

Socially Controlled Growth and Size-Dependent Sex Change in the Anemonefish *Amphiprion frenatus* in Okinawa, Japan

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Abstract Social structure, growth and reproductive experience of a protandrous anemonefish, *Amphiprion frenatus*, were investigated on a coral reef in Okinawa, Japan. In a 67 m × 334 m study area, 24 breeding groups, 10 nonbreeding groups and 2 groups of unknown breeding experience were found around isolated sea anemones. One group usually consisted of 2 or 3 fish. The female in a breeding group was larger than not only her mate but also all males in other breeding groups. The body size and gonadal state of the largest individual in a nonbreeding group were intermediate between the female and male in a breeding group. In both breeding and nonbreeding groups, the largest fish retarded growth of the second largest. After the disappearance or removal of females, their mates took more than 1.5 years to attain the minimum functional female size (about 75 mm in standard length). This delayed sex change can be attributed to strong growth suppression by the female.

The influence of environmental or social conditions on sex change of sequentially hermaphroditic fishes has attracted attention (e.g., Charnov, 1982; Warner, 1984, 1988a, b; Shapiro, 1984, 1989). Anemonefishes (genus *Amphiprion*) are known for socially controlled protandry with a monogamous mating system (Fricke and Fricke, 1977; Moyer and Nakazono, 1978; Ross, 1978a; Fricke, 1979, 1983). Distribution patterns of host sea anemones are the crucial determinant of their social and mating systems (Allen, 1972; Moyer and Sawyers, 1973; Ross, 1978b; Keenleyside, 1979; Moyer, 1980; Thresher, 1984; Ochi, 1986, 1989a, b) and influence their sex change patterns (Ochi and Yanagisawa, 1987; Ochi, 1989a; Hattori and Yanagisawa, in press a, b).

Recently, intensive field studies of *Amphiprion clarkii* were conducted in temperate waters where host density was high (Moyer, 1980; Ochi, 1985, 1986, 1989a, b; Yanagisawa and Ochi, 1986; Ochi and Yanagisawa, 1987; Hattori and Yanagisawa, in press a, b). Its social behavior and sex change pattern greatly differ from those of anemonefishes in coral reef regions, where host density is low. Territories of breeding pairs, each of which includes several separate hosts, are almost contiguous with each other; nonbreeders have home ranges on the outskirts of the pairs' territories; and some nonbreeders become females without passing through a functional male state. These features are attributed to the fact that the fish can move between hosts.

In coral reef regions, social structure and sex change patterns have been studied for some species (Fricke and Fricke, 1977; Moyer and Nakazono, 1978; Ross, 1978a, b; Fricke, 1979, 1983) but many aspects of their ecology remain to be investigated. For example, little is known about the exact distribution pattern of host sea anemones, migration of fish between hosts, and ecological factors related to sex change.

Amphiprion frenatus is one of the common anemonefishes on coral reefs in the Okinawa Islands, Japan. It occurs in a group around a sea anemone. One breeding group usually consists of a female, a male and 1 or 2 small nonbreeders. Females have only ovarian tissue in their gonads, whereas males and nonbreeders have both ovarian and testicular tissues (Moyer and Nakazono, 1978). In the present study, I observed group composition, migration between groups and the growth of individual fish under natural conditions, and conducted field experiments wherein some or all of group members were removed to enable investigation of sex change pattern and the extent of social control of growth.

Materials and methods

Study area and species. The field study was conducted on a fringing reef in front of Sesoko Marine Science Center, University of the Ryukyus at Sesoko Island (26°39'N; 127°57'E), Okinawa,



Fig. 1. The map of study area at Sesoko Island, Okinawa, and the distribution of host sea anemones *Physobranchia ramsayi*. Shaded area indicates shore. Lines indicate reef edges or patch reefs. Areas encircled with a bold line are the study areas. Solid circles indicate locations of sea anemones. Sea anemones with 1, 2 and 3 show that removal experiments 1, 2 and 3 were carried out there, respectively.

Japan. Four species of anemonefishes, *Amphiprion frenatus*, *A. clarkii*, *A. perideraion* and *A. ocellaris*, are distributed in this site. *A. frenatus* was investigated in 2 study areas (Fig. 1): one (Area 1; 67 m × 334 m) was used for the survey of social structure and growth and removal experiments, and the other (Area 2; 67 m × 87 m) for a removal experiment.

Collection of data on host distribution. Maps of the study areas were drawn based on aerial photographs and modified following underwater observations. Locations of the sea anemone *Physobranchia ramsayi*, the host of *A. frenatus*, were plotted on the maps (Fig. 1). The long and short axial lengths of the sea anemones were measured twice in Area 1 in September, 1988. The maximum value of an area that tentacles of a sea anemone covered was estimated by (long axial length) × (short axial length) × 3.14/4, and was used as an index of sea anemone size.

Collection of data on social structure. The social structure of *A. frenatus* was investigated in Area 1 from June to November, 1988. All individuals were captured with hand nets in June and October and their standard lengths measured underwater with a ruler. Individuals larger than 20 mm in standard length (SL) were marked by injecting acrylic paint under the skin (see Thresher and Gronell, 1978). Other specimens could be identified according to their body markings, such as the different numbers and shapes of white bands. Marking of fish was also conducted in Area 2.

Individuals occupying the same host(s) were together defined as a group. Swimming tracks of the largest fish in each group and some others were recorded for 15 min on the map on at least 2 different days during July and September in Area 1. The

outermost traced line in 1 observation was regarded as the border of its home range.

Breeding and nonbreeding groups were defined as groups in which reproduction (spawning or brooding) was observed or not observed, respectively. Fish in each group were designated as α -individuals, β -individuals, γ -individuals and so on, according to their size order in each group. α - and β -individuals in breeding groups were always females and males, respectively (Moyer and Nakazono, 1978); they were called breeders. If an α -breeder (female) disappeared, its mate was named α -nonbreeder until it mated with a new mate. To investigate the breeding condition and migration of fish, I patrolled Area 1 every four days during June and September (4 months) and checked for the presence of fish and egg mass in the vicinity of each sea anemone.

