

Protandrous Hermaphroditism in Six Species of the Anemonefish Genus *Amphiprion* in Japan

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Abstract Population structure and gonad histology were investigated in six species of the anemonefish genus *Amphiprion* occurring in Japanese waters. Species investigated included *Amphiprion frenatus*, *A. clarkii*, *A. polymnus*, *A. perideraion*, *A. sandracinos*, and *A. ocellaris*. All six species occur around their host anemones in small social groups that include a monogamous pair of sexually functional adults and from zero to three sexually non-functional subadults and juveniles. In all species, the single female in a particular social group is larger than her mate, considerably so in some species. Histological examination of gonads indicated that all individuals of all six species are ambosexual as juveniles. At first only testicular tissues mature. Under certain conditions these may later degenerate and the gonads change to ovaries. Field studies supported histological results. A strict dominance hierarchy exists in each social group. The largest fish is always the single female, and the second largest fish is invariably a functional male. Only the two largest fish are sexually functional. Two functional males of *A. clarkii* under study in the field changed to females after the loss of their previous mates. The six *Amphiprion* species in Japan present examples of socially controlled protandrous sex inversion.

In the course of field studies of anemonefishes at Sesoko Island, Okinawa, in 1975, it was found that *Amphiprion frenatus* Brevoort usually appeared around the host anemone in social groupings that included an adult pair and one or two juveniles, and that a remarkable size difference consistently appeared among the adults, with females considerably larger than males. A similar but less obvious female/male size ratio had previously been described for *Amphiprion clarkii* (Bennett) by Moyer and Sawyers (1973). Late in 1976, two individuals of *A. clarkii* at Miyakejima, Izu Islands, were noted to have changed from males to females. These facts indicated the possibility of protandrous hermaphroditism in these species, and led to investigations of other *Amphiprion* species in the Ryukyu Islands. Subsequently, Fricke (1976) and Fricke and Fricke (1977) described protandrous sex inversion in *Amphiprion bicinctus* Rüppell from the Gulf of Aqaba, Red Sea, and in *A. akallopisos* Bleeker from Aldabra in the Indian Ocean. Ross (1978) noted a similar sex change in *A. melanopus* Bleeker from Guam.

The present study describes the social structure of six species of anemonefishes from Japan and reports the results of histological studies

of their gonads.

Materials and Methods

From 1975~1977, field observations of the social behavior and social structures of *Amphiprion frenatus*, *A. clarkii*, *A. polymnus* (Linnaeus), *A. perideraion* Bleeker, *A. sandracinos* Allen, and *A. ocellaris* Cuvier were made using SCUBA or by snorkeling in the waters of Sesoko Island, Okinawa Island, Miyako Island, Ikema Island, and Kuroshima in the Ryukyu Islands, and at Miyake-jima in the Izu Islands. Specimens were collected using hand and screen nets, and were preserved in 10% formalin after weighing and measuring. In specimens larger than 41 mm in standard length, gonads were removed and prepared in 7 μ paraffin sections. In specimens of 40 mm or less in standard length, cross sections of the entire abdominal region were prepared after decalcification in 5% formic acid. The sexes of the fishes were determined under a microscope after staining with haematoxylin and eosin. Fish specimens are deposited at the Tatsuo Tanaka Memorial Biological Station (TMBS), Miyake-jima, and gonad preparations are in the Fishery Research Laboratory of Kyushu University.

Table 1. Body length and sex in six species of *Amphiprion*. The gonads of all specimens were examined histologically. Specimens are grouped under the anemone from which they were collected, as designated by Roman numerals. In most cases not all fish were taken from a particular anemone, and complete social structures per anemone are not represented in this table. F, female; M/F, ambosexual.

