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RESEARCH ARTICLE

When fragmented coral spawn? Effect of size and timing on survivorship and fecundity of fragmentation in *Acropora formosa*

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Abstract In order to determine competency of sexual reproduction and survival rate after fragmentation, the branching coral *Acropora formosa* was fragmented and fragments in three different sizes (ca. 5, 10 and 20 cm long) and three different stages of gametogenesis were transplanted on coral pavement. Their oocyte development and fecundity, as well as spawning were monitored for a 3-year period. The oocyte development was affected by both fragment size and by the developmental stage of oocytes when fragmented. In small fragments, the oocytes were resorbed while in large fragments they continued development. Oocytes in the early vitellogenic stage at the time of fragmentation were resorbed, whereas those in the late stage continued developing. Smaller fragments showed a lower survival rate and histological observations of their gonads revealed resorption of oocytes, suggesting that there was a trade-off of energy between reproduction and survival. Transplanted fragments often spawned one month earlier than the donor colonies in the first year, but spawning occurred in the same month as the donors or did not occur at all in the second year and none spawned in the third year. The risk of colony death may cause the fragments to re-allocate energy for sexual reproduction.

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Introduction

Reef-building corals are organisms that allow parts of colonies to break off, disperse and propagate locally. In order to compensate its disadvantage of propagation, they spawn eggs and disperse the larvae to distant reefs in many cases once a year. Thus, fragmentation is natural repertoire of asexual reproduction. Physical disturbances occur in any season and produce various sizes of fragments (Bruckner and Bruckner 2001). However, little attention has been paid regarding competence of sexual reproduction after fragmentation. Whether fragmented corals spawn at the same time as the donor colony? If not, when they were spawn?

Although there are several reports that naturally or artificially occurring fragments reduce fecundity or cease gonad development, these surveys were conducted only once or a few times after fragmentation (e.g., Smith and Hughes 1999; Zakai et al. 2000). Survivorship and growth of transplanted fragments has been surveyed and discussed (e.g., Rodgers and Cox 2003; Yap 2004). However, spawning of fragments has never been reported. Fecundity may differ depending on the developmental stage of oocytes at fragmentation (e.g., Schreck et al. 2001). It is expected that the reduction in colony size due to fragmentation may

affect reproductive output (Sebens 1982). Connell (1973) postulated that whether a colony joins sexual reproduction or not is determined by size of colony or age of polyps in the colony.

Here, we report oocyte development, fecundity, and survival rate in the fragments of *Acropora formosa* (Dana 1846) for a 3-year period after transplantation. *A. formosa* has an open arborescent colony shape. They are known fragmenters (reviewed by Highsmith 1982) and occur abundantly in Okinawa, Japan. We examined two factors, timing of fragmentation and fragment sizes. Detailed data on the reproductive ability and survival rate of fragments would lead to increased knowledge of the reproductive biology of modular animals (Lewis 1991; Bruno 1998). Also, it will be of great help for recent restoration approaches such as coral nursery in situ and ex situ, and for the forecast of reef recovery after ship grounding, tsunami, typhoon, etc.

Materials and methods

We conducted this study at Majanohama beach, Akajima Island, Okinawa, Japan. The experimental station was the same as that of Okubo et al. (2005). The surrounding waters are relatively enclosed with weak currents; turbidity and sedimentation rates are relatively high compared to other sites at this island.

In Okinawa, oocytes of *A. formosa* begin to appear in the mesoglea in October and spawning takes place in May or June (Hayashibara et al. 1993; Okubo 2004). Hence, the coral has oocytes with a small volume in early vitellogenesis in November, it has oocytes with a large volume in late vitellogenesis in March, but has no oocytes in August.

A. formosa were fragmented artificially on 7 November 1999, 15 March 2000 and 11 August 2000 by means of wire cutters from the branch tips of 6 donor colonies (hereafter referred to as the donors) each measuring about 1 m high and 4.5 m^2 in cross-section. From each donor colony, 9–13 fragments were prepared, and polyps of 3 cm from the top were examined in the laboratory if there were oocytes. Using a ruler in situ, they were grouped into three size classes as small (5 ± 0.06 cm in length), medium (about 10 ± 0.07 cm), and large (about 20 ± 0.40 cm). Then, 511 fragments in all were transferred 15–20 m away from the donors and transplanted at a depth of 2–3 m. We drove 8-cm-long concrete nails into the substrate of coral pavement, leaving 5 cm protruding from the surface with a 20 cm space between nails. Each fragment was fastened along the nail using one or two polyethylene cable ties.

Oocyte development of three fragments from the donors and three fragments from each of the three size classes of the transplants was examined every 2 weeks after fragmentation. The oocyte samples from the donors were collected at a position >50 cm distant from the position where transplanted fragments were taken in order to avoid the effect of lesion (Van Veghel and Bak 1994). There was no significant difference in the oocyte development among the donors, and between the donors and three undisturbed colonies nearby (see Results). The six undisturbed small colonies, which had 7–11 branches with length of 19–26 cm, in the experimental station were also examined to confirm the adult size of this species.