An immigrant and recruit were defined as an individually discriminated fish which migrated from another host in the study area, and a fish which was newly found in the study area, respectively.

Removal experiments. I conducted 3 removal experiments in November, 1988: 1) the removal of all group members (17 cases in Area 1); 2) the removal of a female and a male from a group (6 cases in Area 1); and 3) the removal of a female from a group (4 cases in Area 2). The purposes of these experiments were to determine whether or not replacements filled the vacant posts (all experiments) and when remaining or replacement individuals reproduced after the removal (experiments 2 and 3), and to compare the growth patterns of α - and β -individuals before and after the removal (experiments 1 and 2). Experimental groups were selected at random in the study area (Fig. 1).

To investigate the occurrence of reproduction and migration of fish after the removal, I patrolled Areas 1 and 2 every day for a week after the removal and subsequently once a week in December, 1988 and every four days in March, May, June, October and November, 1989 and June and November, 1990. Standard lengths of the fish were measured in June and November, 1989 and June and November, 1990 in Area 1 and in November, 1989 and June and November, 1990 in Area 2.

Growth. Growth increments over 4 months under natural conditions were calculated based on standard length data from Area 1 in June and October, 1988. After the removal experiments, standard lengths of all α - and β -individuals in Area 1, including new α - and β -individuals, were measured in June and November, 1989 and June, 1990.

Gonad histology. All fish removed in experiments 1 and 2 (N=64) were fixed and preserved in Bouin's solution. The gonads of specimens larger than 30 mm SL and the whole bodies of specimens smaller than 30 mm SL were embedded in paraffin and sectioned. Serial cross sections (6 or 8 μ m thick) from 2 sections of each sample were stained with haematoxylin and eosin, and gonad structures were examined under a microscope.

Results

Distribution of host sea anemones. Thirty-eight and 11 host sea anemones were found in Areas 1 and 2, respectively. These sea anemones were sparsely distributed (Fig. 1, see also Hirose, 1985). Their average density was 0.17 individuals per 100 m², and the average distance between nearest neighbours was 9.3 m \pm 5.7 SD (N=49, range=0.6–23.3 m). Their size was 663 cm² \pm 393 SD on average (N=36, Table 1). The size of 2 sea anemones could not be measured, owing to the complex coral structure around them. Neither death nor recruitment of sea anemones occurred during June and October, 1988.

Social groups of anemonefish. Thirty-six groups were found in Area 1. Each group was found around an isolated sea anemone except for 1 group which used 3 sea anemones close to one another (\bar{x} =0.7 m \pm 0.2 SD, N=3).

The anemonefish swam around the host sea anemone. The average home range of α -individuals was 6.8 m² \pm 5.4 SD (N=66). Home ranges of other members (N=66) were always included within the α -individual's home range. γ - and δ -individuals

rarely swam out from the sea anemone. In the group which used 3 sea anemones, the home range of the female (29.8 m²) covered 3 sea anemones but the home ranges of the male and 6 nonbreeders were usually restricted to 1 of the 3 hosts.

Out of 34 groups in which reproduction was examined, 24 (70.6%) were breeding groups and the rest nonbreeding (Table 2). Reproduction in 2 groups was not examined, since the coral structure around their hosts was so complex that the presence or absence of an egg mass could not be confirmed. A female produced 2 to 10 clutches during the observation period from 1 June to 30 September (\bar{x} =6.8 \pm 2.2 SD, N=24).

Breeding groups all occupied sea anemones larger than 800 cm² (Table 1), and used larger sea anemones than nonbreeding groups (U-test, U=53, P<0.01, N₁=24, N₂=10). Group size varied from 2 to 8 individuals (Table 2) with an average of 3.0 \pm 1.1 SD. Group size was positively correlated with the size of sea anemone (r =0.39, P<0.05, N=34), but the sum of body sizes of all fish in a group were even more closely correlated (r =0.55, P<0.01, N=34).

Table 1. Size distributions of host sea anemones *Physobranchia ramsayi* which the anemonefish used for spawning or not.

Size class (cm ²)	Number of host sea anemones		Total
	Used for spawning	Not used for spawning	
0– 200	1	4	5
200– 400	5	5	10
400– 600	4	1	5
600– 800	1	1	2
800–1,000	7	0	7
1,000–1,200	2	0	2
1,200–1,400	4	0	4
1,400–1,600	1	0	1
Total	25	11	36

Table 2. Group size of the anemonefish in October, 1988.

Group size	Number of breeding groups	Number of nonbreeding groups	Total
2	4	5	9
3	17	2	19
4	2	3	5
8	1	0	1
Total	24	10	34

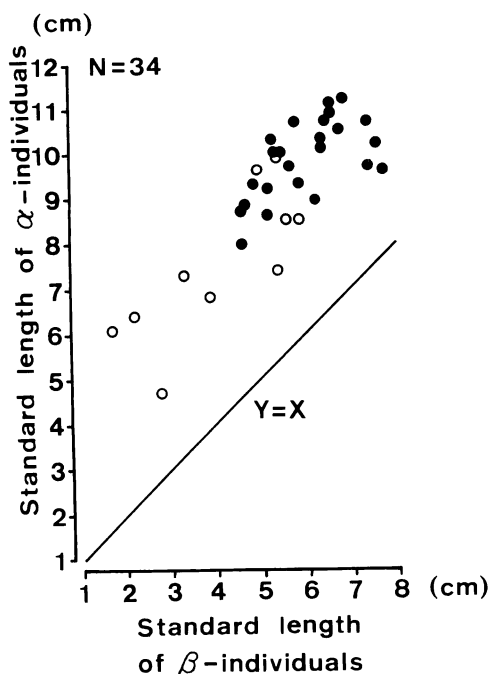


Fig. 2. Relationship between body sizes of α - and β -individuals in one group. Solid and open circles indicate breeders and nonbreeders, respectively. Data were collected in October, 1988.

Although group size did not differ significantly between breeding and nonbreeding groups (U-test, $U=101$, $P>0.05$, $N_1=24$, $N_2=10$), the sum of body sizes of all fish in a breeding group was larger than that in a nonbreeding group (U-test, $U=2.49$, $P<0.05$, $N_1=24$, $N_2=10$).