Species and social group	First ranking fish			Second ranking fish			Third or lower ranking fish			Locality	Date
	No.	SL (mm)	Sex	No.	SL (mm)	Sex	No.	SL (mm)	Sex		
<i>A. frenatus</i>											
I.	760600-3	85	F	760600-4	57	M/F	760600-5	21	M/F	Kuroshima	Jun. 1976
II.	760600-1	83	F	760600-2	50	M/F				Kuroshima	Jun. 1976
III.	751225-02A	77	F	751225-02B	57	M/F				Kuroshima	Dec. 1975
IV.	770626-4	75	F	770626-5	64	M/F	770626-6	18	M/F	Miyako-jima	Jun. 1977
V.	760600-6	74	F	770600-7	58	M/F				Kuroshima	Jun. 1976
VI.	760600-8	71	F				760600-9	35	M/F	Kuroshima	Jun. 1976
VII.	760219-01A	70	F				760219-01B	32	M/F	Okinawa	Feb. 1976
VIII.	751225-02C*	69	F							Kuroshima	Dec. 1975
IX.	760600-10***	55	M/F	760600-11	34	M/F				Kuroshima	Jun. 1976
X.	760600-12***	53	M/F	760600-13	28	M/F				Kuroshima	Jun. 1976
<i>A. clarkii</i>											
I.	760700-1	109	F							Miyake-jima	Jul. 1976
II.	761125-3	109	F	761125-2	93	M/F				Miyake-jima	Nov. 1976
III.	761125-1	101	F	761125-4	75	M/F				Miyake-jima	Nov. 1976
IV.	760905-1	101	**M/F	760905-2	72	M/F				Miyake-jima	Sep. 1976
V.	761125-5	69	F							Miyake-jima	Nov. 1976
<i>A. polymnus</i>											
I.	771006-6	106	F	771006-5	102	M/F				Okinawa	Oct. 1977
II.	771006-7	77	F	771006-8	76	M/F	771006-9	24	M/F	Okinawa	Oct. 1977
<i>A. perideraion</i>											
I.	771006-1	76	F	771006-2	45	M/F				Okinawa	Oct. 1977
II.	770702-3	61	F	770702-2	33	M/F				Okinawa	Jul. 1977
III.	771006-3	60	F	771006-4	49	M/F				Okinawa	Oct. 1977
IV.	771008-5	60	F	771008-6	40	M/F				Okinawa	Oct. 1977
V.							770630-1	30	M/F	Okinawa	Jun. 1977
<i>A. sandracinos</i>											
I.	770626-2	61	F	770626-3	43	M/F				Yaeyama Lagoon	Jun. 1977
<i>A. ocellaris</i>											
I.				770625-1	44	M/F				Miyako-jima	Jun. 1977
II.	771008-3	42	F	771008-4	35	M/F				Okinawa	Oct. 1977
III.	771008-1	41	F							Okinawa	Oct. 1977
IV.				770626-1	41	M/F				Miyako-jima	Jun. 1977
V.				771007-1	35	M/F				Okinawa	Oct. 1977
VI.							771007-2	28	M/F	Okinawa	Oct. 1977

* Solitary female, no other fish in anemone.
functional male present. See text for details.

** This individual had intermediate color pattern on its caudal fin.

*** No female or

Results

***Amphiprion frenatus*.** This species is rather common in coral reefs from Okinawa Island to the Yaeyama Islands. Social groups usually consist of from 3 to 4 fish, with all social behavior centering around the host sea anemone. Field studies at Okinawa, Sesoko, Miyako, and Kuroshima Islands indicated that these groups invariably consisted of two sexually active adults and 1~2 juveniles or subadults. Consistently, the female adult was considerably larger than the male (Table 1). A large carpet of clustering anemones covering an area of approximately 15 m² was discovered in the lagoon near Ikema Island, Miyako Group, in June, 1977. This anemone colony sheltered twelve *A. frenatus*, consisting of a single large, dominant female, a smaller adult male, and ten small subadults and juveniles. The adults displayed typical *Amphiprion* courtship behavior (Allen, 1972; Fricke, 1974; Moyer and Bell, 1976). Subadults were aggressively chased by both adults, but more frequently by the male.