All 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 polyp (e.g., Chornesky and Peters 1987), 10–30 polyps from each fragment size from a similar position to the donors, i.e., at 7–8 cm from the branch tip in November and 12–13 cm from the branch tip in March were examined respectively. In the small fragments, however, those from randomly selected positions were examined as not all polyps possessed oocytes. From August 2000 to June 2001, the second gametogenesis for November- and March-transplants and the first gametogenesis for August transplants, polyps of all fragments formed new oocytes. We examined the condition of polyps from the top of the fragments and, ten polyps with oocytes were selected at random from 2–3 cm lower than the position where oocytes were found for the first time. The oocytes were approximated as spherical and, their volume was calculated by the formula, $V = 4/3\pi(X/2)(Y/2)^2$, where X is the maximum diameter and Y is the minimum diameter of the oocyte. Oocyte development was quantitatively shown by three indices: (a) number of oocytes, i.e., average number of oocytes per polyp, (b) oocyte volume, i.e., average volume of an oocyte, (c) the fecundity, i.e., average volume of total oocytes in a polyp. Histological sections were made in samples in which evidence of oocyte resorption was found. They were dehydrated in an ethanol series, cleared in xylene, embedded in paraffin at 60°C, sectioned of 10 μm thickness, and stained either with hematoxylin and eosin or with Giemsa.

The conditions and survival rates of the transplants were regularly monitored using SCUBA. *A. formosa* is hermaphroditic and the eggs and sperm within each polyp are compressed into one or more egg–sperm bundles. The spawning index (%) was expressed as the number of fragments having bundles in all fragments. During the mass spawning period in May and June in 2000–2002, occurrence of the bundles in the mouth of polyps as well as spawning were recorded for all fragments, the donors, and other untouched colonies at

night (2100–2300 hours) by means of SCUBA. These observations were conducted for 11 days before and after the full moon night.

Data analysis

The percentage survival rate, three indices of oocyte development and the spawning index were analyzed statistically using Kruskal–Wallis test. Where significant differences were established, Dunn's multiple comparison tests were employed to determine which group(s) differed. The oocyte volume in November-transplanted large fragments and the donors was analyzed using Mann–Whitney's U test.

Results

Survival rates of fragments

Almost of all large fragments survived in the first year and, their survival rates did not differ among the different timings of fragmentation (Table 1). In the November- and March-transplants the survival rates of large fragments showed significantly higher survivorship than small fragments. The timing effect was apparent in the smaller fragments. The August- and March-transplants survived significantly better than the November-transplants after 1 year (Fig. 1 and Table 1).

All the March- and August-transplants survived in the second year, except a small fragment (Fig. 1). In comparison, the survival rate of the November-transplants was low. That of the large and medium fragments was relatively higher than the small fragments (Table 1).

November-transplants with oocytes in early vitellogenesis

In November 1999, both the donors and fragments had small oocytes in early vitellogenesis. Mean number of oocytes was 6.6 (SD = ±2.3, $n = 10$), oocyte volume was 0.05×10^{-2} mm^3 (SD = $\pm 0.04 \times 10^{-2}$, $n = 66$), and fecundity was 0.33×10^{-2} mm^3 (SD = $\pm 0.25 \times 10^{-2}$, $n = 10$). Table 2 shows the results of statistical tests examining oocyte development. Number of oocytes of the donors did not significantly vary until their spawning in June 2000 (Kruskal–Wallis test, $H = 12.6$, $P > 0.08$; Fig. 2a). On the contrary, oocytes disappeared within two months in the medium fragments and within one month in the small fragments. In the large fragments, oocytes developed as the donors (Fig. 2c). Except for January oocyte volume did not differ significantly from the donors until April. In May, however, oocyte volume of the large fragments sharply increased and became even statistically larger than the oocytes of the donors (Fig. 2b). Such a sharp increase occurred in June in the donors. There was no significant difference in three indices among the donors

Table 1 *Acropora formosa*. Results of statistical tests examining the effect of timing of fragmentation and fragment size on annual survival rate (%) of transplanted fragments after 1 year

Comparing among sizes per season								
Season	Annual survival rate (%)			Kruskal–Wallis test		Dunn's multiple comparison test		
	Large	Medium	Small	H	P	L vs. M	L vs. S	M vs. S
First year								
November	98.3 ± 4.1	83.5 ± 10.4	48.3 ± 12.2	14.3	<0.001*	0.10*	<0.001*	0.01*
March	100	93.3 ± 7.8	86.6 ± 1.0	6.5	0.04*	0.13	0.04*	0.13
Second year								
November	74.7 ± 10.0	69.4 ± 16.8	29.2 ± 29.7	7.3	0.03*	0.23	0.05*	0.07*
August	100	100	96.4 ± 7.1	2.0	0.37	–	–	–

Comparing among seasons per size					
Fragment size	Kruskal–Wallis test		Dunn's multiple comparison test		
	H	P	Nov vs. Mar	Mar vs. Aug	Nov vs. Aug
First year					
Large	1.3	>0.51	–	–	–
Medium	6.3	<0.05*	0.14	0.18	0.08*
Small	11.7	<0.003	0.01*	0.12	<0.001*

L = 20 cm, M = 10 cm, and S = 5 cm. Means are given ±SD

*Significant difference ($P < 0.05$)

