp. Polyps with no oocytes were quantified as 0 in (a). Fragments lacking oocytes and testes were confirmed by histological sections which were paraffin-embedded and stained with hematoxylin and eosin (Okubo et al. 2007).

All fragments, their donor colonies, and other untouched colonies were examined at night (21:00-23:00) while scuba diving for the presence of egg-sperm bundles and spawning. These observations were conducted at night for 11-14 d before and after the full moon (21-31 May and 20-30 June 2002 and 11-25 May and 9-19 June 2003). Since both species are hermaphroditic and the eggs and sperm within each polyp are

compressed into 1 or more egg-sperm bundles, the spawning of fragments was expressed as the percentage of all fragments examined which had bundles (Table 1).

RESULTS

Survival rates

In July 2001, about 1 mo after transplantation, many fragments exhibited bleaching. The survival rate was higher in small (80%) compared to large (71.4%) fragments (Fig. 1). However, 1 yr later, the small fragments had gradually died, and the ultimate survival rate was lower compared to that of the large fragments (Table 1). The daily growth rate from 27 Sept. to 6 Nov. 2001 was 0.02 ± 0.03 g for small fragments and 0.09 ± 0.07 g for large fragments. The survival rate of fragments transplanted in Feb. 2002 was higher in large compared to small fragments during the observation period. An effect of the timing of fragmentation was also apparent. The survival rate of Feb.-transplanted fragments was consistently higher compared to July-transplanted fragments, regardless of size (Fig. 1, Table 1).

Oocyte development and spawning

July-transplanted fragments

No oocytes were found in the mesoglea of donor colonies in July 2001 after the mass spawning (Table 1). In Sept. 2001, however, 33% of the large fragments and 67% of the donor colonies produced oocytes, whereas no oocytes were found in small fragments (Fig. 2). Statistically, oocyte numbers and fecundity did not significantly differ; however, a significant difference was observed in oocyte volume between the large fragments ((0.02.5 ± 1.4) × 10^{-4} mm^3, n = 23) and donor colonies ((4.0 ± 2.5) × 10^{-4} mm^3, n = 57) (Fig. 2; Mann-Whitney U-test, U = 57, $p > 0.11$ for oocyte number; U = 892.0, $p < 0.02$ for oocyte volume; and U = 59, $p > 0.08$ for fecundity in

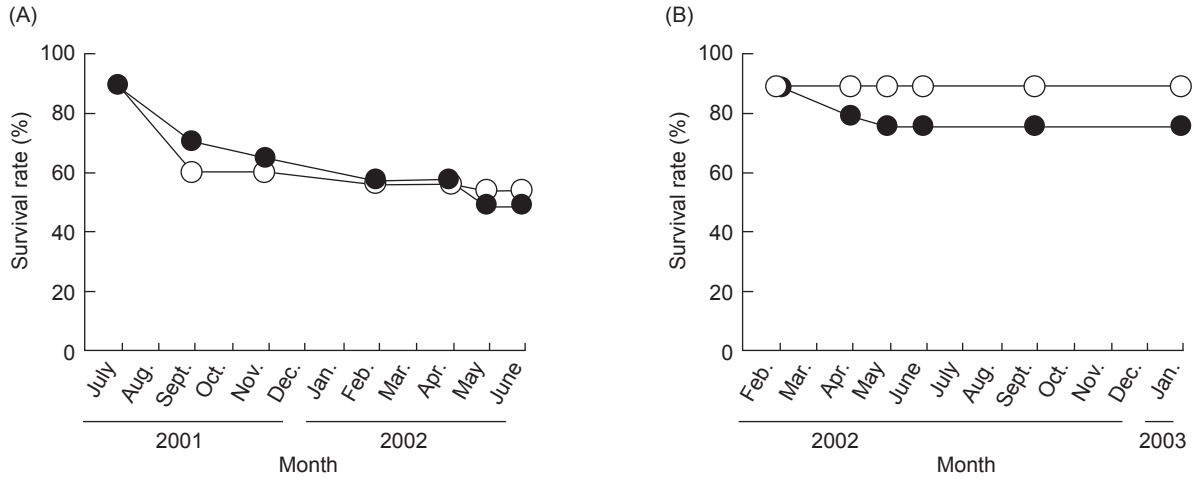

Fig. 1. Survival rate (%) of fragments transplanted on (A) 13 July 2001 (n = 126) and (B) 20 Feb. 2002 (n = 120). Solid circles, small fragments; open circles, large fragments.

