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Original Article

Determinants of body size composition in limited shelter space: why are anemonefishes protandrous?

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I examined why anemonefishes, *Amphiprion ocellaris*, *Amphiprion frenatus*, and *Amphiprion perideraion*, which inhabit single host anemones, are monogamous and protandrous. Because they live in small groups ($\leq$6 individuals) with a size hierarchy, they might have the environmental potential for polygyny. If the dominant fish were male, he could monopolize mating opportunities, and subordinates should be female. Female fecundity increases with body size, so that group body size composition largely influences his reproductive success. First, I developed an optimization model to predict the body size composition based on the carrying capacity of the host (C) and a fixed body size ratio (γ) or difference (δ) between individuals adjacent in rank. The γ and δ were assumed to be necessary for subordinates to avoid fatal eviction from the group. The model using δ and C could predict the body size composition of the 3 species. Next, I incorporated nonlinear female fecundity functions into the model to evaluate whether polygyny or monogamy was the better mating system for the dominant fish. I examined relationships between gonad weight and body size of *A. frenatus* and *A. perideraion* to estimate the functions. Assuming large δs and the nonlinear functions, dominant fish could have higher reproductive success in monogamous mating systems. The model also indicated that where C is limited, a larger δ resulted in one large, one small, and several very small subordinate individuals. This combination of body size composition model and nonlinear female fecundity functions can explain the function of protandry and monogamy under limited shelter space. *Key words:* carrying capacity, conflict avoidance, group structure, protandry, sex change. *[Behav Ecol]*

INTRODUCTION

Sequential hermaphroditism (sex change) is widely found in plants and animals (Policansky 1982; Warner 1988a; Munday et al. 2006), especially in marine fishes with protogyny (from female to male) predominating (Kuwamura and Nakashima 1998; Munday et al. 2006). If the expected reproductive success differs between the sexes with body size, an individual that can change sex at the proper size will have more offspring than one that remains exclusively male or female (Ghiselin 1969; Charnov 1982; Warner 1988a, 1988b). This size-advantage hypothesis (SAH) predicts that protogyny will be found in species with polygynous mating systems, where larger males can monopolize mating opportunities, and the expected reproductive success of males will increase rapidly with increasing body size much more than in females (Charnov 1982; Warner 1988a, 1988b; Kuwamura and Nakashima 1998).

Protandry (male to female) is expected to occur in species with mating systems in which the expected reproductive success of males is less sensitive to their body size, whereas that of females increases with increasing body size (Ghiselin 1969; Charnov 1982; Warner 1988a, 1988b). In promiscuous mating systems of some pandalid shrimps and lizard flatheads (fish), for instance, pairing randomly occurs with respect to body size, so large males do not have an advantage in mating (Charnov 1982; Shinomiya et al. 2003). Mating systems of protandrous species have been studied in some crustaceans, fishes, and mollusks (Warner 1988a; Wright 1989; Tsai et al. 1999; Correa and Thiel 2003; Chen et al. 2004; Bauer 2006; Collin 2006). Although the SAH has been highly successful in explaining the adaptive significance of various sex-change strategies, especially for protogynous species (Munday et al. 2006), information is still lacking for a complete understanding of protandry in relation to mating systems.

Anemonefishes (genus *Amphiprion*, Perciformes: Pomacentridae) have symbiotic associations with sea anemones. The fishes often inhabit isolated single hosts and form small groups with a size hierarchy (Allen 1975; Fautin and Allen 1992). The largest fish is female, and the second-ranked fish is a male who takes care of demersal eggs (Fricke HW and Fricke S 1977; Moyer and Nakazono 1978; Kuwamura and Nakashima 1998); when the female disappears from the group, this male changes sex, and the third-ranked fish inherits the male breeding position and territory (Buston 2004a, 2004b; Mitchell 2005). Several authors have suggested that pair formation in anemonefishes occurs randomly with respect to body size (Fricke HW and Fricke S 1977; Kuwamura and Nakashima 1998) because larval recruitment to a sparsely distributed host anemone was considered to occur randomly, postrecruitment movement between isolated hosts was thought to be difficult (Allen 1975; Fricke HW and Fricke S 1977), and only 2 adults could inhabit a single host due to the small carrying capacity of the host anemone (Fricke HW and Fricke S 1977; Fautin and Allen 1992; Hattori and Yamamura 1995; Kuwamura and Nakashima 1998). However, recent studies demonstrated that larval recruitment to a single host is not random (Elliott et al. 1995; Schmitt and Holbrook 1999;

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Elliott and Mariscal 2001; Buston 2003b; Hattori 2005; Mitchell 2005), individuals often move between hosts to obtain larger hosts or mates if the opportunity arises (Hattori 1994, 2005; Hirose 1995), and there are usually third- and lower-ranked fish which are often larger than breeders in other groups (Hattori 1995; Buston 2004a, 2004b; Mitchell 2005). Whether they form pairs randomly is unclear. It has also been suggested that protandry in anemonefishes functions to secure mates without the risk of adult movement between sparse hosts and to ensure that the larger member of a breeding pair is always female, which ensures higher fecundity for both partners (Fricke HW and Fricke S 1977; Kuwamura and Nakashima 1998; Munday et al. 2006). However, no explanations sufficiently explain why anemonefishes are monogamous while living in small groups.