The size distributions of males (46–77 mm SL) and females (80–112 mm SL) were discrete (Table 3). α -nonbreeders occupied an intermediate size between males and females, and β -nonbreeders were smaller than males. The average body sizes of females, α -nonbreeders, males and β -nonbreeders differed significantly from one another (t-test of all pairwise combinations, $P<0.01$).

Body sizes of α - and β -individuals in a group were positively correlated (Fig. 2, $r=0.83$, $P<0.01$, $N=34$). Their size difference was great ($\bar{x}=36.8$ mm \pm 8.7 SD, $N=34$). The size difference between β - and γ -individuals in a group was also great ($\bar{x}=35.6$ mm \pm 8.3 SD, $N=25$), but the sizes were not significantly correlated ($r=0.37$, $P>0.05$, $N=25$). The size difference between γ - and δ -individuals in a

group was small ($\bar{x}=13.5$ mm \pm 8.3 SD, $N=6$), with the sizes positively correlated ($r=0.93$, $P<0.01$, $N=6$).

Gonad phase and size order in a group. The gonad structure in *A. frenatus* was categorized into 6 phases based on the state of spermatogenesis and oocyte development and on the presence of spermatocyte cysts, an epithelium, an ovarian cavity and ovigerous lamellae (Table 4, Fig. 3). Oogonia and spermatogonia could not be discriminated between in this study. The criteria used for gonad categorization basically follows Hattori and Yanagisawa (in press a).

A) Undevelopment phase: The gonad could not be detected in serial cross sections of the whole body.

B) Pre-ripe male phase I: The gonad had a few spermatocyte cysts, spermatids and sperm, and perinucleolus oocytes, but did not have an epithelium, an ovarian cavity, ovigerous lamellae or a complex

Table 3. Standard length (mm) of the anemonefish in relation to size order in a breeding group and in a nonbreeding group.

	Size order	N	Standard length (mm)		
			Mean	SD	Range
Breeding group	α (female)	24	98.6	8.6	80–112
	β (male)	23	60.5	9.9	46– 77
	γ	21	25.1	11.6	12– 68
	$< \gamma$	7	20.1	13.8	8– 50
Nonbreeding group	α	10	75.2	16.2	47– 99
	β	11	39.5	15.7	17– 59
	γ	4	21.3	12.7	12– 40
	$< \gamma$	3	12.7	4.0	9– 17

Table 4. Gonad phases of the anemonefish determined by the gonad features. O3, yolk oocytes; O2, cortical alveolus oocytes; C, ovarian cavity; L, lamella structure; O1, perinucleolus oocytes; Cy, spermatocyte cysts; S1, spermatocytes; S2, spermatids and/or sperm; E, epithelium structure; Cys, complex structure consisted of many cysts; +, present; –, absent.

Gonad phase	Features of gonad									
	O3	O2	C	L	O1	Cy	S1	S2	E	Cys
Undevelopment	–	–	–	–	–	–	–	–	–	–
Pre-ripe male I	–	–	–	–	+	+	+	+	–	–
Pre-ripe male II	–	–	–	–	+	+	+	+	+	–
Ripe male	–	–	–	–	+	+	+	+	+	+
Ripe female I	–	+	+	+	+	–	–	–	+	–
Ripe female II	+	+	+	+	+	–	–	–	+	–