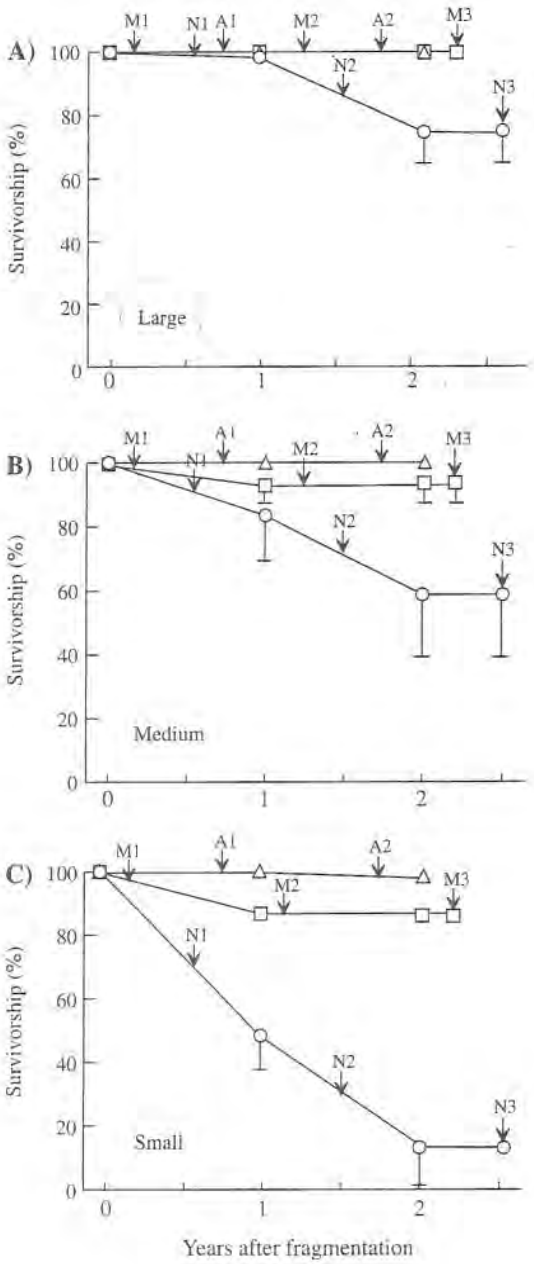

Fig. 1 Survivorship of fragments (%). **a** Large (20 cm) fragments, **b** medium (10 cm), and **c** small (5 cm). *Circles* transplanted in November, *squares* March, and *triangles* August. Spawning time is indicated by *arrows*. Abbreviations are *N* November-transplanted fragments, *M* March, *A* August, *1* spawning in the first year after fragmentation, *2* spawning in the second year, *3* spawning in the third year. *Error bar* is SD. The survivorship at 2.5 year is calculated using the data of 0.5 year

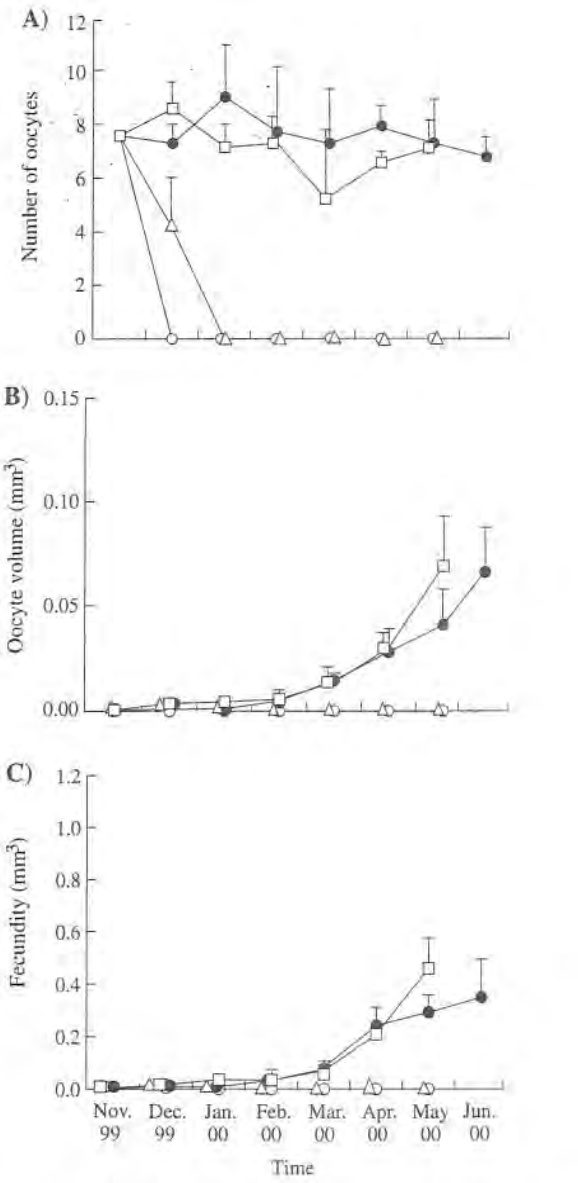

Fig. 2 Oocyte development in November-transplants. **a** Oocyte number, **b** oocyte volume, and **c** fecundity. *Filled circles* donors; *squares* large fragments; *triangles* medium fragments; and *open circles* small fragments. *Error bar* is SD

(Kruskal–Wallis test for number of oocytes, $H = 2.40$, $P > 0.29$; for oocyte volume, $H = 3.90$, $P > 0.14$; for fecundity, $H = 1.87$, $P > 0.39$; in March 2000), and between the donors and undisturbed colonies (Kruskal–Wallis test for oocyte number, $H = 2.15$, $P > 0.54$; for oocyte volume, $H = 6.29$, $P > 0.09$; for fecundity, $H = 0.74$, $P > 0.86$; in March 2000). The six undisturbed small colonies examined to confirm the adult size of this species had no oocytes throughout the observation period.

The donors spawned at 2230 hours on 17 June 2000. Five out of ten undisturbed colonies having similar size to the donors spawned in May and the rest spawned in June. On the other hand, half of the large fragments spawned on 25 and 26 May and the rest spawned on 17 June (Fig. 4a); a few medium fragments spawned but only on 25 and 26 May (Fig. 4d), whereas none of small fragments spawned (Fig. 4g).