Table 1. Transplantation of *Acropora nasuta*. Fragments were taken from 42 donor colonies. +, fragments with oocytes at transplantation; -, no oocytes present. Survival rates were measured 1 yr after transplantation

Date of transplantation	Oocytes present	Fragment size	Number of fragments	Survival rate (%)	Spawning (%)
13 July 2001	-	Small	70	57.0	0
	-	Large	56	64.0	43.8
20 Feb. 2002	+	Small	60	86.7	70.0
	+	Large	60	100	100

Sept. 2001; large fragments < donor colonies). In large fragments, oocyte development continued, and 1 mo before mass spawning occurred (Apr. 2002), the difference in oocyte volume between large fragments and donor colonies had further increased (Mann-Whitney *U*-test, $U = 536.5$, $p < 0.02$; $n = 21$ for large fragments, $n = 78$ for donor colonies; Fig. 2). In Feb. 2002, oocytes were observed in one of 3 small fragments, but subsequently disappeared (Fig. 2). On 29 May 2002, donor colonies and 44% of the large fragments began to release bundles at between 22:15 and 23:00, but none of the small fragments spawned. After this event, no gametes were left in the polyps of the fragments. In the 2nd year, all donors spawned in July 2003, but no fragments spawned. In the 3rd year of gametogenesis (2003-2004), no gametes were observed in small and large fragments.

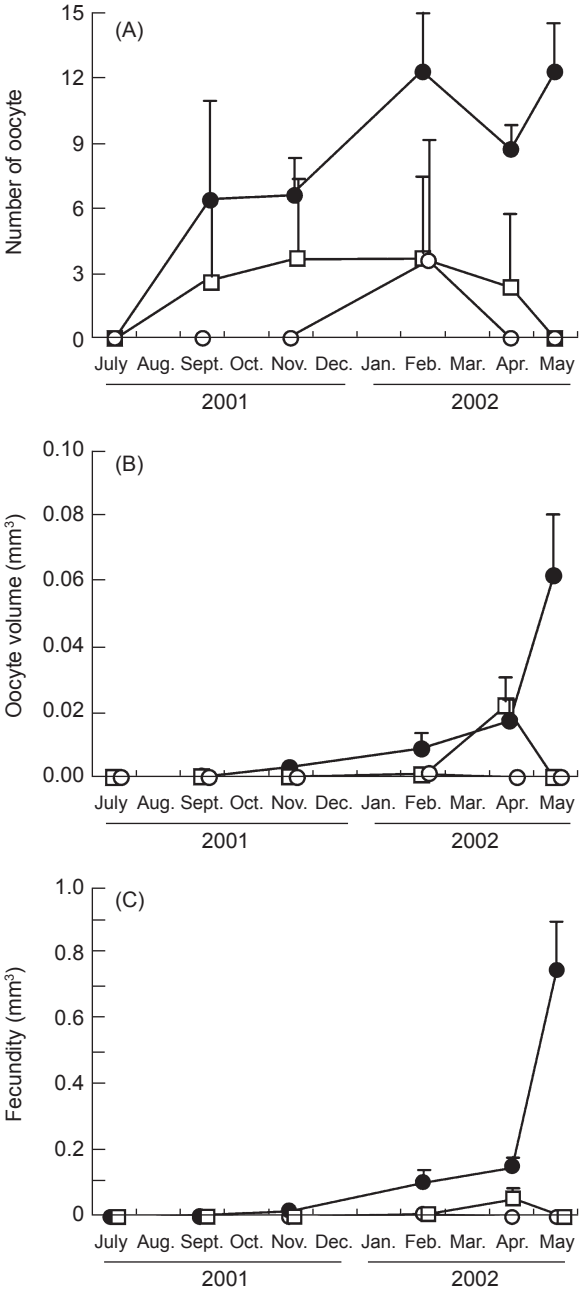

Fig. 2. Oocyte development in fragments transplanted on 13 July 2001. (A) Average oocyte number per polyp ($n = 9$); (B) average oocyte volume ($n = 0$-110); (C) fecundity (average of total oocyte volume per polyp; $n = 9$). Filled circles, donors; squares, large fragments; open circles, small fragments. The error bar represents the standard deviation.