Histological studies of the gonads of this species revealed that the largest individuals in social groups had ovaries (Table 1, Fig. 1A). All smaller fish, from the second ranking member to the smallest, had male and female germ cells within the same gonads (Fig. 1B, C). In these ambosexual gonads (Reinboth, 1970), the testicular parts were observed in various developmental stages, but the oocytes were in young stages, with the most developed oocytes in the peri-nucleolus stage. Even fish in the smallest size group (18~28 mm in standard length) had ambosexual gonads

(Fig. 1D).

Both fish of juvenile pairs with no large fish on the same anemone had ambosexual gonads during the spawning season (Anemone No. IX and X in Table 1). The largest fish of both these pairs, 55 mm and 53 mm in standard length, showed reduced testicular parts, but no maturation of ovarian tissues. Small size may prevent maturation of oocytes.

The present field studies and histological examinations suggest that the oocytes in ambosexual gonads mature when the fish grows larger than 55 mm in standard length, and when the fish attains the largest size in a particular social group. When the oocytes begin to mature, the testicular parts of ambosexual gonads disappear. Many brown bodies (Reinboth, 1970) usually seen in the ovary may be an indication of degenerated testicular tissues.

***Amphiprion clarkii*.** This species is common from Miyake-jima to the Yaeyama Islands. At Miyako Island and Kuroshima, host anemones usually sheltered a sexually active pair of adults and two small juvenile or subadult fish. Invariably the female was somewhat larger than the male. At Okinawa Island, *A. clarkii* often shared its host anemone with *Amphiprion perideraion*. In all examples where adults of both species occurred in the same anemone, only a single juvenile *A. clarkii* was present, suggesting interspecific competition for shelter.

Social behavior and social structures of *A. clarkii* at Miyake-jima are quite different from what has been observed in the Ryukyu Islands. Frequently, large clusters of anemones at Miyake-jima contain more than one breeding pair (Moyer, 1976) (Table 2). For example,

Table 2. Comparison of *Amphiprion clarkii* adult populations per anemone host at Sesoko Island and Miyake-jima, and comparison with *Amphiprion akallopisos* and *Amphiprion bicinctus*.

Species	Location	No. of anemone host	No. of adults* per anemone host
<i>A. clarkii</i>	Miyake-jima	39	3.2
<i>A. clarkii</i>	Sesoko Island	33	1.7
<i>A. akallopisos</i> **	Aldabra	82	2.6
<i>A. bicinctus</i> **	Gulf of Aqaba	228	1.1

* females and functional males.