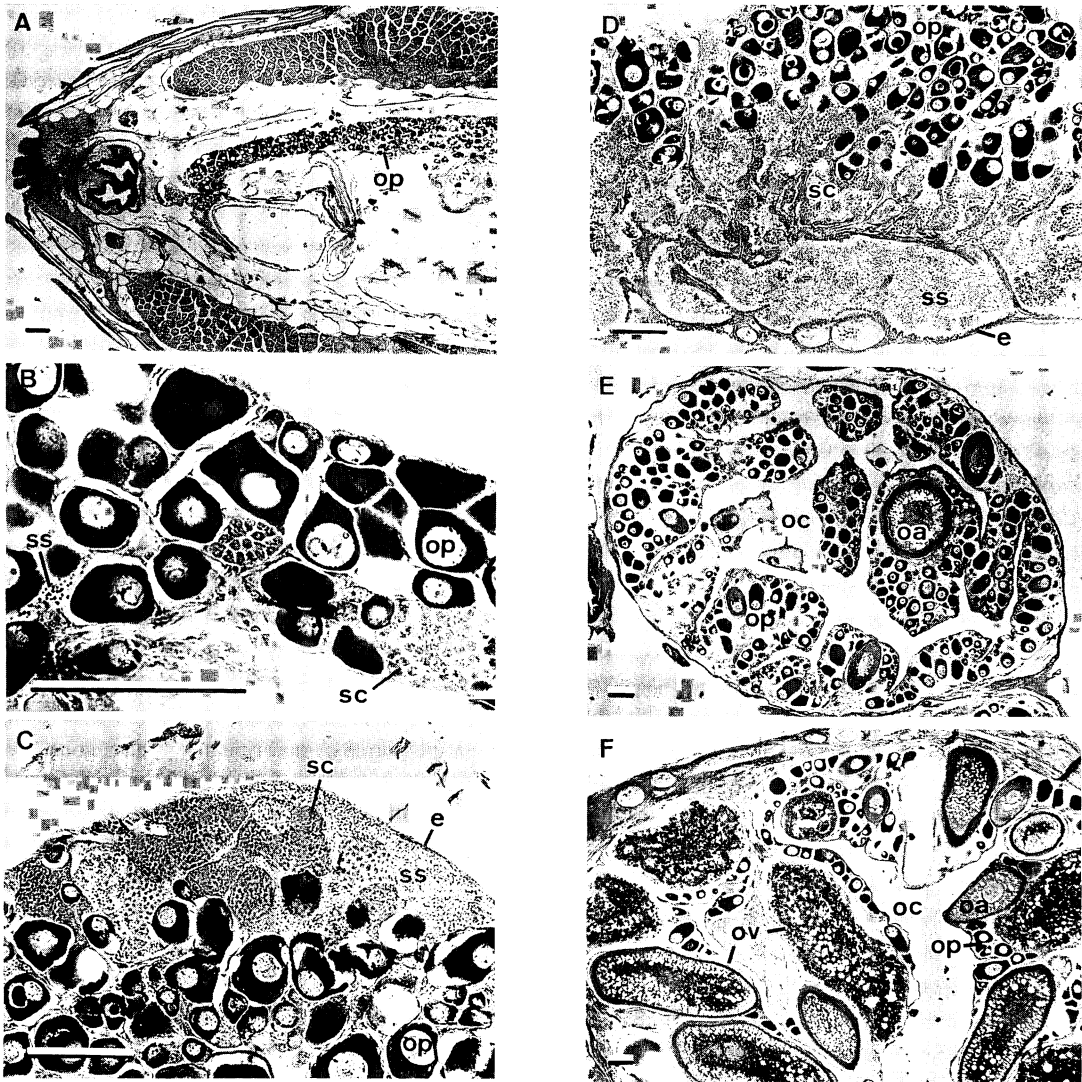


Fig. 3. Gonad phases of the anemonefish. Scales indicate $100\mu\text{m}$. A, pre-ripe male I phase gonad (20 mm SL, γ -nonbreeder) which seems to have only oocytes in the perinucleolus stage (op). B, pre-ripe male I phase gonad (magnification of photograph A), showing many oocytes in the perinucleolus stage (op), a few spermatocyte cysts (sc) and spermatids and/or sperm (ss) but no epithelium and no complex structure consisting of many spermatocyte cysts, spermatids and/or sperm. C, pre-ripe male II phase gonad (28 mm SL, γ -nonbreeder), showing many oocytes in the perinucleolus stage (op), a few spermatocyte cysts (sc), spermatids and/or sperm (ss) and an epithelium (e) but no complex structure consisting of many spermatocyte cysts, spermatids and/or sperm. D, ripe male phase gonad (66 mm SL, male), showing complex structure consisting of many spermatocyte cysts (sc), spermatids and/or sperm (ss) and an epithelium (e). Oocytes in the perinucleolus stage are also seen (op). E, ripe female I phase gonad (64 mm SL, α -nonbreeder), showing an ovarian cavity (oc) and oocytes in the perinucleolus stage (op) and the cortical alveolus stage (oa). F, ripe female II phase gonad (109 mm SL, female), showing an ovarian cavity (oc) and oocytes in the perinucleolus stage (op), the cortical alveolus stage (oa) and the vitellogenesis stage (ov).

structure consisting of many spermatocyte cysts (Fig. 3A, B).

C) Pre-ripe male phase II: The gonad had a few spermatocyte cysts, spermatids and sperm, perinucleolus oocytes and an epithelium, but did not have an ovarian cavity, ovigerous lamellae or a complex structure consisting of many spermatocyte cysts (Fig. 3C).

D) Ripe male phase: The gonad had a complex structure consisting of many spermatocyte cysts at various stages of spermatogenesis, perinucleolus oocytes and an epithelium, but did not have an ovarian cavity or ovigerous lamellae (Fig. 3D).

E) Ripe female phase I: The gonad had an epithelium, an ovarian cavity and ovigerous lamellae with perinucleolus and cortical alveolus oocytes, but did not have any spermatocytes, spermatids or sperm (Fig. 3E).

F) Ripe female phase II: The gonad had an epithelium, an ovarian cavity and ovigerous lamellae with perinucleolus, cortical alveolus and yolk oocytes, but did not have any spermatocytes, spermatids or sperm (Fig. 3F).

All γ - and δ -individuals had gonads of the pre-ripe male or the undevelopment phase (Table 5). Half of the β -nonbreeders had ripe male phase gonads, whereas the remainder had pre-ripe male phase. All β -breeders (males) had ripe male phase gonads. Gonads of α -nonbreeders included 4 phases, although more than half were at the ripe female phase I. Most α -breeders (females) had ripe female phase II gonads, and the remainder ripe female phase I.

The relationship between the body size and gonad phase (Fig. 4) shows that the gonads develop from

the undevelopment phase through male phases to female phases: i.e., undevelopment phase \rightarrow pre-ripe male phase I \rightarrow pre-ripe male phase II \rightarrow ripe male phase \rightarrow ripe female phase I \rightarrow ripe female phase II.

Stability of group members and sex change. Two each of the 36 α -individuals (5.6%) and the 36 β -individuals (5.6%) and 9 of the 23 lower-ranking individuals (39.1%) disappeared during the 5-month observation period prior to the removal experiments. During the same period, 27 individuals were recruited to the groups, 5 of them (18.5%) subsequently disappearing. All the recruits were smaller than 10 mm SL when they were first found (N=27, measurement by eye).

Inter-group migration occurred only once during the 5-month observation period: 44 days after the disappearance of a male from a breeding group, it was replaced by an α -nonbreeder (61 mm SL) from the nearest group (6 m apart) (Table 6).

Following the disappearance of α -individuals, no former mates undertook female reproductive roles during the study period (see Table 6). Gonads of the remaining fish after such a disappearance were examined in 2 groups. In the first (examined 75 days after disappearance of the female), a new α -individual (74 mm SL) had ripe male phase gonads and a new β -individual (54 mm SL) pre-ripe male II gonads. In the second group (examined after 135 days), a new α -individual (47 mm SL) had pre-ripe male phase II gonad and a new β -individual (28 mm SL) pre-ripe male phase I gonads.

Gonads of 7 individuals, which had been α -nonbreeders from the beginning of the study (for at least 156 days), were examined. Five specimens (96, 86, 85, 68 and 64 mm SL) had ripe female phase I gonads and the 6th (73 mm SL) pre-ripe male phase II gonads. Only the largest specimen (99 mm SL) had ripe female phase II gonads. By contrast, gonads of 6 individuals which had been β -nonbreeders from the beginning of the study (for at least 156 days) were mostly at the ripe male phase: the largest specimens (59, 56, 54 and 50 mm SL) had ripe male phase gonads and the 2 smallest (39 and 33 mm SL) pre-ripe male phase gonads.