Animals living in small groups with size hierarchies tend to have the environmental potential for polygyny (EPP, Emlen and Oring 1977): If the dominant member in the group is male, he can monopolize mating opportunities. Anemonefishes inhabiting isolated single hosts can be considered to have the EPP. Group members are confined to sparsely distributed hosts, and the third-ranked fish are often of reproductive size and can mature quickly after a dominant adult disappears, suggesting that their reproductive status is socially, not ontogenetically, constrained (Fricke HW and Fricke S 1977; Moyer and Nakazono 1978). Thus, the third-ranked fish may be able to reproduce as a female if sexual maturation was not inhibited by the social influence of the higher ranking members. In addition, males take care of demersal eggs (Allen 1975). If the dominant fish in a group were male, he could monopolize mating opportunities by taking care of many eggs, like the males in several polygynous damselfishes and gobies (Whiteman and Côté 2004). Monogamy and protandry in the anemonefishes may be one possible alternative to polygyny and protogyny that have evolved in small groups.

Under the EPP, monogamy usually occurs when biparental care, cooperative territorial defense, and/or extended mate guarding are necessary to increase the reproductive success of the dominant members (Emlen and Oring 1977; Correa and Thiel 2003; Whiteman and Côté 2004). However, the anemonefishes and some other habitat-specialist fishes that are confined to limited shelter space and form small groups with a size hierarchy show monogamy without cooperative territorial defense, mate guarding, or biparental care. In addition, the intraspecific competition for food, mates, spawning sites, and/or living space in these fishes is potentially or actually intense (e.g., anemonefishes, Buston 2003a; Mitchell 2005; a coral croucher, Wong et al. 2005; and coral-dwelling gobies, Thompson et al. 2007). In the anemonefish *Amphiprion percula*, which inhabits a single host with limited shelter space, subordinates are found to delay their own growth, maintaining a large body size ratio between adjacently ranked group members. This allows the subordinates to coexist and avoid being forcibly evicted from the host by the dominant fish because of potentially intense intraspecific competition (Buston 2003a, 2003b). Intraspecific competition involving intrasexual competition is associated with the evolution of monogamy within groups (Emlen and Oring 1977). However, monogamy often involves size-assortative mating, which does not favor protandry (Ghiselin 1969; Charnov 1982; Warner 1988a, 1988b). In order to understand how protandry and monogamy are related, we need to examine the relationships between the body size compositions of group members and their potential fecundity.

In anemonefishes, limited shelter space seems to determine both the summed body lengths (or total biomass) of group members (Allen 1975; Hattori 1991, 2005; Buston 2003a; Mitchell 2003) and group size (see Mitchell and Dill 2005). Because large fish use the most resources, such as foods and space, growth of the dominant fish may retard the growth of subordinates when shelter space is limited (Allen 1975; Buston 2003b). Hattori (2005) hypothesized that under limited shelter space, the expected reproductive success via male function will not increase with increasing body size, although that via female function is expected to correlate positively with body size. If anemonefish lived in haremic groups with dominant males, growth of males would retard the growth of females, and consequently, the total fecundity of the small females might be lower than the potential "female" fecundity of the dominant fish. However, Hattori's verbal model did not incorporate the degree of the size ratio between individuals adjacent in rank within the group and the relationships between female fecundity and body size. Fecundity of females may increase exponentially with body size, and the shape of the function between female fecundity and body size may directly influence the fecundity of the dominant fish in the haremic groups (Muñoz and Warner 2004). If group members need a minimum body size difference between adjacently ranked individuals to stay in the group under limited shelter space and if female fecundity increases almost linearly, the dominant fish would have higher fecundity in polygyny than in monogamy because the expected fecundity of the dominant male is the sum of the relatively "large" females' fecundities.

In the present study, first, I developed a mathematical optimization model to predict the group body size composition based on the carrying capacity of the host and a fixed body size ratio between individuals adjacent in rank. However, the model could not predict the group body size composition of *A. ocellaris*, which is closely related to *A. percula* (Allen 1975), or of *A. frenatus*, both of which live in small groups in single isolated hosts (see Results). Then, I adopted another criterion, a fixed body size difference between group members adjacent in rank. As a result, the group body size compositions of all subject species were predictable. Next, I incorporated nonlinear female fecundity functions into the group body size composition model to evaluate whether polygyny or monogamy was the better mating system for the dominant fish. In order to estimate the functions, I used the relationships between gonad weight and body size of *A. frenatus* and *A. perideraion*. I discuss why the anemonefishes are monogamous and protandrous under conditions of limited shelter space.

MATERIALS AND METHODS

Group body size composition model and the predictions

In anemonefish inhabiting a single isolated host, a small group consists of a breeding pair and 0–4 nonbreeders (Hattori 1991; Buston 2003a; Mitchell 2003). The summed body lengths of the group members are closely correlated with the size (area or diameter) of the host anemone (Hattori 1991, 2005; Buston 2003a; Mitchell and Dill 2005). Accordingly, I assumed that the summed length of all group members was constant for a given shelter size, that is, host anemones of the same size:

$$\sum b_i = H\left(x\right),$$

where b is the body length of each group member of rank 1–6 ($b_1 > b_2 > b_3 > b_4 > b_5 > b_6$) and H is a function of x, the area of the host anemone.