Table 2 November-transplants. Results of non-parametric tests examining differences of number of oocytes (number), oocyte volume (volume), polyp fecundity (fecundity) in the donors and the three sizes of fragments

	$U^{\blacklozenge}$	H^{D}	P	Dunn's multiple comparison test					
				D vs. *L*	*D* vs. *M*	*D* vs. *S*	*L* vs. *M*	*L* vs. *S*	*M* vs. *S*
Number									
Dec	–	30.4	<0.001*	0.09*	0.08*	0.03*	0.03*	0.009*	0.08*
Jan	–	29.5	<0.001*	0.11	0.003*	0.003*	0.005*	0.005*	0.14
Feb	–	29.3	<0.001*	0.12	0.014*	0.014*	0.02*	0.02*	0.14
Mar	–	44.4	<0.001*	0.05*	0.005*	0.005*	0.05*	0.05*	0.14
Apr	–	31.1	<0.001*	0.06*	<0.001*	<0.001*	<0.001*	<0.001*	0.14
May	–	29.5	<0.001*	0.12	<0.001*	<0.001*	<0.001*	<0.001*	0.14
Volume									
Dec	–	117.3	<0.001*	<0.001*	0.15	–	<0.001*	–	–
Jan	5482.5	–	0◆*	–	–	–	–	–	–
Feb	1924.5	–	>0.91◆	–	–	–	–	–	–
Mar	5125.5	–	>0.93◆	–	–	–	–	–	–
Apr	2870.5	–	>0.28◆	–	–	–	–	–	–
May	4544.0	–	<0.001◆*	–	–	–	–	–	–
Fecundity									
Dec	–	32.0	<0.001*	0.05*	0.10	0.10	0.03*	0.03*	0.13
Jan	–	32.8	<0.001*	0.01*	0.07*	0.07*	0.003*	0.003*	0.14
Feb	–	29.4	<0.001*	0.13	0.08*	0.08*	0.08*	0.08*	0.14
Mar	–	41.2	<0.001*	0.10	0.03*	0.03*	0.08*	0.08*	0.14
Apr	–	29.6	<0.001*	0.11	0.006*	0.006*	0.01*	0.01*	0.14
May	–	31.5	<0.001*	0.06*	0.01*	0.01*	0.002*	0.002*	0.14

D = donors, *L* = 20 cm, *M* = 10 cm, and *S* = 5 cm

◆ Mann–Whitney's *U* test for *D* versus *L*

D Kruskal–Wallis test

*Significant difference ($P < 0.05$)

During the second gametogenesis in May 2001, oocytes were found at the position of 3.7cm from the tip of the large fragments, but no oocytes occurred in the medium and small fragments. Oocyte volume of the large fragments was not significantly different from that of the donors (Table 3), but the number of oocytes and fecundity were significantly lower than the donors. The donors, large and medium fragments spawned at 2200–2230 hours on 6 or 7 June 2001. The spawning index was significantly higher in the large fragments than the medium fragments (Fig. 4a, d, g).

In the third spawning season in May and June 2002, none of the transplants had oocytes and they did not spawn (Fig. 4), although the donors spawned on 29 May 2002.

March-transplants with oocytes in late vitellogenesis

In March 2000, both the fragments and the donors carried large oocytes in late vitellogenesis at the time of fragmentation. The number of oocytes was 8.5 (SD = ±2.1, $n = 30$), oocyte volume was 4.3×10^{-2} mm^3 (SD = $\pm 1.6 \times 10^{-2}$, $n = 255$), and fecundity was 35×10^{-2} mm^3 (SD = $\pm 7 \times 10^{-2}$ mm^3, $n = 30$). Table 4 shows the results of statistical tests examining oocyte development. Except for 13 April the number of oocytes of the large fragments was not significantly different from that of the donors (Fig. 3a). Oocyte volume of the large fragments increased, similar to the donors until 26 April. However, it increased significantly within two weeks and became bigger than that of the donors before the spawning on 23 May. Thus, the fecundity was statistically higher in large fragments than the donors (Fig. 3c).

In the medium and small fragments, the number of oocytes decreased (Fig. 3a). Two thirds of the medium fragments lost oocytes 2.5 months after fragmentation, and one third of the small did so within 1.5 months after transplantation. The residual oocytes of both sizes developed for spawning. Oocyte volume of the medium fragments was smaller than that of the donors until 26 April. Then it increased significantly within one month and became significantly bigger than that of the donors before spawning on 23 May (Fig. 3b).

All transplants spawned on the same calendar day as those transplanted in November 1999. About half of the large fragments spawned in May, and the rest in June, while all of the medium and small fragments spawned only in May (Fig. 4b). The spawning index of large fragments was significantly higher than the medium and small fragments (Kruskal–Wallis test: $H = 7.8$, $P < 0.03$, Dunn's multiple comparison test: large vs. medium, $P < 0.001$; large vs. small, $P < 0.001$; medium vs. small, $P = 0.24$; Fig. 4). In the first year the March-transplants spawned significantly more than the November- and August-transplants in all sizes (Fig. 4, see Okubo et al. 2005).

In the second and third gametogenesis 2000–2002, however, no oocyte or spermary was observed in all transplants, and thus spawning did not occur (Fig. 4b, e, h).