Removal experiments. a) **Experiment 1 (removal of all members, Table 7):** In all 17 experiments, no recognized fish from other groups reoccupied the vacant sites during the 2-year study period. In 14 experimental groups (82.4%), recruits were found during the study period. Except 2 specimens (34 and 60 mm SL), they were all smaller than 10

Table 5. Relationship between gonad phases and size order in a breeding or nonbreeding group.

Gonad phases	Number of individuals							
	Breeding group				Nonbreeding group			
	Size order				Size order			
	α	β	γ	$<\gamma$	α	β	γ	$<\gamma$
Undevelopment		1	3			2	2	
Pre-ripe male I		4	1			3	1	0
Pre-ripe male II			3	1		2	1	1
Ripe male		14				1	4	
Ripe female I	4					5		
Ripe female II	10					1		
Total	14	14	8	5	9	8	4	2

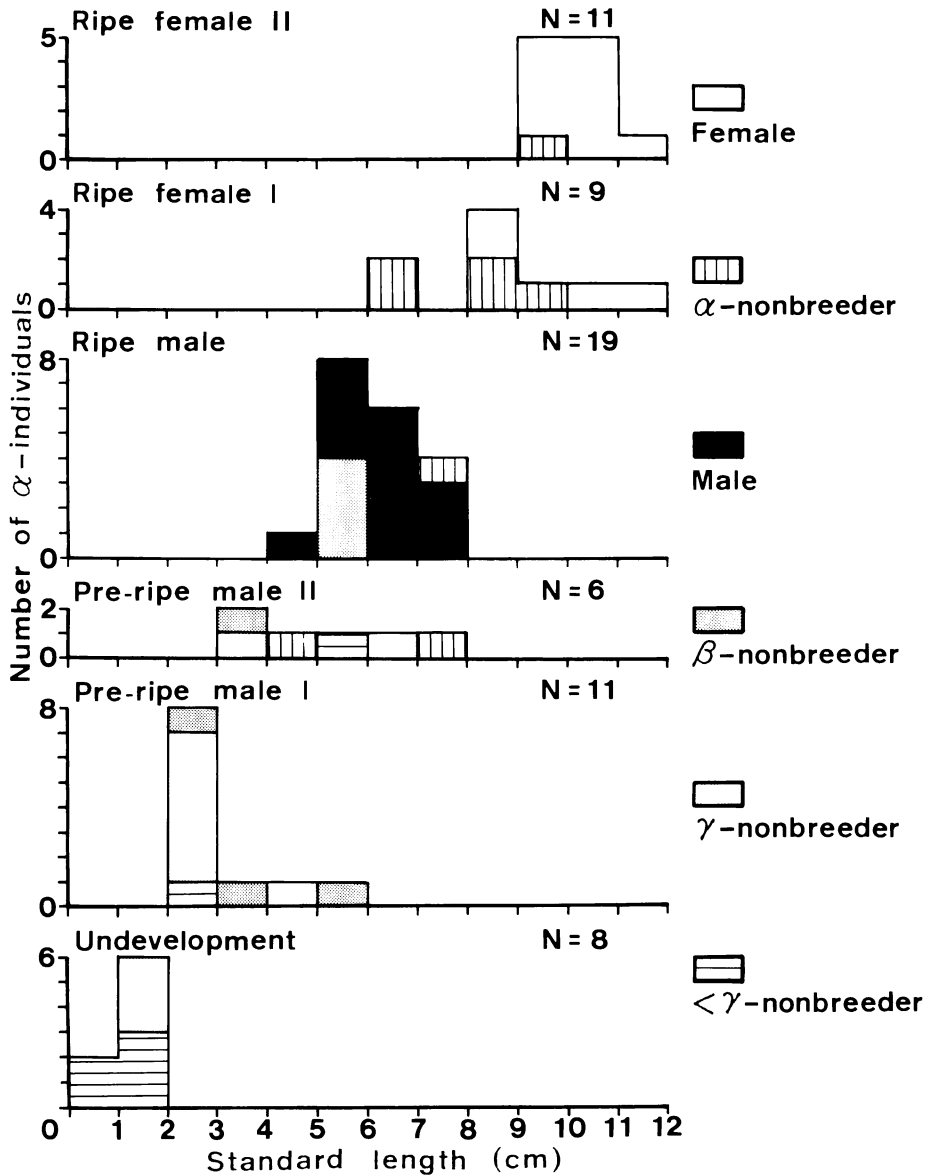


Fig. 4. Size-frequency distributions of individuals in relation to gonad phases, breeding experience and size order in a group.

mm SL (measurement by eye) when first found. The 2 larger fish may have been immigrants from unknown sea anemones in the study area, although they were included in recruits in this paper. In 5 experiments, sea anemones disappeared with or without settlement of juveniles (Groups 13–17).

New α - and β -individuals, which were all smaller than 75 mm at the end of the study period, did not reproduce.

b) Experiment 2 (removal of a breeding pair, Table 8): Following removal of a breeding pair, all members which remained in the experimental groups stayed with their hosts. Of 6 experimental groups, only 1 (Group 18) was replaced by members of a non-experimental group. They were a female (90 mm SL) and a β -nonbreeder (61 mm SL) which migrated from the nearest group (6 m apart) 3 and 4 days after the removal, respectively.

Table 6. Disappearance of α - or β -individuals during June and November, 1988.

Date of disappearance	Fish disappeared			Remaining fish			Events after disappearance until November, 1988
	Size (mm SL)	Size order	Breeding experience	Size (mm SL)	Size order	Breeding experience	
Aug. 20	110	α	female	71	β	male	—
				50	γ	nonbreeder	
				36	δ	nonbreeder	
June 25	82	α	nonbreeder	38	β	nonbreeder	—
				17	γ	nonbreeder	
Sept. 17	63	β	male	90	α	female	Immigration of α -nonbreeder (61 mm SL) in the nearest group
				22	γ	nonbreeder	
Nov. 3	22	β	nonbreeder	64	α	nonbreeder	—

Table 7. Body size (mm SL) and reproduction of recruits to vacant sea anemones after the removal of all group members. *, a recruit after the previous survey.