The body size difference between individuals adjacent in rank is large, and the difference is maintained by growth modification of each individual (Buston 2003a; Buston and Cant 2006). I assumed that the size ratio (γ) or difference (δ) between group members adjacent in rank is the same within a population of a species irrespective of their specific rank:

$$\gamma = b_i/b_{i+1} \text{ or } \delta = b_i - b_{i+1}.$$

If group members coexist by maintaining γ or δ and if their summed length is determined by the carrying capacity of the host (C), the body size of each individual of rank i (b_i) can be predicted from the 2 values (γ or δ, and C, where C is the expected summed length in a host of a given size). The optimum body lengths of group members (b_i) were calculated to maximize the body size of each fish using an optimization tool (Excel Solver tool, Microsoft Office Excel 2003) under the following restrictions [$b_1 \geq 0$, $b_2 \geq 0$, $b_3 \geq 0$, $b_4 \geq 0$, $b_5 \geq 0$, $b_6 \geq 0$, $b_1 + b_2 + b_3 + b_4 + b_5 + b_6 \leq C$, and ($\gamma\, b_1 \geq b_2$, $\gamma\, b_2 \geq b_3$, $\gamma\, b_3 \geq b_4$, $\gamma\, b_4 \geq b_5$, $\gamma\, b_5 \geq b_6$) or ($b_2 + \delta \leq b_1$, $b_3 + \delta \leq b_2$, $b_4 + \delta \leq b_3$, $b_5 + \delta \leq b_4$, $b_6 + \delta \leq b_5$)].

If the anemonefishes use fixed values of γ or δ in local populations (Buston 2003a), the model predicts the body size of individuals of each rank based on C, which can be tested by field data.

Evaluation of the better mating system for the dominant fish

Fecundity increases with body size in female anemonefishes (Ochi 1989b; Mitchell 2003). Because relationships between potential female fecundity and body size can be described by exponential equations in fishes (Muñoz and Warner 2004), I assumed that the fecundity of a female (f) would be exponentially proportional to its body size:

$$f_i = \alpha b_i^{\beta},$$

where b_i is the body size of an individual of rank i, and α and β are coefficients. If these coefficients cannot be statistically determined, the nonlinear female fecundity functions cannot be used for determining the better mating system for the dominant fish.

In the case of protandrous sex changers, the dominant fish must be female, and its reproductive success (F) would be represented as follows:

$$F = f_1.$$

In contrast, in the case of protogynous sex changers, the dominant fish must be male, and its reproductive success (M) would be represented as follows:

$$M = f_2 + f_3 + f_4 + f_5 + f_6.$$

M and F were calculated using the predicted or actual b_i, and the estimated α and β, to compare a monogamy–protandry system with a polygyny–protogyny system in terms of reproductive success.

Verification of the assumptions and test of the model

The assumptions of the model were examined with field data on the 3 anemonefishes, *A. ocellaris*, *A. frenatus*, and *A. perideraion*, that inhabit single isolated host anemones: 1) whether the size of the host can be used to predict the summed lengths of the group members; and 2) whether there are no significant differences in average size ratio (b_i/b_{i+1}) or average size difference ($b_i - b_{i+1}$) between individuals of different size categories (i).

When the assumptions were valid, b_i was calculated using field data (C and γ or δ). The average value of the body size of group members and the average value of the size ratio or difference between b_1 and b_2 were regarded as C and γ or δ, respectively. The predicted body size of each group member b_i was compared with the average body size measured in situ using estimated 95% confidence intervals (Figures 1 and 2). One-sample t-tests were conducted (Tables 1 and 2). When the model predicted small values, such as 4.0 mm, this usually meant that there were no fish in the rank (the smallest fish was about 10 mm). When no fish was found in a given rank, the body size of that fish was considered to be 0 mm.

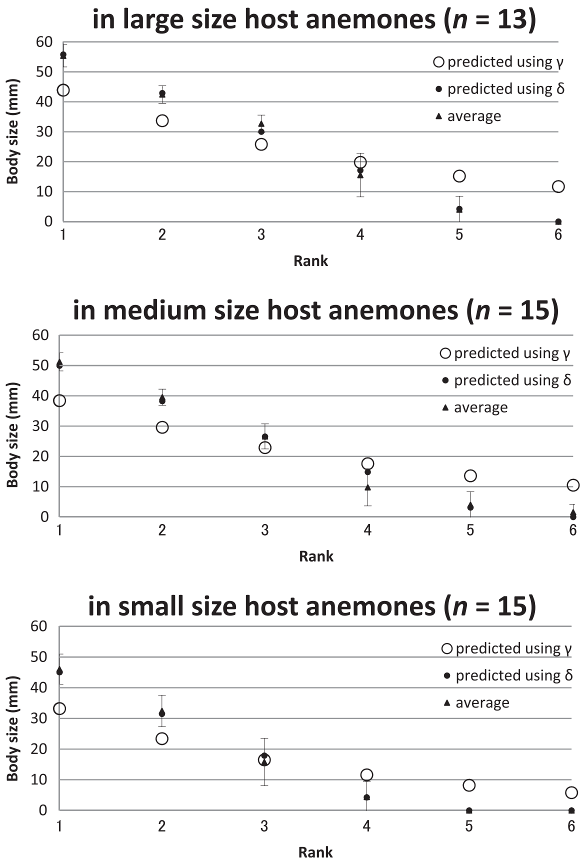

Figure 1
The average body sizes (SLs) of group members from rank 1 to rank 6 of *Amphiprion ocellaris*, according to the 3 host size categories (large, medium, and small), and the predicted body size of each group member (assuming a maximum group size of 6) calculated from the optimization model. The model was based on the average values of body size ratio (γ) or difference (δ) between the largest and the second largest fish in the group and the average values of the summed length of group members; 95% confidence intervals are shown.