Feb.-transplanted fragments

In Feb. 2002, all transplanted fragments, as well as the donor colonies, possessed developing oocytes in the late vitellogenic stage. The oocyte number was 12.2 (SD = 2.8, $n = 9$), volume was 0.009 mm^3 (SD = 0.004 , $n = 117$), and fecundity was 0.106 mm^3 initially (SD = 0.034 mm^3, $n = 9$). In Apr., 2 mo after transplantation, the oocyte number had decreased in all transplanted fragments as well as donors (Fig. 3). Oocytes occurred in 33% of the small fragments, but the volume was significantly larger than that of the donor colonies (0.022 ± 0.008 mm^3 for small ($n = 18$) vs. 0.017 ± 0.004 mm^3 ($n = 78$) for donors; Kruskal-Wallis test, $H = 14.2$, $p < 0.001$; Dunn's multiple-comparison test, $p < 0.05$); the oocyte volume of large fragments (0.015 ± 0.007 mm^3, $n = 43$) did not differ from that of donor colonies in Apr. (Dunn's multiple-comparison test, $p > 0.05$). Oocytes of both small and large fragments continued to develop, and the oocyte volume sharply increased until May (Fig. 3). Before the mass spawning on 24 May, the respective oocyte volumes did not significantly differ from the donor colonies (Kruskal-Wallis test, $H = 3.5$, $p > 0.17$). However, the oocyte number and fecundity were highest in the donor colonies (Fig. 3; Dunn's multiple-comparison test, $p < 0.05$ for all comparisons, donor colonies > large fragments > small fragments). On 29 May 2002, 70% of the small ($n = 60$) and 100% of the large Feb.-transplanted fragments ($n = 60$) spawned on the same calendar day as the July-transplanted fragments and donors.

In the 2nd gametogenesis after May 2002, neither oocytes nor spermary were observed in any Feb.-transplanted fragments. Donors spawned in July 2003, but all fragments failed to do so, except for 1 large fragment that spawned on

the same calendar day as the donors. In the 3rd gametogenesis, no gametes were observed in any of the fragments.

DISCUSSION

In the 1st year, large fragments had a higher survival rate and produced more gametes than did small fragments, indicating that fragment size affected the survival rate and sexual reproduction in *A*. *nasuta*. Both large and small fragments, however, ceased oocyte development and spawning in the 2nd year and thereafter. A similar trend was reported in *Acropora* of branching and tabular forms, i.e., *A*. *formosa* and *A*. *hyacinthus*. Fragments apparently reallocate energy resources after fragmentation and infertility occurs for a certain period of time (Nonaka et al. 2003). In the experiment with *A*. *formosa*, fragment transplantation resulted in resorption of oocytes in the early vitellogenic stage, while oocytes in the late stage continued to develop (Okubo et al. 2005). These results suggest that colony size may be the main factor in sexual reproduction in *Acropora* corals rather than polyp age, although the fragments may have been in a poor condition or in various stages of recovery. This trend was also found in the massive coral *Favites chinensis*, while in other massive corals like *Goniastrea favulus* (Kojis and Quinn 1985) and *G*. *aspera* (Kai and Sakai 2008), polyp age may affect sexual reproduction, indicating that the reproductive strategy might not be defined by coral morphology. The daily growth rates were 0.13 ± 0.09 g in *A*. *formosa* and 0.02 ± 0.03 g in *A*. *nasuta* (Okubo unpublished data), while spawning ratios for similar sizes were 20.1% ± 7.9% in *A*. *formosa* (Okubo et al. 2005) and 70% in small fragments of *A*. *nasuta*. *Acropora nasuta* might use a higher amount of energy for reproduction rather than growth compared to *A*. *formosa* in which repeated fragmentation occurs (Highsmith 1982).