Group	1 month after removal			7 months			1 year			1.5 year			2 years			Reproduction of α - and β -individuals
	α	β	γ	α	β	γ	α	β	γ	α	β	γ	α	β	γ	
1	34*	—	—	49	15*	—	59	32	—	67	41	—	74	47	—	no spawning
2	—	—	—	—	—	—	48*	23*	—	59	35	—	72	47	—	no spawning
3	—	—	—	—	—	—	40*	26*	—	53	33	—	64	42	—	no spawning
4	—	—	—	32*	—	—	46	22*	—	51	27	—	62	36	—	no spawning
5	—	—	—	—	—	—	<10*	—	—	<29	—	—	60*	40	—	no spawning
6	—	—	—	<20*	—	—	<45	15*	—	58	28	—	<60	<30	—	no spawning
7	—	—	—	—	—	—	30*	—	—	40	<10*	—	58	<10	—	no spawning
8	—	—	—	<10*	—	—	30	—	—	43	26*	—	56	41	—	no spawning
9	—	—	—	<10*	—	—	30	—	—	42	—	—	53	<10*	—	no spawning
10	—	—	—	14*	—	—	28	—	—	38	—	—	47	<10*	—	no spawning
11	—	—	—	—	—	—	<10*	—	—	33	—	—	47	<10*	—	no spawning
12	—	—	—	—	—	—	17*	—	—	31	—	—	46	<10*	—	no spawning
13	<10*	—	—	39	—	—	48	25*	—	52	29	—	Host disappeared.			
14	—	—	—	—	—	—	30*	—	—	40	—	—	Host disappeared.			
15	Host disappeared.															
16	Host disappeared.															
17	Host disappeared.															

Table 8. Body size (mm SL) and reproduction of the remaining and replacement fish after the removal of a breeding pair from a group. *, an immigrant; **, a recruit.

Group	Just after removal			1 month			7 months			1 year			1.5 year			2 years			Reproduction of α - and β -individuals
	α	β	γ	α	β	γ	α	β	γ	α	β	γ	α	β	γ	α	β	γ	
18	20	—	—	90*	61*	20	94	62	23	97	63	23	98	63	24	98	63	24	May, 1989
19	19	—	—	—	—	—	—	—	—	<10**	<10**	—	36	21	—	53	35	—	no spawning
20	15	—	—	22**	15	—	40	29	—	55	37	—	62	40	—	73	45	—	no spawning
21	33	—	—	33	17**	—	51	28	—	65	38	—	73	42	—	81	45	—	November, 1990
22	28	14	—	28	14	—	43	26	—	51	31	—	60	35	—	71	41	—	no spawning
23	12	—	—	<20**	12	—	44	25	—	52	31	—	59	33	—	62	35	—	no spawning

In 4 other experimental groups (66.7%), recruits were found during the 2-year study period. In 3 of the 4 groups (Groups 20, 21 and 23), 1 recruit was found within about 1 month following the removal. The recruits settled 3, 7 and 33 days after the removal, respectively, 2 of them being larger than the remaining juveniles. The 2 larger fish may have been immigrants from unknown sea anemones in the study area. In another group (Group 19), 2 recruits were found about 1 year after the removal. They were smaller than 10 mm SL (measurement by eye).

Only 2 pairs of new α - and β -individuals reproduced during the 2-year study period. In one pair (Group 18), an immigrant female and an immigrant β -individual reproduced 6 months after the removal experiment. In the other (Group 21), 1 resident, which had been a juvenile (33 mm SL) at the beginning of the study, reproduced as a female with a recruit 2 years after the removal experiment. This female was 81 mm SL when it first reproduced.

c) Experiment 3 (removal of a female, Table 9): Two α -individuals from non-experimental groups migrated to 2 out of 4 experimental groups. In one group (Group 27), a member (105 mm SL, breeding experience unknown) from the nearest group, 6 m apart, was found 1 day after the removal. In the other (Group 24), a member (a little larger than 90 mm SL, breeding experience unknown) from the nearest group, 7 m apart, was found 4 days after the removal. In these 2 groups, reproduction of the immigrant fish was observed 6 months after the removal. During the 2-year study period, a recruit was found in only 1 experimental group (Group 26).

In the above 2 non-experimental groups from which α -individuals had emigrated, one new α -individual reproduced as a female when it reached 75 mm SL and the other new α -individual (60 mm SL) disappeared along with the host 7 months later.

Growth under natural conditions. Growth increments of both α - and β -individuals over a 4

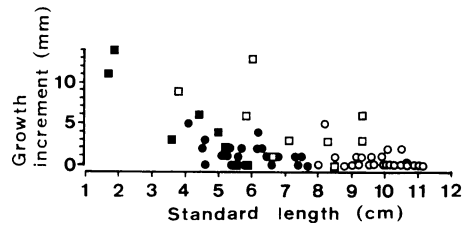


Fig. 5. Relationships between body size of α - (open) and β -individuals (solid) in June, 1988 and their growth increments during 4 months. Circles and squares indicate that the individuals were in breeding and nonbreeding groups, respectively.

month period were negatively correlated with their body size (Fig. 5, $r = -0.72$, $P < 0.01$, $N = 33$, for α -individuals; $r = -0.78$, $P < 0.01$, $N = 31$, for β -individuals). There was a significant difference in growth increment between females ($\bar{x} = 0.6 \text{ mm} \pm 1.1 \text{ SD}$, $N = 24$) and α -nonbreeders ($\bar{x} = 4.9 \text{ mm} \pm 4.1 \text{ SD}$, $N = 9$; U-test, $U = 24.5$, $P < 0.01$), and between males ($\bar{x} = 1.4 \text{ mm} \pm 1.3 \text{ SD}$, $N = 23$) and β -nonbreeders ($\bar{x} = 5.0 \text{ mm} \pm 5.1 \text{ SD}$, $N = 8$; U-test, $U = 49.5$, $P < 0.05$). Growth increments of α - and β -individuals in a single group were positively correlated ($r = 0.89$, $P < 0.01$, $N = 31$). There was no significant difference in growth increments between all α - and β -individuals (U-test, $U = 398$, $P > 0.05$, $N_1 = 33$; $N_2 = 31$). Growth of α -individuals was usually greater than in β -individuals in a range (40–72 mm) where their sizes overlapped (Fig. 5).