Field data collection

To test the model, a field study on *A. ocellaris*, which inhabits a single species of host anemone, *Stichodactyla gigantea*, was performed in July and August 2009 on Shiraho Reef, Ishigaki Island (lat 24°22′N, long 124°15′E), Okinawa, Japan. A high-resolution digital color aerial photograph (taken by Pasco on 20 September 2006: 1/10 000, C19-1608, ground resolution = 10 cm) was used as a field map (see Hattori and Kobayashi 2009; Hattori and Shibuno 2010). All *S. gigantea* could be individually identified by their locations on the map. Each individual anemonefish was captured with deep hand nets of cotton-like thread, rolled up in the net to prevent movement, and measured with a ruler (standard length [SL], the straight-line distance from the tip of the snout to the base of the caudal fin, in millimeter) in situ. All individuals were rapidly returned to their anemones. Anesthetic chemicals, use of

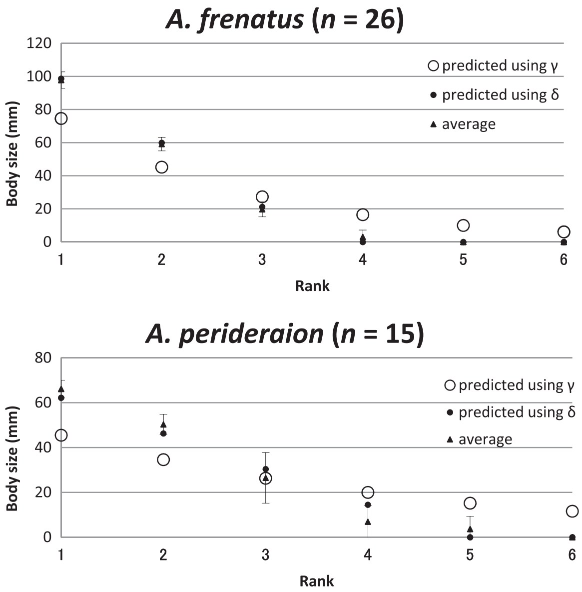

Figure 2
The average body sizes (SLs) of group members from rank 1 to rank 6 of 2 anemonefishes, *Amphiprion frenatus* and *Amphiprion perideraion*, which live in isolated single host anemones, and the predicted body size of each group member (assuming a maximum group size of 6) calculated from the optimization model based on the average values of body size ratio (γ) or difference (δ) between the largest and the second largest fish in the group and the average values of the summed length of group members. Only breeding groups were selected; 95% confidence intervals are shown.

which is prohibited in the prefecture of Okinawa because of harmful effects on fishes and the environment, were not used. In my preliminary studies, I observed several individuals moving among several hosts when those hosts were close to one another (see Hattori and Kobayashi 2009); for this study, only data of members of 43 groups that did not include these migrants were used. The oral disk of *S. gigantea* was considered to be an oval, and the long and short axial lengths were measured on 3 occasions in July 2009 to estimate area. As several different individuals of *S. gigantea* seemed to shrink between measurements for undetermined reasons, I used the data set with the smallest standard deviation of the calculated area for analyses. The body size composition was expected to differ by host size, so *S. gigantea* were classified into 3 size categories (small [<400 cm^2, n = 15], medium [400–800 cm^2, n = 15], and large [>800 cm^2, n = 13]). The average value of the summed lengths of group members was calculated in each category.

Structure and body size composition of groups of *A. frenatus* and *A. perideraion*, both of which use single isolated hosts, have already been published (Hattori 1991, 1995). Assumption 1 was satisfied; the regression line between the SL of group members and the area of the host anemone was statistically significant (Hattori 1991, 1995). To verify assumption 2, original published data from October 1988 (Hattori 1991, 1995) were used, excluding groups that used multiple hosts and those that did not include any breeders. SLs of group members were used for the test.

Estimation of nonlinear female fecundity functions

No study has reported on the relationships between fecundity and gonad weight in anemonefishes. However, gonad weight linearly reflects potential female fecundity in some fishes (Rhodes and Sadovy 2002; Sivakumaran et al. 2003). For simplicity, I assumed that the fecundity of a female (f) would be linearly proportional to its gonad weight. I used the regression curves between gonad weight and SL for *A. frenatus* and *A. perideraion* reported by Hattori (1991, 2000) to estimate α and β of the functions of female fecundity and body size. I could not collect data on gonad weight of *A. ocellaris* because Shiraho Reef was designated a marine protected area in 2007. If the regression curves are not statistically significant, the functions cannot be applied to the model.

Comparison of the 2 mating systems for the dominant fish

M and F were calculated as αb_1^{β} and $\alpha\left(b_2^{\beta}+b_3^{\beta}+b_4^{\beta}+b_5^{\beta}+b_6^{\beta}\right)$, respectively. Predicted and actual b_i values were used. Estimated values of α and β for *A. perideraion* were applied to the model for *A. ocellaris* because their body sizes were similar to those of *A. perideraion.*

Table 1
Comparison of body size ratio and body size difference between individuals adjacent in rank