During gametogenesis, oocyte volumes of large July-transplanted fragments, which had experienced bleaching, and small Feb.-transplanted fragments increased compared to those of the donor colonies. This suggests that stress may result in a specific stimulation to maintain oocyte quality and enhance spawning. Okubo et al. (2005 2007) reported that in the 1st year, oocyte volume increased and oocyte number decreased for *A*. *formosa*, especially in smaller fragments, and > 50% of fragments spawned 1 mo earlier than did the donor colonies. Although no coral-related information is available, it is known that stress in late vitellogenesis can result in early spawning in fish (Contreras-Sanchez et al. 1998).

Timing of fragmentation also affected the survival rate and sexual reproduction. In fact, all Feb.-transplanted fragments survived, whereas none of the July-transplanted fragments did. The low survival rates of July-transplanted fragments in 2001 might be attributable to the unusually high temperatures that followed transplantation. Water temperatures exceeded 30°C, and bleaching was observed in many coral colonies, including fragments (Taniguchi 2002, Okubo et al. 2005). In

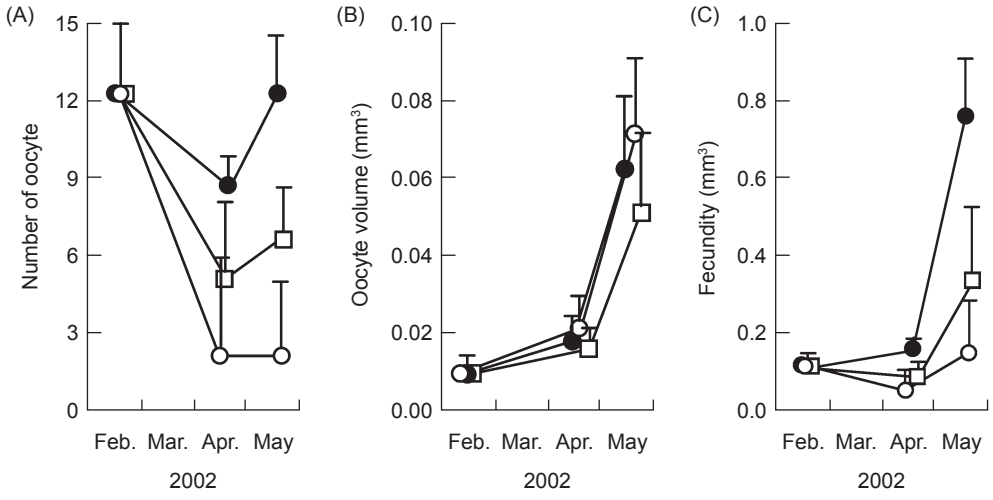

Fig. 3. Oocyte development in fragments transplanted on 20 Feb. 2002. (A) Average oocyte number per polyp (n = 9); (B) average oocyte volume (n = 18-110); (C) fecundity (average of total oocyte volume per polyp; n = 9). Filled circles, donors; squares, large fragments; open circles, small fragments. The error bar represents the standard deviation.

the Philippines, it was reported that the survival and growth rates of the branching coral *A. pulchra* decreased during warm periods (> 30 ºC) (Yap et al. 1984 1998). In the present study, the survival rate of large Feb.-transplanted fragments was 100%, suggesting that transplantation should be conducted during cooler periods.

In conclusion, transplantation of larger fragments during the cooler season resulted in an increased survival rate and spawning ratio in the 1st year after transplantation in *A. nasuta*.

Acknowledgments: We thank the staff of the Akajima Marine Science Laboratory, Okinawa, Japan.

REFERENCES

Chornesky EA, EC Peters. 1987. Sexual reproduction and colony growth in the scleractinian coral *Porites astreoides*. Biol. Bull. **172:** 161-177.

Connell JH. 1973. Population ecology of reef-building corals. *In* OA Jones, T Endean, eds. Biology and geology of coral reefs. New York: Academic Press, pp. 205-245.

Contreras-Sanchez WM, CB Schreck, MS Fitzpatrick, CB Pereira. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod. **58:** 439-447.