Table 3 Results of Mann–Whitney's *U* test examining the number of oocytes (number), oocyte volume (volume) and polyp fecundity (fecundity) between November-large fragments and the donors in May 2001 prior to the second spawning

	Donors	November-large fragments	Mann–Whitney's *U* test	
			U	*P*
Number	7.3 ± 2.4 (30)	4.6 ± 2.6 (30)	670.5	0.002*
Volume (mm^3)	0.047 ± 0.020 (218)	0.045 ± 0.015 (131)	14330.5	0.95
Fecundity (mm^3)	0.338 ± 0.099 (30)	0.199 ± 0.138 (30)	712	0.001*

Value ± SD (number of sample)

*Significant difference ($P < 0.05$)

Table 4 March-transplants. Results of Kruskal–Wallis test and Dunn's multiple comparison test examining differences of number of oocytes (number), oocyte volume (volume), polyp fecundity (fecundity) in the donors and the three sizes of fragments

	Kruskal–Wallis test		Dunn's multiple comparison test					
	H	*P*	*D* vs. *L*	*D* vs. *M*	*D* vs. *S*	*L* vs. *M*	*L* vs. *S*	*M* vs. *S*
Number								
29 Mar	7.5	0.058	–	–	–	–	–	–
13 Apr	30.2	<0.001*	0.09*	0.07*	0.04*	0.11	0.08*	0.11
26 Apr	64.9	<0.001*	0.13	0.07*	0.01*	0.08*	0.01*	0.04*
9 May	51.2	<0.001*	0.14	0.76*	0.02*	0.08*	0.02*	0.07*
23 May	66.0	<0.001*	0.11	0.02*	0.005*	0.02*	0.004*	0.08*
Volume								
29 Mar	142.1	<0.001*	0.11	0.04*	0.01*	0.07*	0.03*	0.08*
13 Apr	53.6	<0.001*	0.10	0.07*	0.04*	0.10	0.06*	0.10
26 Apr	40.5	<0.001*	0.12	0.07*	0.07*	0.09*	0.09*	0.13
9 May	59.3	<0.001*	0.10*	0.13	0.05*	0.09*	0.09*	0.05*
23 May	209.9	<0.001*	<0.001*	0.04*	0.13	0.05*	0.07*	0.10*
Fecundity								
29 Mar	27.8	<0.001*	0.11	0.11	0.06*	0.13	0.10*	0.10*
13 Apr	34.2	<0.001*	0.09*	0.06*	0.04*	0.10	0.07*	0.11
26 Apr	63.1	<0.001*	0.12	0.06*	0.02*	0.07*	0.02*	0.07*
9 May	48.1	<0.001*	0.13	0.10	0.03*	0.11	0.03*	0.05*
23 May	70.3	<0.001*	0.02*	0.06*	0.02*	0.004*	<0.001*	0.08*

D = donor colonies, *L* = 20 cm, *M* = 10 cm, and *S* = 5 cm

*Significant difference ($P < 0.05$)

August-transplants without oocyte at transplantation

Neither donor colonies nor fragments had oocytes in August 2000, two months after the mass spawning. Oocytes still did not occur in the following year (8 May 2001) in the medium and small fragments. However, in only 2 out of 30 polyps of the large fragments carried 1 and 2 oocytes respectively. Oocyte volume was $4.5 \times 10^{-2}\ mm^3$ (SD = 1.5×10^{-2}, $n = 3$) and fecundity $0.5 \times 10^{-2}\ mm^3$ (SD = 1.7×10^{-2}, $n = 30$). Oocyte volume was not significantly different from that of the donors (Mann–Whitney's *U* test, $U = 329$, $P > 0.98$), whereas the number of oocytes and fecundity was significantly lower (Mann–Whitney's *U* test for number of oocytes, $U = 0.5$, $P < 0.001$; for fecundity, $U = 1$, $P < 0.001$). They spawned at 2200–2230 hours on 6 and 7 June 2001, at the same time with donors (Fig. 4).

In the second gametogenesis 2001–2002, no oocytes or spermary was observed in all transplants (Fig. 4c, f, i). In the third gametogenesis, due to two typhoons that directly hit the experimental station on 9 and 19 September 2002, most of the transplants were broken. Again, none of transplants spawned in May and June 2003 and, the data were not included in this study.

Histological study

The primary gastrodermal structures in Anthozoa are mesenteries. Their main functions include reproduction, digestion, and circulation (Fautin and Mariscal 1991). In the donors and untouched colonies, developing oocytes were found in a row attached to a single mesentery (Fig. 5a). Histological sections revealed that their gametogenic areas contained normal oocytes with chromatin in their nucleus (Fig. 5e). One month after fragmentation, the November-medium, March-small, and March-medium fragments showed a different appearance in gonads from those of the donors. Through dissection under the microscope we saw that the number of oocytes had decreased and some oocytes looked translucent in the center (Fig. 5b,