				Statistical test results					Statistical test results		
Species (statistical test)	Body size ratio	n	Average	Test statistic	Effect size	P	Body size difference	Average (mm)	Test statistic	Effect size	P
Amphiprion ocellaris	Rank 1/rank 2	43	0.75	F = 2.7	0.27	0.049	Rank 1 − rank 2	12.7	F = 1.3	0.19	0.278
(ANOVA)	Rank 2/rank 3	38	0.70				Rank 2 − rank 3	11.5			
	Rank 3/rank 4	20	0.65				Rank 3 − rank 4	11.1			
	Rank 4/rank 5	8	0.61				Rank 4 − rank 5	8.75			
Amphiprion frenatus	Rank 1/rank 2	26	0.60	F = 40.1	1.24	<0.001	Rank 1 − rank 2	38.8	F = 30.9	1.09	<0.001
(ANOVA)	Rank 2/rank 3	23	0.37	Excluding the last category			Rank 2 − rank 3	37.5	Excluding the last category		
(*t*-test)	Rank 3/rank 4	6	0.60	t = 9.19	2.63	<0.001	Rank 3 − rank 4	10.5	t = 0.54	0.16	0.58
Amphiprion perideraion	Rank 1/rank 2	15	0.76	F = 1.28	0.38	0.301	Rank 1 − rank 2	15.9	F = 1.11	0.35	0.360
(ANOVA)	Rank 2/rank 3	10	0.75				Rank 2 − rank 3	13.3			
(*t*-test)	Rank 3/rank 4	4	0.63	Excluding the last 2 categories			Rank 3 − rank 4	14.8	Excluding the last 2 categories		
	Rank 4/rank 5	2	0.76	t = 0.12	0.05	0.900	Rank 4 − rank 5	7.0	t = 0.97	0.39	0.340

Results of one-way analysis of variance (ANOVA) or *t*-test are shown for *A. ocellaris*, *A. frenatus*, and *A. perideraion*, which live in single isolated host anemones. Analyses were conducted using all categories and excluding the last 1 or 2 categories when those categories had few data points.

Table 2

Results of one-sample *t*-tests between predicted and observed body sizes (SLs in mm) of group members from rank 1 to rank 6 of *Amphiprion ocellaris* in the 3 host size categories (large, medium, and small)

Host size category	Size rank	Average body size	Predicted body size (using body size difference, δ)	One-sample *t*-test		
				t	Effect size	*P*
Large ($n = 13$)	1	55.4	55.8	−6.71	0.07	0.80
	2	42.5	42.9	0.34	1.88	0.74
	3	32.7	30.0	2.03	0.88	0.07
	4	15.5	17.1	−0.47	0.22	0.65
	5	4.0	4.2	−0.10	1.49	0.92
	6	0	0	—	—	—
	Sum	150.1	150.0			
Medium ($n = 15$)	1	51.2	49.9	0.42	2.14	0.68
	2	39.5	38.2	0.40	1.79	0.69
	3	26.6	26.5	0.59	0.49	0.57
	4	9.8	14.8	−1.75	0.25	0.10
	5	4.0	3.1	0.44	1.33	0.66
	6	1.6	0.0	1.34	2.27	0.20
	Sum	132.7	132.5			
Small ($n = 15$)	1	46.1	45.1	0.43	1.33	0.68
	2	32.5	31.5	0.41	0.87	0.69
	3	15.8	17.9	−0.58	0.10	0.57
	4	4.4	4.3	0.04	0.80	0.97
	5	0	0	—	—	—
	6	0	0	—	—	—
	Sum	98.8	98.8			

The predicted body size of each group member (assuming a maximum group size of 6) was calculated from the optimization model (based on the average body size difference between the first- and second-ranked fish in the group and the average summed length of the group members). When a group had no fish of a given rank, that rank was recorded as having an SL of 0 mm.

RESULTS

Verifying the assumptions and test of the model

The summed length of group members of *A. ocellaris* was significantly correlated with the area of the host *S. gigantea* (Pearson product moment correlation coefficient: $r = 0.723$, $T = 6.70$, $n = 43$, $P < 0.001$, 95% confidence interval: 0.540–0.841). The summed length could be predicted from the area of the host using a regression line (Summed length [mm] = 65.2444 + 0.0009 Anemone area [mm^2], $R^2 = 0.5233$, $F_{41} = 45.0$, $n = 43$, $P < 0.0001$]). There was a significant difference in γ among the size categories (*i*), but no substantial difference was found in δ (Table 1).

In *A. frenatus*, there was a significant difference in γ and in δ among the size categories (*i*). However, when the last category was excluded (because most groups consisted of only 3 fish), there was still a significant difference in γ, but no substantial difference was found in δ (Table 1). In *A. perideraion*, no substantial difference was found in either γ or δ among the size categories (Table 1).

Using γ, the predicted SLs of group members did not correspond to their actual average SLs for different ranks in the 3 host size categories of *A. ocellaris* (Figure 1). Using δ, however, there was no substantial difference between the predicted and average SLs of group members ordered by rank (Table 2).

Similarly, in *A. frenatus*, there was a substantial difference between the predicted and actual SLs of each group member using γ (Figure 2), but using δ, the predicted SL of a group member ordered by rank did not significantly differ from the actual average SL of the group member (Figure 2, Table 3). In *A. perideraion*, using δ, the predicted SL of a group member ordered by rank did not differ substantially from the average SL of the group member except for rank 4 (Figure 2, Table 3), where the predicted value was slightly lower than the 95% lower confidence interval of the observed value (Figure 2). Using γ, however, there was a large difference between predicted and actual SLs of group members ordered by rank, except for rank 3 (Figure 2, Table 3).

Nonlinear female fecundity and the better mating system for the dominant fish

Figure 3 shows well-fitted regression curves between gonad weight and SL in *A. frenatus* and *A. perideraion*. In order to estimate the functions between female fecundity and body size, α and β were obtained from the regression curves. Table 4 shows *F* and *M* calculated from body sizes obtained in the model and those calculated from SLs measured in situ. Calculated *F* was much larger than calculated *M* in all cases in the 3 species, and there were highly significant differences between *F* and *M* in the real fish (Table 4).