** after Fricke and Fricke (1977).

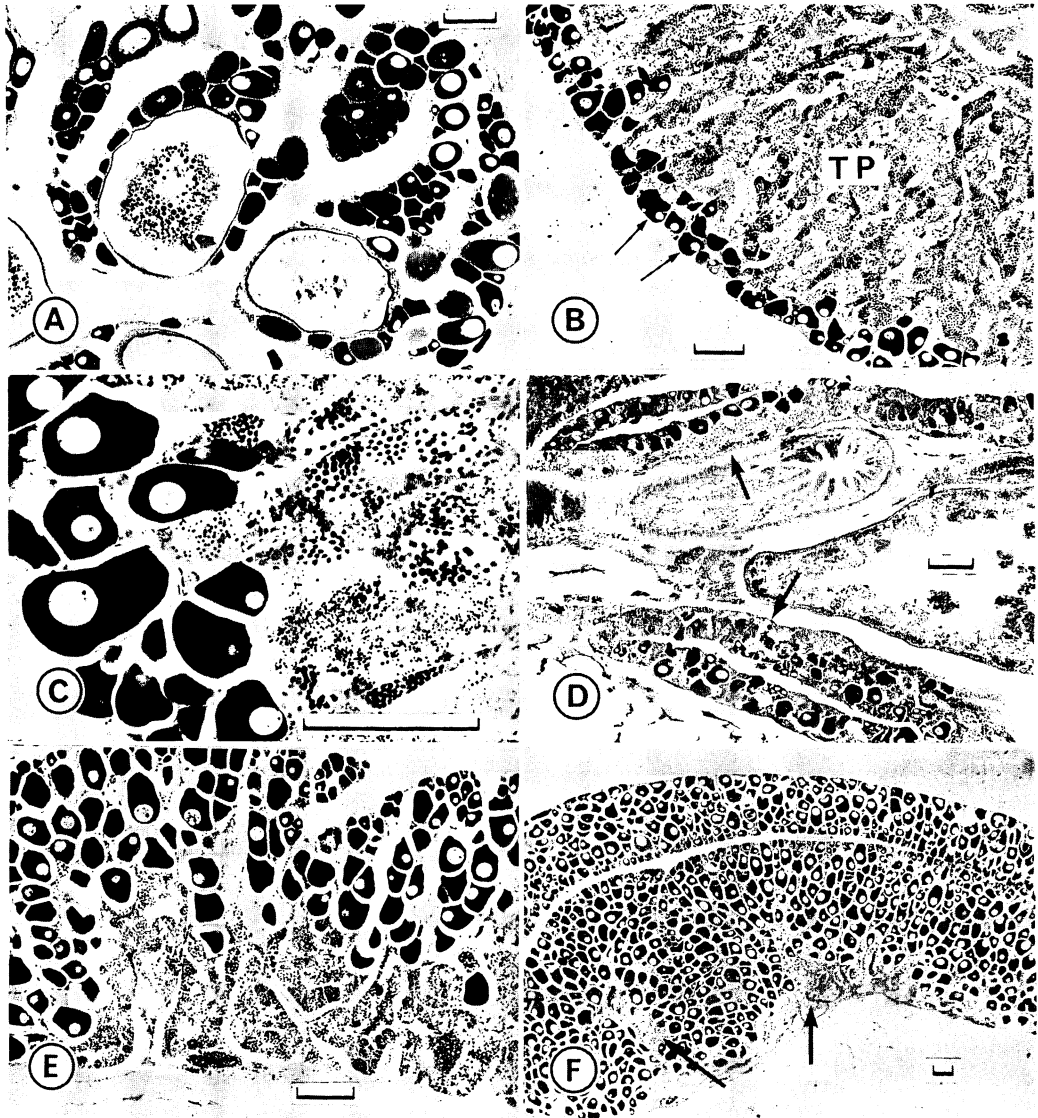


Fig. 1. Photomicrographs of the gonads of *Amphiprion frenatus* (A-D) and *A. clarkii* (E, F). Scales show 100 μ . A: Ovary of *A. frenatus* (760600-8). B: Ambosexual gonads of *A. frenatus* (760600-4) with a functional testicular part (TP) and young oocytes (arrows). C: Higher magnification of B. D: Ambosexual gonads of young *A. frenatus* (760600-13, 28 mm in standard length). Arrows show the left and right lobes. E: Ambosexual gonad of *A. clarkii* (761125-4). F: Gonads of *A. clarkii* (760905-1) with intermediate caudal fin color (Moyer, 1976). The number of oocytes is increasing and testicular parts (arrows) are undergoing reduction.

as many as four breeding pairs resided in a single anemone colony of approximately 14 m². Migrations of dominant females to new nesting sites and different mates have been occasionally noted, and lengthy migrations of up to 64 m to new anemone sites by adult males were often seen after the breeding season.

In the winter of 1976, two females from pairs studied at Miyake-jima by Bell (1976), Moyer (1976), and Moyer and Bell (1976) disappeared. By late May, 1977, the former male from each pair had changed into female, mating and spawning with formerly subdominant, sexually inactive males.

Histological studies supported observations on sex change in the field. Functional males had ambosexual gonads (Table 1, Fig. 1E). A larger individual from a pair (Anemone No. IV, *A. clarkii*, in Table 1) had an intermediate color pattern on its caudal fin (Moyer, 1976). The gonads of this fish were undergoing reduction of testicular parts and displayed young oocytes, indicating the fish was changing from a functional male to a female (Fig. 1F).