Cowen RK, KMM Lwiza, S Sponaugle, CB Paris, DB Olson. 2000. Connectivity of marine populations: open or closed? Science **287:** 857-859.

Cumming RL. 2002. Tissue injury predicts colony decline in reef-building corals. Mar. Ecol.-Prog. Ser. **242:** 131-141.

Forsman ZH, B Rinkevich, CL Hunter. 2006. Investigating fragment size for culturing reef-building corals *(Porites lobata* and *P. compressa*) in *ex situ* nurseries. Aquaculture **261:** 89-97.

Hayashibara T, K Shimoike, T Kimura, S Hosaka, A Heyward, P Harrison, K Kudo, M Omori. 1993. Patterns of coral spawning at Akajima Island, Okinawa, Japan. Mar. Ecol.-Prog. Ser. **101:** 253-262.

Highsmith RC. 1982. Reproduction by fragmentation in corals. Mar. Ecol.-Prog. Ser. **7:** 207-226.

Kai S, K Sakai. 2008. Effect of colony size and age on resource allocation between growth and reproduction in the corals *Goniastrea aspera* and *Favites chinensis*. Mar. Ecol.-Prog. Ser. **354:** 133-139.

Kojis BL, NJ Quinn. 1985. Puberty in *Goniastrea favulus*: age or size limited? *In* C Gabrie, B Salvat, eds. Proceedings of the 5th International Coral Reef Symposium, Vol. **4:** pp. 289-293.

Mathews ML. 2007. Evidence for restricted gene flow over small spatial scales in a marine snapping shrimp *Alpheus angulosus*. Mar. Biol. **152:** 645-655.

Nonaka M, AH Baird, T Kamiki, HH Yamamoto. 2003. Reseeding the reefs of Okinawa with the larvae of captive-bred corals. Coral Reefs **22:** 34.

Okubo N, T Motokawa, M Omori. 2007. When fragmented coral spawn? Effect of size and timing on survivorship and fecundity of fragmentation in *Acropora formosa*. Mar. Biol. **151:** 353-363.

Okubo N, H Taniguchi, T Motokawa. 2005. Successful methods for transplanting fragments of *Acropora formosa* and *Acropora hyacinthus*. Coral Reefs **24:** 333-342.

Petersen D, M Hatta, M Laterveer, D van Bergen. 2005. *Ex situ* transportation of coral larvae for research, conservation, and aquaculture. Coral Reefs **24:** 510-513.

Rodgers KS, EF Cox. 2003. The effects of trampling on Hawaiian corals along a gradient of human use. Biol. Conserv. **112:** 383-389.

Schreck CB, W Contreras-Sanchez, MS Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture **197:** 3-24.

Sebens KP. 1982. Limits to indeterminate growth: an optimal size model applied to passive suspension feeders. Ecology **63:** 209-222.

Smith LD, TP Hughes. 1999. An experimental assessment of survival, re-attachment and fecundity of coral fragments. J. Exp. Mar. Biol. Ecol. **235:** 147-164.

Taniguchi H. 2002. Coral bleaching occurred around Akajima Island in 2001– comparison with event in 1998. Midoriishi **13:** 30-33. (in Japanese)

Wallace CC. 1999. Staghorn corals of the world: a revision of the coral genus *Acropora* (Scleractinia; Astrocoeniina; Acroporidae) worldwide, with emphasis on morphology, phylogeny and biogeography. Melbourne, Australia: CSIRO Publishing.

Yap HT. 2004. Differential survival of coral explants on various substrates under elevated water temperatures. Mar. Pollut. Bull. **49:** 306-312.

Yap HT, RM Alvarez, HM Custodio, RM Dizon. 1998. Physiological and ecological aspects of coral transplantation. J. Exp. Mar. Biol. Ecol. **229:** 69-84.

Yap HT, ED Gomez. 1984. Growth of *Acropora pulchra*. Responses of natural and transplanted colonies to temperature and day length. Mar. Biol. **87:** 209-215.

Zakai D, O Levy, NE Chadwick-Furman. 2000. Experimental fragmentation reduces sexual reproductive output by the reef-building coral *Pocillopora damicornis*. Coral Reefs **19:** 185-188.