General tendency of the body size composition model

The body size composition model with δ indicated that for the same summed length ($\sum b_i$ or the same shelter size), larger δ caused relatively larger b_1 but smaller b_3 and smaller group size (Figure 4).

DISCUSSION

Determinants of body size composition under limited shelter space

The results of the present study indicated that body size composition can be determined by 2 factors: 1) the carrying capacity of the host and 2) the fixed body size differences

Table 3

Results of one-sample *t*-tests between predicted and observed body sizes (SLs in mm) of group members from rank 1 to rank 6 of *Amphiprion frenatus* and *Amphiprion perideraion*, which live in isolated single host anemones

				One-sample *t*-test				One-sample *t*-test		
Species	Size rank	Average body size	Predicted body size (using body size ratio, γ)	*t*	Effect size	*P*	Predicted body size (using body size difference, δ)	*t*	Effect size	*P*
A. frenatus (n = 26)	1	97.8	—	—	—	—	98.6	−0.43	0.08	0.67
	2	59.1	—	—	—	—	59.9	−0.35	1.53	0.73
	3	19.7	—	—	—	—	21.2	−0.84	0.16	0.41
	4	3.1	—	—	—	—	0	2.19	2.25	0.05
	5	0	—	—	—	—	0	—	—	—
	6	0	—	—	—	—	0	—	—	—
	Sum	179.7					179.7			
A. perideraion (n = 15)	1	66.1	45.5	11.3	2.93	<0.001	62.2	2.18	2.93	0.05
	2	50.3	34.6	7.32	1.89	<0.001	46.3	1.86	1.89	0.08
	3	26.5	26.3	0.03	0.01	0.975	30.4	−0.74	0.01	0.47
	4	6.9	20	−3.90	1.01	0.001	14.5	−2.26	1.01	0.04
	5	3.6	15.2	−4.30	1.12	<0.001	0.0	1.34	1.12	0.20
	6	0.0	11.6	—	—	—	0.0	—	—	—
	Sum	153.4	153.2				153.4			

The predicted body size of each group member (assuming a maximum group size of 6) was calculated from the optimization model (based on the average values of either body size ratio or body size difference between the largest and the second largest fish in the group and the average values of the summed length of group members). Only breeding groups were selected. When a group had no fish of a given rank, that rank was recorded as having a SL of 0 mm.

(δ) between group members adjacent in rank. The data on *A. ocellaris*, *A. perideraion*, and *A. frenatus* statistically supported the assumptions of the model, and there were no substantial differences between predicted values of body size and average body size of ordered group members in these species. Mitchell and Dill (2005) proposed the group structure hypothesis to explain how group size of the anemonefishes is determined. According to the hypothesis, the largest fish in a group retards the growth of subordinates, and consequently, the body size of the largest fish sets the upper limit of group size, irrespective of host size. The size of the largest fish might be able to determine the summed length of subordinates because the body size difference was a constant in the present study. In such a case, we can still use the model because it uses the summed length for the predictions. However, it is unclear why the largest fish determines the summed length of subordinates irrespective of host size, although it is logical that the carrying capacity of the host determines the summed length due to resource limitation. Coefficient of determination in the regression analyses suggested that unknown factors influenced the summed length. Sea anemones do not have a fixed morphology, so that the size, or carrying capacity, was difficult to estimate. New indices to express 3D anemone shapes may be necessary to predict the summed length correctly.

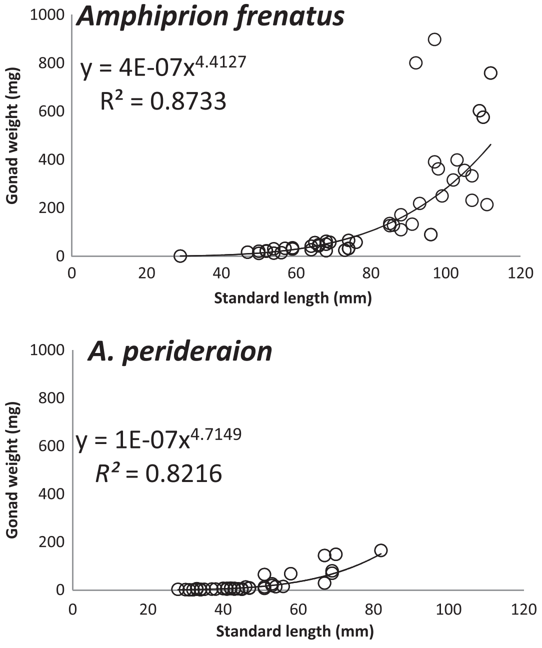

Figure 3
The relationships between body size (SL) and gonad weight of *Amphiprion frenatus* and *Amphiprion perideraion*. The predictions of nonlinear regression functions are shown.