An average growth increment of γ - and δ -individuals was $2.3 \text{ mm} \pm 3.4 \text{ SD}$ (mode=0, range=0–12, $N = 15$). Their growth increments were positively correlated with body size ($r = 0.81$, $P < 0.01$, $N = 15$).

Growth after removal experiments. Growth patterns of new α - and β -individuals after the removal experiments were similar to those of α - and β -

Table 9. Body size (mm SL) and reproduction of the remaining fish after the removal of a female from a group. *, an immigrant fish; **, a recruit; n, body size not measured.

Group	Just after removal			1 month			7 months			1 year			1.5 year			2 years			Reproduction of α - and β -individuals
	α	β	γ	α	β	γ	α	β	γ	α	β	γ	α	β	γ	α	β	γ	
24	50	<10	—	>90*	50	—	n	n	—	n	n	—	n	n	—	n	n	—	March, 1989
25	44	23	—	n	n	—	n	n	—	62	44	10	69	47	10	Host disappeared.			
26	44	20	—	n	n	—	n	n	—	60	39	—	65	42	—	72	24**	—	no spawning
27	60	23	<10	105*	60	23	n	n	n	n	n	n	n	n	n	n	n	n	March, 1989

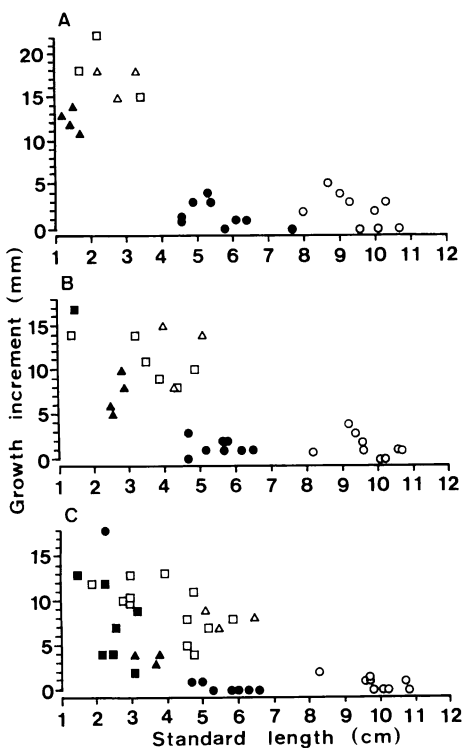


Fig. 6. Relationships between body size of α - (open) and β -individuals (solid) after the removal experiments and their growth increment during October, 1988 and June, 1989 (A), during June and November, 1989 (B) and during November, 1989 and June, 1990 (C). Circles indicate non-experimental groups. Triangles and squares indicate the remaining fish and recruits in experimental groups, respectively.

individuals under natural conditions (Figs. 5, 6). In the same size class, growth of α -individuals was nearly always greater than that of β -individuals (Fig. 6). Growth of α -individuals in experimental groups was clearly greater than that of β -individuals in non-experimental groups for the same size class (Fig. 6). There was no significant difference in the growth increments of all α - and β -individuals in the experimental groups (U-test, $P > 0.05$, throughout the study period, except between October, 1988 and June 1989).

Discussion

Isolated social groups. Anemonefishes in coral reef regions have been believed to live in isolated

groups (Fricke and Fricke, 1977; Moyer and Nakazono, 1978; Fricke, 1979; Keenleyside, 1979; Moyer, 1980; Thresher, 1984; Warner, 1984; Ross, 1990). However, detailed examinations of the spatial distribution of groups and the frequency of fish movement between groups have not been carried out. At the study site, host sea anemones of *A. frenatus* were sparsely distributed (Fig. 1), and home ranges of *A. frenatus* were always restricted to the vicinity of a sea anemone. Prior to the removal experiments, both adults and juveniles rarely moved between sea anemones during the 5-month study period. These results clearly indicate that the groups were isolated from one another.

Isolated groups can be generally categorized into 2 types: spatially isolated and socially isolated. The former can occur when the host sea anemones are so sparsely distributed that the anemonefish can not move between the hosts, whereas the latter can occur when members of one group inhibit immigration from another group. In this study of *A. frenatus*, even after the removal of group members, fish movement between groups rarely occurred during the 2-year observation period (Tables 7-9). Any movement, if it occurred, was only limited to between sea anemones whose distance apart was shorter than the average separation distance of nearest neighboring sea anemones in the study area. Therefore, the groups of *A. frenatus* in this study site can be regarded as spatially isolated.

Adults of *A. clarkii* in a temperate population are suggested as having an inhibitory effect on larval settlement in their sea anemones (Ochi, 1985). In *A. frenatus*, after the removal of part of or all group members, the recruitment of juveniles stopped when the group size attained 2 (Tables 7-9). This suggests that the settlement of juveniles is socially controlled by the residents.

Social control of growth. Among individuals in the same size class, growth increments were nearly always larger in α -individuals than in β -individuals (Figs. 5, 6), indicating that change from a β -individual to an α -individual was accompanied by an increase in growth rate. This means that the growth of β -individuals is suppressed by the presence of α -individuals. It is this growth suppression which causes the great size difference between α - and β -individuals observed in *A. frenatus*. In several anemonefish species in coral reef regions, growth of subordinates is also socially controlled by the dominant fish in a group (Allen, 1972; Fricke and Fricke,

1977; Fricke, 1979). However, the size difference between males and females is generally smaller than that found for *A. frenatus* in this study (Allen, 1972; Fricke and Fricke, 1977; Moyer and Nakazono, 1978). Growth suppression may be more severe in *A. frenatus* than in other anemonefishes.