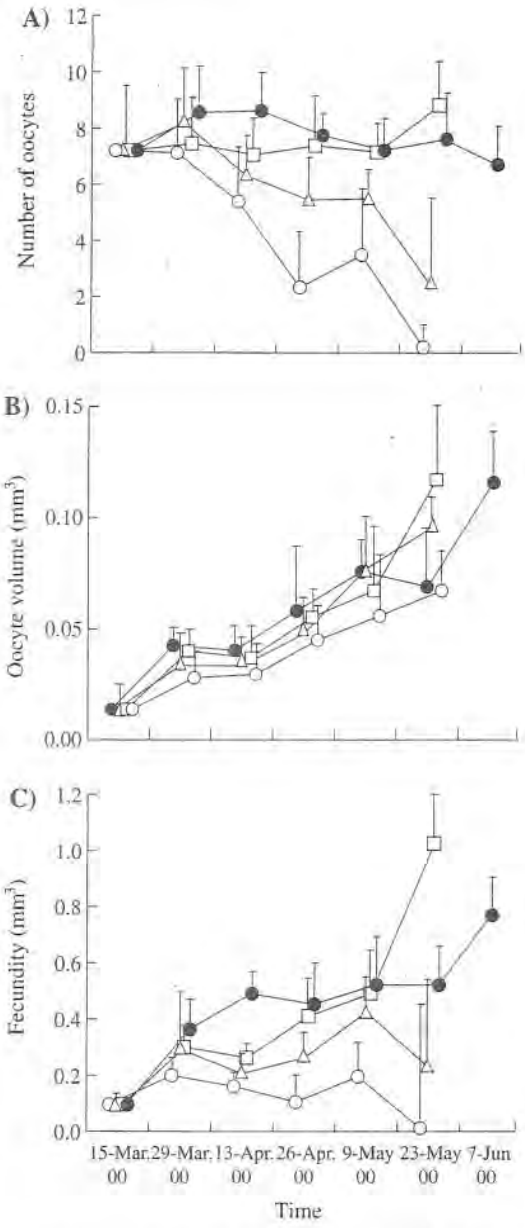

Fig. 3 Oocyte development in March-transplants. **a** Oocyte number, **b** oocyte volume, and **c** fecundity. *Symbols* are as in Fig. 2

inset). Such oocytes were hollow inside (Fig. 5f). The sections also showed the presence of small, round structures. All of them had no nucleus. Because they were found in the place where once oocytes had occupied, they were very likely degenerating. These oocytes, however, were not a sign of dying polyps. Although they were found in almost every polyp of the November-medium and March-medium and small fragments, most of the polyps in these fragments survived. In the transplants from which all oocytes disappeared several months after fragmentation, the gonads looked similar to those of the donors without oocytes (Fig. 5d).

The basal parts of the transplants showed characteristic changes in the gonads within one month after fragmentation. In every polyp in the basal section regardless of whether it carried oocytes or not, all mesenteries fused together to make a little-tapered structure with some indentations and short protrusions (Fig. 5c). Neither ovary nor testis was found. In the normal mesenterial gastrodermis, as in anthozoans in general, cnidocytes and granulocytes are scattered among the epithelial cells that form a distinctive and unique basal layer (Fig. 5h). The fused mesenteries kept this structure, too. Their characteristic features were the presence of empty spaces and the abundant distribution of cells that were strongly stained with eosin (Fig. 5g). Two types of cells were distinguished. The type I was slender with hyaline cytoplasm filled with numerous eosinophilic granules whose diameter was about 1–1.5 μm. They were found side by side with epithelial cells (Fig. 5g, thin arrows and lower inset). This type of cell was weakly stained in the normal mesenteries of donors and undisturbed colonies (Fig. 5h, arrows); however, strongly stained cells were also found in the mesenteries having many empty spaces like the fused mesenteries in them. On the other hand, the type II cells were round about 10 μm diameter with many eosinophilic granules as those of the type I (Fig. 5g, thick arrows and upper inset). The cells were found randomly in the mesenterial gastrodermis, but they were seldom observed both in the donors and undisturbed colonies. The polyps in the basal part of transplants died within 4 months after fragmentation. Polyps with fused mesenteries eventually died.

Discussion

The present study revealed that the development of oocytes was affected by both size of fragment and developmental stage of oocytes when transplanted. There were two general trends: (1) oocytes were resorbed in small fragments while they continue developing in large fragments; (2) oocytes in the early vitellogenic stage at the time of fragmentation were resorbed while oocytes in the late stage continued developing. Smaller fragments showed low survivorship and could not afford to invest in sexual reproduction. It is reasonable that small fragments resorb oocytes and convert their energy for survival, because a fair amount of energy is needed for further oocyte development. Okubo et al. (2005) described that the transplanted small fragments of *A. formosa* did not spawn but their growth rates were four times higher than larger fragments that spawn. Colonies suffering from stress due to the fragmentation may abandon