Buston and Cant (2006) demonstrated that in *A. percula* (in a Papua New Guinean population), the body size ratio (not the body size difference) of group members adjacent in rank was constant. In *A. ocellaris* and *A. frenatus* in the present study, the size difference (not the size ratio) was constant, and the size difference and the size of shelter space determined the group body size compositions. The large size difference, as well as the large size ratio, may minimize costly conflicts among individuals that live in small groups (Buston et al. 2007; Buston and Zink 2009). Indeed, in *A. percula*, *A. ocellaris*, *A. frenatus*, and *A. perideraion*, the home ranges of subordinates are confined to single hosts, but few aggressive interactions were observed among group members (Hattori 1991, 1995, 2000, 2005; Buston and Cant 2006; Hattori A, unpublished data). In contrast, in *A. bicinctus*, *A. akallopisos*, and *A. clarkii*, the home ranges of which are not confined to single hosts (they often use multiple ones), aggressive interactions were frequently observed among neighboring individuals, and the body size difference was very small (Fricke HW and Fricke S 1977; Fricke 1979; Hattori and Yanagisawa 1991). This implies that the body size difference or ratio may vary with social structure. At present, it is not clear whether individuals actually maintain a specific size difference or ratio in a population

Table 4

Comparisons between *F* (expected reproductive success when the dominant fish is female in a monogamous mating system) and *M* (expected reproductive success when the dominant fish is male in a polygynous mating system) in the 3 *Amphiprion* anemonefishes, which inhabit single isolated hosts. Predicted body size (using γ or δ) and actual body size were used for the calculations

	Using predicted body size calculated with body size ratio (γ)			Using predicted body size calculated with body size difference (δ)			Using actual body size				Paired *t*-test		
Species	*M*	*F*	*F*/*M*	*M*	*F*	*F*/*M*	*M*	*F*	*F*/*M*	*n*	*t*	*P*	Effect size
Amphiprion frenatus	—	—	—	28.16	252.18	9.0	31.55	257.44	8.2	26	−13.23	<0.0001	87.0
Amphiprion perideraion	2.48	6.57	2.6	8.14	28.68	3.5	16.84	42.00	2.5	15	−8.39	<0.0001	11.6
Amphiprion ocellaris	—	—	—	3.28	11.04	3.4	4.71	12.89	2.7	43	−9.07	<0.0001	5.9

because strong statistical evidence has not been obtained. If the body size difference between adjacently ranked individuals is variable, the model cannot determine the group body size composition. This may limit the applicability of the model to other systems. Recent work on a protogynous angelfish *Centropyge bicolor*, which forms small groups with a size hierarchy but is less confined to shelters, revealed that where home ranges of group members somewhat spatially segregated within groups, body size differences between adjacently ranked individuals were small and groups were unstable (Ang and Manica 2010a, 2010b). Similar phenomena are known in several marine fishes (e.g., *Dascyllus aruanus*, Asoh 2003; *Trimma okinawae*, Manabe et al. 2007). Flexible body size differences between adjacent ranked group members may result from alternative reproductive strategies, including sex change of subordinates in unstable groups.

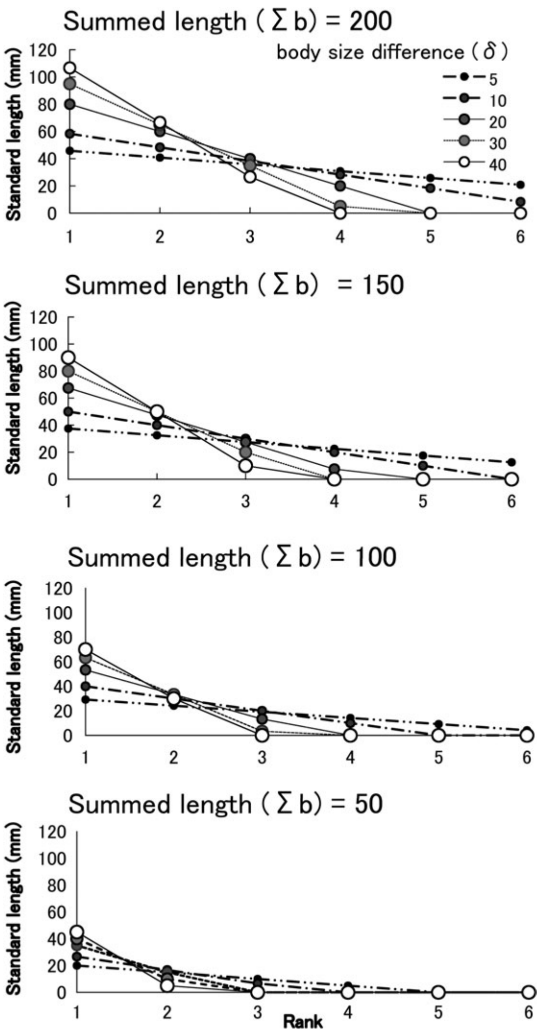

Figure 4
Body sizes of group members from rank 1 to rank 6 (assuming a maximum group size of 6) predicted from the optimization model based on the fixed body size difference among individuals adjacent in rank (δ) and the summed body length of group members (Σ*b*), which is assumed to be determined by the shelter size (carrying capacity of the host anemone).

Monogamy and high fecundity of the dominant fish under limited shelter space

Assuming large size differences (δ) between members adjacent in rank and nonlinear female fecundity functions, the model indicated that dominant fish would have higher reproductive success in monogamous systems than in polygynous systems in small groups under the EPP. In the present study, *A. frenatus* had the largest body size difference between the sexes, and most groups consisted of only 3 fish (Hattori 1991, 2005; Hirose 1995); the expected reproductive success of a monogamous dominant fish (*F*) was 8 times as large as that simulated for a polygynous dominant fish (*M*, Table 4). The other 2 species, *A. perideraion* and *A. ocellaris*, both had relatively small body size differences between adjacently ranked members, and the expected reproductive success of monogamous females (*F*) was significantly larger than that of polygynous males (*M*, Table 4).