Since anemonefishes depend on host sea anemones, distribution patterns of hosts strongly influence their social structure and growth. For example, in a temperate population of *A. clarkii* whose host sea anemones are densely distributed, each individual can move between sea anemones. Some nonbreeders and solitary males have home ranges outside females' territories (Ochi and Yanagisawa, 1987; Ochi, 1986, 1989a, b; Hattori and Yanagisawa, in press a, b), so that females cannot suppress their growth. Since breeding pairs have territories that include several sea anemones, males can use different hosts from females (Ochi and Yanagisawa, 1987; Ochi, 1986, 1989a, b; Hattori and Yanagisawa, in press a) and can therefore escape suppression by females. In contrast, among juveniles of 0-year olds of *A. clarkii*, which were confined together in a sea anemone, dominants strongly suppressed the growth of subordinates (Ochi, 1986). In a population of *A. melanopus*, individuals were also confined together in a colony of sea anemones, the total body size of individuals in social group being highly correlated with the total size of sea anemones which the group occupied (Ross, 1978b), as in *A. frenatus* in the present study. Ross (1978b) suggests that the extent of social suppression of growth depends on the carrying capacity of the host. These facts suggest that growth suppression in anemonefishes occurs whenever individuals are isolated in a small group.

Field observations and experiments in several fishes have revealed that the growth of subordinates is suppressed by the dominant individual in a group. This is attributed to behavioral interactions or resource competition between members in a group (Borowsky, 1973, 1987; Brett, 1979; Farr, 1980; Rubenstein, 1981; Jones, 1987; Forrester, 1990; Wootton, 1990). This phenomenon has been well established in laboratory experiments on fishes (Nagoshi, 1967; Yamagishi et al., 1974; Sohn, 1977; Koebele, 1985; Wootton, 1990). Growth suppression of subordinates may be common to fishes that are confined together for a long period in a limited space.

Size-dependent sex change and socially controlled growth. In a temperate population of *A. clarkii*, a

proportion of nonbreeders have been reported to become female without passing through a functional male state (Ochi and Yanagisawa, 1987; Ochi, 1989a; Hattori and Yanagisawa, in press a). Direct transition to female from a juvenile or subadult is referred to as prematurational sex change (defined by Warner and Robertson, 1978) or femininity differentiation in the nonbreeder state (Hattori and Yanagisawa, in press a). The possibility of femininity differentiation in the nonbreeder state has also been suggested in other anemonefishes (Fricke and Fricke, 1977; Fricke, 1979; Thresher, 1984; Hattori and Yanagisawa, in press a). In *A. frenatus* in this study, femininity differentiation in the nonbreeder state was observed in one remaining individual after the removal of a pair. This means that females can be recruited from not only males but also juveniles. Ten α -nonbreeders observed in this study were transitional individuals either from a male to a female or from a juvenile to a female.

In anemonefishes studied so far, males and nonbreeders usually become females within several weeks or months after they became the dominant individuals. For example, a remaining male of *A. bicinctus* changed sex in 26 days after the removal of his mate (Fricke and Fricke, 1977). A nonbreeder of *A. clarkii* in a temperate population became a functional female in 20 days after pair formation with a nonbreeder (Hattori and Yanagisawa, in press a). In contrast, a male of *A. frenatus* still had male gonads several months after the disappearance of his mate. Moreover, most of the specimens which had been α -nonbreeders from the beginning of the present study possessed gonads that were less mature than functional female gonads or were still male, while most of their mates (β -nonbreeders) possessed gonads at the same developmental stage as functional males (Table 5). These results indicate that delayed reproduction of new pairs can be attributed to the physiological unreadiness of α -nonbreeders. In the removal experiments, only 2 individuals (1 male and 1 γ -nonbreeder), which reached 80 mm and 75 mm, became female within 2 years. Moreover, the smallest size of functional females under natural conditions was 80 mm SL. In *A. frenatus*, therefore, reaching 75–80 mm SL is a prerequisite for becoming a functional female. In other words, the timing of femininity differentiation in this species is more or less size-dependent.

In anemonefishes, females are generally larger than their mates (Allen, 1972; Fricke and Fricke,

1977; Moyer and Nakazono, 1978; Ross, 1978a; Fricke, 1979). Larger body size is apparently advantageous to females because their fecundity generally depends upon body size (Fricke and Fricke, 1977; Fricke, 1979; Ochi, 1989a). The growth rate of α -nonbreeders of *A. frenatus* in this study was higher than that of females, indicating that after α -individuals began to reproduce, their growth rate decreased. The timing of first reproduction of α -individuals, therefore, strongly affects their future fecundity. Accordingly, there must exist a smallest mature size that accords females high life-time reproductive success. Under circumstances wherein social suppression of growth is so strong that males can not grow larger than the mature size, sex change will occur after they have attained the above minimum size. On the other hand, under circumstances wherein social suppression of growth is so weak that males can grow larger than mature size, they can become functional females soon after the disappearance of their mates. Size-dependent sex change observed in *A. frenatus* can be attributed to strong growth suppression.

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ハマクマノミの成長抑制とサイズ依存的性転換

服部昭尚

雄性先熟魚であるハマクマノミの社会構造と、個体の成長および繁殖経験を、沖縄県瀬底島の裾礁において1988年6月から1990年11月まで観察した。ハマクマノミはほとんどの場合、1つの宿主イソギンチャクに2-3個体から成るグループを形成していた。67m×334mの調査域に、36のグループがまばらに分布し、グループ間での個体の移動はほとんどなかった。24グループでハマクマノミは産卵したが(繁殖グループ)、10グループでは産卵しなかった(非繁殖グループ)。繁殖グループでの最大個体は雌で、次に大きい個体は雄であった。雄と雌の体長差はきわめて大きく、すべての雄はどの雌よりも小さかった。非繁殖グループでの最大個体は雌よりも小さく、雌よりも未発達あるいは雄的な両性生殖腺を持っていた。また、その次に大きい個体は雌よりも小さく、雄的な両性生殖腺を持っていた。どのグループでも最大個体は次に大きい個体の成長を強く抑制していた。雌を除去すると雄は成長するが、雄が性転換して雌として繁殖する最小サイズ(約75 mm SL)に達するまでに1年半以上かかった。このサイズ依存的な性転換は、雌の雄にたいする強い成長抑制の結果であろうと推察された。

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