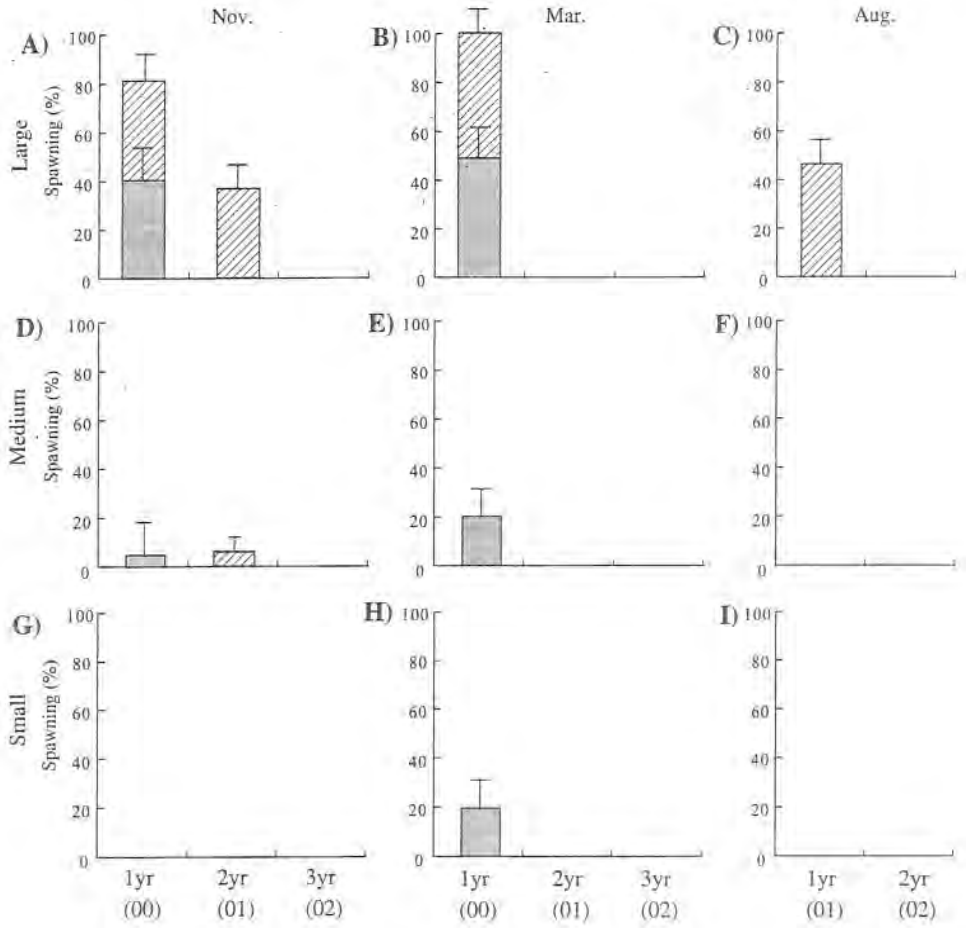

Fig. 4 Spawning index (%) in transplants in the 3 years after fragmentation. *Left column* is for the November-transplants, *middle* is for the March, and *right* is for the August. The *upper row* is for large fragments, *middle* is for medium, and *lower* is for small. *Filled bar* spawning in May and *hatched bar* spawning in July. *Error bar* is SD. Spawning was not observed where no bars are given

developing such oocytes. The present study suggests that the trade-off between reproduction and survival/growth in modular animals occur as is observed in unitary ones (e.g., Giekmann et al. 2004).

The sharp increase in oocyte volume just before spawning became evident 1 month earlier in the transplanted fragments than the donors. Although there is so far no information whether fragmentation affects the timing of the sharp increase and spawning in cnidarians and other modular organisms, the present results indicate that stress from fragmentation may prompt gametogenesis in *A. formosa*, because the tendency was more evident in smaller fragments than larger ones. It is known that stress applied in late vitellogenesis results in early spawning in rainbow trout (Contreras-Sanchez et al. 1998).

The present study suggests that whether a colony joins sexual reproduction or not is determined by size of colony and age of polyps in the colony. The colony size is clearly the main factor, because most of the medium and small fragments did not spawn from the second year. However, some of November-large and medium fragments in the second year and half of the August-large ones in the first year formed new oocytes and spawned, in spite of the fact that the small undisturbed colonies which were larger than the transplants formed no oocytes. Kojis and Quinn (1985) described in their study on *Goniastrea favulus* that the age of polyps might affect sexual reproduction. We consider two possible interpretations other than the age of polyps for the results. The first one is that the fragments had residual germ cells or interstitial cells that had already developed beyond a critical point to become oogonium in the previous year. The second one is that the fragments are still experiencing moderate adverse effects of fragmentation (stress) to spawn, while those with low stress shift their resources mostly to growth.

The size of oocytes of the transplants was similar to that of donors although the number of oocytes per polyp decreased, especially in small fragments (Fig. 3a). In this connection, Hall and Hughes (1996) found that in undisturbed natural colonies of *Acropora hyacinthus* and *A. nana* the number of oocytes per polyp remain constant while the oocyte size increase with colony size. The discrepancy between their study and ours may be derived from difference between undisturbed natural colonies and transplanted fragments that were under considerable stress. In the transplants, the resources