Anemonefishes often inhabit densely distributed host anemones, where they are monogamous and size differences between the partners are very small. Why are they monogamous? In areas of high host density, the fish use multiple hosts, have cooperatively defended territories almost contiguous with those of others, and subadults have home ranges in the outskirts of pairs' territories (Fricke 1979; Moyer 1980; Ochi 1986, 1989a, 1989b; Yanagisawa and Ochi 1986; Kobayashi and Hattori 2006). After all, they do not have the EPP: Individuals are not confined to single hosts (except small juveniles) and large subadults, which are of reproductive size, pair with each other size-assortatively, and move between hosts to establish their territories (Ochi 1989a, 1989b; Hattori and Yanagisawa 1991; Hattori and Yamamura 1995). If they were polygynous, the dominant male might not have high fecundity due to the high cost of territorial defense. An example is the monogamous longnose filefish, *Oxymonacanthus longirostris*, in which males defend territories so as not to decrease females' feeding rates and fecundity (Kokita and Nakazono 1999).

In addition to anemonefishes, other habitat-specialist marine fishes that inhabit small sparsely distributed shelters often have monogamous mating systems (Whiteman and Côté 2004; Wong et al. 2005, 2008; Thompson et al. 2007). In some of these fishes, males are larger than females, and they show haremic groups where they inhabit large or densely distributed shelters (e.g., *D. aruanus*, Asoh 2003; *Caracanthus unipinna*, Wong et al. 2005; *T. okinawae*, Manabe et al. 2007). Although their group size may be dependent on the shelter size, the summed length of group members may be independent of it. In addition, the body size differences between adjacently ranked fish are small and/or variable (Asoh 2003; Wong et al. 2005; Manabe et al. 2007). In anemonefishes, the body size composition model of the present study indicated that where C was limited, larger body size differences resulted in relatively larger b_1 but smaller subordinates (Figure 4). Groups consisting of large females, small males, and smaller subordinates can be formed where 1) the carrying capacity of shelters determines the summed length (or biomass) of group members, probably because of intraspecific resource competition, 2) large body size differences (or ratios) between group members adjacent in rank are necessary for subordinates to avoid fatal eviction, and 3) fecundity increases nonlinearly in females. It should be noted that pair formation of this type does not require cooperative territorial defense, extended mate guarding, or biparental care of offspring.

Nonrandom pair formation and protandry under limited shelter space

Fricke HW and Fricke S (1977) suggested that protandry means that the larger member of a pair is always female, which ensures high fecundity of the pair. However, they did not see the degree of body size differences among adjacently ranked members in the small groups. When the size difference is small, a polygynous and protogynous system could be better for the dominant fish in terms of expected reproductive success. According to the SAH, protandry can occur in species with mating systems in which the expected reproductive success of males is less sensitive to their body size, whereas that of females increases with increasing body size (Ghiselin 1969; Charnov 1982; Warner 1988a, 1988b). In the case of random pair formation with respect to body size, large males cannot gain a mating advantage from a large body size (Ghiselin 1969; Charnov 1982; Warner 1988a, 1988b). However, the model presented here indicated that body size composition is predictable: that is, pair formation does not occur randomly. In the body size composition model with nonlinear female fecundity, the expected reproductive success via male function will not increase with body size, whereas the expected reproductive success of females increases exponentially with increasing body size, as suggested by Hattori (2005). This clearly supports the SAH. In the temperate anemonefish, *A. clarkii*, which inhabits high-density hosts to which this model does not apply, adults have pair territories and mate size-assortatively, so that sex changers are actually rare (Ochi 1989a, 1989b; Hattori and Yanagisawa 1991; Hattori and Yamamura 1995).

Besides fishes, some habitat-specialist gastropods, a parasitic isopod, and a shrimp that are confined to isolated hosts or shelters show protandry and form small groups that consist of large females, small males, and no or a few very small subordinates (e.g., the shelf limpet, *Crepidula norrisiarum*, Warner et al. 1996; the parasitic isopod, *Ichthyoxenus fushanensis*, Tsai et al. 1999; the coral-dwelling snail, *Coralliophila violacea*, Chen et al. 2004; the semiterrestrial shrimp, *Merguia rhizophorae*, Baeza 2010; the hingebeak shrimp, *Rhynchocinetes uritai*, Bauer and Thiel 2011). Although these small subordinates are often regarded as males due to their possession of a penis or appendix masculina, how they mate with females in small groups is unknown. It is not clear whether males' fecundity is independent of body size (Warner et al. 1996; Tsai et al. 1999; Chen et al. 2004; Bauer 2006; Collin 2006; Baeza 2010). If the small subordinates are not functional males, then ecological circumstances are very similar to the anemonefishes in which small subordinates have ambosexual gonads that have a few spermatocyte cysts, spermatids, and sperm but are not functional males (Hattori 1991, 1994; Hattori and Yanagisawa 1991). The combination of the body size composition model and nonlinear female fecundity functions may be able to explain their protandry.

Conclusions

The results of the present study indicated that body size composition of anemonefishes inhabiting single isolated hosts is determined by the carrying capacity of the host and the body size differences among group members adjacent in rank. This body size composition is presumably a strategy for coexisting with limited shelter space. In these systems, we can predict the body size composition of a new pair; pair formation cannot be regarded as random with regard to body size. Incorporating nonlinear female fecundity functions into the model, monogamy and protandry were predicted to be better than polygyny and protogyny in terms of expected reproductive success for the dominant fish. The body size composition model with nonlinear female fecundity supports the SAH and explains the function of monogamy and protandry when shelter space is limited.

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