***Amphiprion polymnus*.** This species is rare in the Ryukyu Islands. We were able to find it only in the vicinity of Tsuken Island, Nakagusuku Bay, Okinawa, where it is rather common. Social groupings consist of a pair of adults and usually two subadults or juvenile fish. Unlike *A. clarkii* and *A. frenatus*, the female is only slightly larger than her mate (Table 1).

Histological studies indicated that the largest fish in each social group had ovaries and smaller fish had ambosexual gonads identical to the previous two species (Table 1).

***Amphiprion perideraion*.** At Okinawa and Sesoko Islands, *A. perideraion* was commonly observed, usually sharing the anemone host with *A. clarkii*. An adult pair and usually 2~3 subadults or juveniles inhabit the anemone. In this species, the female is considerably larger than her mate.

Social groups included one large female and a smaller functional male with ambosexual gonads (Table 1).

***Amphiprion sandracinos*.** This species is rather rare, occurring from Okinawa to the southern Ryukyu Islands. In all of our observations, a pair of breeding adults and 2~3 subadults and juveniles inhabited the anemone. As in most other *Amphiprion* species, the single female was noticeably the largest fish. Smaller fish had ambosexual gonads (Table 1).

***Amphiprion ocellaris*.** This species is common in the shallow lagoons of the southern Ryukyu Islands, where it lives around its anemone host in groups that include a pair of breeding adults and 2~3 subadults and juveniles. Typically the female is slightly larger than her mate. Functional males and subdominant fish had ambosexual gonads (Table 1).

Discussion

Histological studies of the six Japanese species of *Amphiprion* indicated that all individuals in all six species are ambosexual as very small juveniles. At first, only testicular tissues mature. These later degenerate and the gonads change to ovaries. Field studies on the social structures of each species agree well with our histological results. A strict dominance hierarchy exists in each social group. The largest fish of each social group was always the single female and that fish holding the second size rank was invariably a functional male. Furthermore, field studies showed that in two cases functional males of *A. clarkii* changed to females after the loss of the previous mates. It can be concluded that *Amphiprion clarkii*, *A. frenatus*, *A. polymnus*, *A. perideraion*, *A. sandracinos*, and *A. ocellaris* are all protandrous hermaphrodites.

The sizes of females within a particular species of *Amphiprion* differed between social groups in different anemones (Table 1). This strongly suggests that sex change in these species is not size dependent, but takes place when the functional male attains the highest social rank at the death or disappearance of the former female. In some cases a female at a particular anemone may be smaller than a male of the same species at another anemone, e.g., *A. clarkii*, *A. polymnus*, and *A. ocellaris* in Table 1.

Fricke (1976) and Fricke and Fricke (1977) hypothesized protandry as a universal adaptation of members of the genus *Amphiprion*. Heavy predator pressures restrict anemonefishes to the immediate vicinity of their respective anemones, thus preventing migration for the purpose of locating a mate. Protandry guarantees reproductive success, as long as two or more fish are present at a given anemone. Our findings support the above authors' hypothesis.

The presence of numerous multi-adult populations of *Amphiprion clarkii* inhabiting large anemone clusters at Miyake-jima contrasts with the situation with other species of *Amphiprion* and with conspecifics in the Ryukyu Islands, where only one female and one functional male are present at a given anemone.

The Miyake-jima situation seems to represent an unstable *Amphiprion* social structure. This problem is currently under investigation.

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日本産クマノミ属6種の雄性先熟性転換

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日本産のハマクマノミ, クマノミ, トウアカクマノミ, ハナビラクマノミ, セジロクマノミ, カクレクマノミの6種について, 社会構造を潜水観察するとともに, 性転換を調べた。これらの6種では, 宿主イソギンチャク毎に, 大型の成熟雌1尾とそれより小型の雄1尾および未成魚0~3尾よりなる社会構造が認められた。雄および未成魚は, すべて両性生殖腺を持っており, 雄性先熟の性転換を行なうことを明らかにした。さらに, これらのクマノミ類の社会構造と性転換の関係について検討した。

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