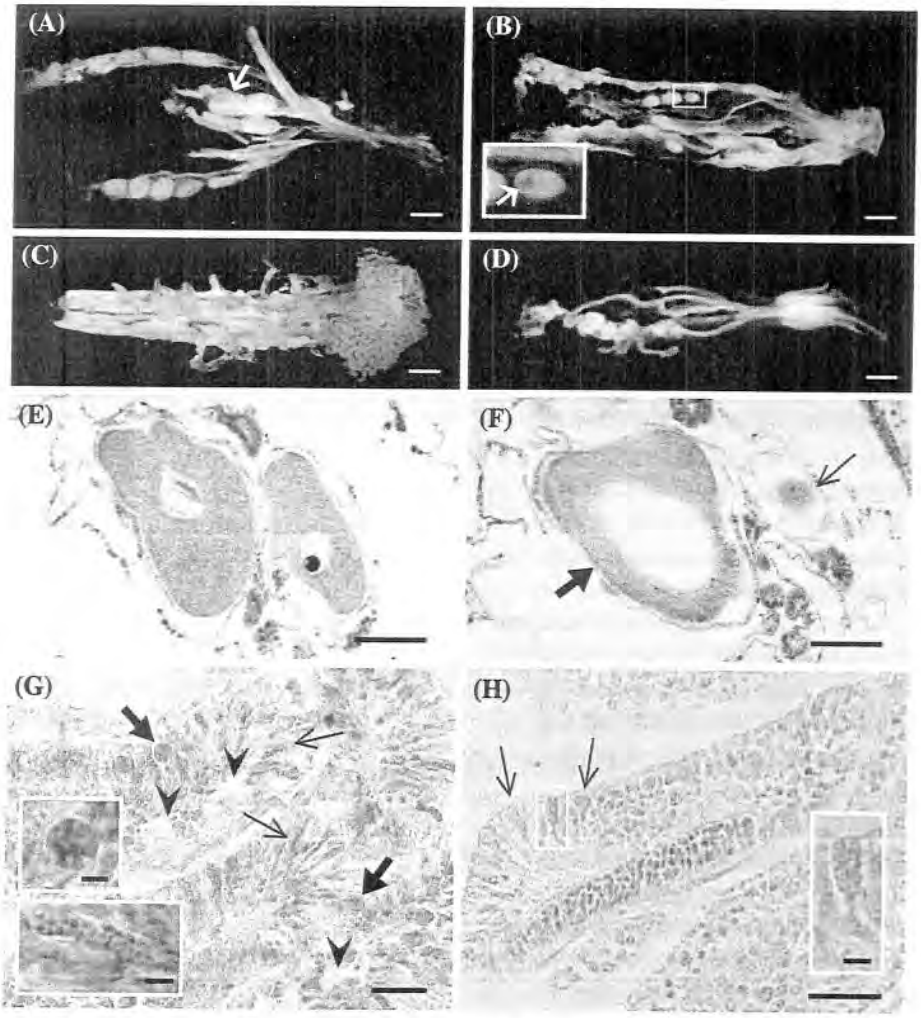

Fig. 5 Gonads of *Acropora formosa* and their histological sections. **a** Normal gonad with developing oocytes in the donor in April 2000. Strands of mesentery, with oocytes (*arrow*) attached in a row, were bound together at the oral end (to the right in **a**, **b**, **c** and **d**). **b** Gonad with oocytes in the March-small fragments in April 2000, 1 month after fragmentation. The center of an oocyte looked translucent, which is shown in higher magnification in inset. **c** Gonad with fused mesenteries in the polyps in the basal parts of fragments. **d** Gonad without oocytes in the donor in August 2000, 2 months after spawning. **e** Section of gonads shown in (**a**). Normal oocytes with nucleus in the donor colony. **f** Section of gonads shown in (**b**). The left oocyte (*thick arrow*) has hollow inside and the right one (*thin arrow*) shrinks and separate from the cells, which would play a role as the nutritive cells, around oocytes. **g** Section of fused mesenteries shown in (**e**). Numerous eosinophilic cells are found in the mesenterial gastrodermis. *Thin arrows* and *lower inset* show type I cells. *Thick arrows* and *upper inset* show type II cells. *Arrowheads* show empty spaces where cells are lacking. **h** Sections of normal mesenteries of the donor in early May 2000. *Arrows* show granulocytes with granules weakly stained with eosin (*inset* shows the higher magnification). **e**, **f** Giemsa staining; **g**, **h** hematoxylin and eosin staining. *Bar* 0.5 mm (**a**, **b**, **c**, **d**), 100 μm (**e**, **f**), 30 μm (**g**, **h**), 5 μm (*insets* in **g** and **h**)

usable for reproduction were no doubt limited. The total reproductive effort is defined as number of eggs times egg size (Smith and Fretwell 1974). In our case, resource limitation in transplants resulted in the decrease in the number of oocytes in a polyp.

The decrease in number of oocytes suggested that some oocytes were resorbed after transplantation. We found two ways of degeneration of gonads. Firstly, in the medium and small fragments the hollow oocytes and small round structures were found in the degenerating gonads after transplantation. The oocyte may become hollow first and then may collapse to small structures that are eventually resorbed. The number of resorbed oocytes was apparently fewer in the late vitellogenesis than in the early stage. Thus, there seems to be a critical stage in vitellogenesis after which oocytes are no longer resorbed. Secondly, the polyp, in which gonadal degeneration was observed, gradually died after the degeneration. Those mesenterial gastrodermis contained cells with eosinophilic granules, and they may play some roles in tissue degradation by phagocytosis and/or by releasing lysins (Hutton and Smith 1996). This type of degeneration was found typically at the basal part of all transplants; thus, the injuries of cut surface and burial in

accumulated sediments would be causes of the reduction of fecundity (e.g., Okubo et al. 2005).

Coral reefs have been degrading over the past few decades at an alarming rate throughout the world (Bryant et al 1998). Recent assessment suggested that 11% of historical extent of coral reefs is already lost, while a further 16% is severely damaged (Wilkinson 2002). In order to remedy man-induced damage caused by such events as ship groundings, mining, and harbor construction, transplantation of coral fragments or of coral heads has been conducted (reviewed by Harriott and Fisk 1988; Okubo and Omori 2001; Rinkevich 2005). To date, the reef rehabilitations by transplantation of corals have been evaluated only on the basis of the survival and growth of fragments (e.g., Custodio and Yap 1997). However, the competence of sexual reproduction should be also considered to enhance the development of restoration technologies. Here, we suggest the preferable timing and fragment size of the corals that broadcast eggs in case of transplantation needed for shore embankment. The first priority is to avoid the warmest months since most of the transplants do not survive at higher water temperature (Okubo et al. 2005). Corals are normally under more stress during the warmest time, and bleaching tends to occur in the transplants. Then, transplantation after spawning would be preferable. After that, the minimum size with 100% survival rate should be transplanted. Tissue degradation by the injuries of cut surface occurs mostly at the basal part of transplants. This problem can be reduced if fragments are hanged on the net or rope in the water column during healing after fragmentation. Once spawning ceased, it will take a few year that the transplants to resume spawning. It was recently reported that a number of transplanted fragments of *Acropora muricata* in the open water aquarium spawned once again after 7 years (Nonaka et al. 2003). We are convinced that, in combination with the various reef-recovery strategies such as McManus et al. (1997), Omori (2005) and Fox et al. (2005), the present study add some useful data for technical development of restoration of coral reefs.

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