

NOTE

Species and sexual differences in human-oriented behavior of anemonefish at Okinawa Island, Japan

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ABSTRACT: Anemonefish are one of the most popular and frequently encountered fish groups by divers, and it is necessary to know how the approaches of humans affect them to maintain a balance between utilization and conservation in coral reefs. Agonistic behaviors toward divers were analyzed for 130 individuals of 4 species of anemonefish (*Amphiprion ocellaris*, *A. clarkii*, *A. perideraion*, and *A. frenatus*) on the west coast of Okinawa Island, Japan. Divers' approaches differently affected each species of anemonefish, even though the fish inhabited the same area. The duration of agonistic behavior in female *A. frenatus* was observed to be longest of the 4 species. Sexual differences in agonistic behavior were prominent in *A. perideraion* and *A. frenatus*. When human observers stayed at a distance of 2.0 m from host anemones, no responses were observed in any of the species. Agonistic behavior increased when the distance of observers decreased to 1.0 m in females of *A. frenatus* and *A. clarkii*, and to 0.3 m in *A. perideraion*. During observation periods of 3 min, anemonefish behavior did not return completely to pre-observation behavior. We hypothesize that frequent human approaches could affect behavior and stress levels of some species of anemonefish. Thus, the distance and frequency of human approaches around anemonefish may need to be considered in order to protect anemonefish, as they are popular and commonly observed in diving tourism.

KEY WORDS: Agonistic behavior · *Amphiprion* · Divers · Conservation · Nature-based tourism

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1. INTRODUCTION

Numbers of diving tourists are increasing on many coral reefs around the world (e.g. Musa & Dimmock 2012). While diving is a nature-based tourism activity with significance in environmental education, coral reefs are often negatively affected in places frequently used by divers (e.g. Roche et al. 2016). The negative influence of divers on the survival and behavior of fish has been demonstrated (e.g. Watson & Harvey 2007, Titus et al. 2015, Nunes et al. 2018).

Anemonefish (Pomacentridae) strongly depend on specific sea anemone individuals as hosts and defend these host anemones as a territory from conspecific and heterospecific intruders (Moyer & Sawyers 1973). Interspecific territorial behavior has been observed in reef fish, and its significance has been discussed (e.g. Myrberg & Thresher 1974, Itzkowitz

1990). As territory defense involves large energy expenditures, territory owners should discriminate against intruders and change their territorial behavior based on the incurred disadvantage, such as predation risk or resource competition (e.g. Matsumoto & Kohda 2004, Lehtonen et al. 2010). An experimental study using jewel damselfish *Plectroglyphidodon lacrymatus* indicated that experiences or sensitization toward the intruding species might play an important role in determining the degree of agonistic behavior (Di Paola et al. 2012).

Territorial defense by *Amphiprion clarkii* is known to be directed toward human observers (Moyer & Sawyers 1973). Therefore, it is expected that frequent approaches and other actions by divers, may affect anemonefish behavior. In 2 other species of anemonefish (*A. mccullochi* and *A. latezonatus*) in eastern Australia, variation and repeatability of

behaviors such as boldness, sociability, and aggression greatly varied between species and social ranks (Wong et al. 2017). Therefore, the reactions of fishes to divers may differ among species and social ranks, even in the same local area.

Okinawa Prefecture in southern Japan is visited by 9.57 million tourists annually, of whom over 600 000 participate in diving activities (SCUBA diving, snorkeling), and both of these numbers are increasing rapidly (Okinawa Prefectural Government 2017). Anemonefish are distributed in shallow waters and reef slopes and are one of the fish groups that are most popular and frequently encountered by divers around the Ryukyu Archipelago. In order to maintain a balance between utilization and conservation in coral reefs, it is necessary to know how the approaches of humans affect each sex and species of anemonefish. In the present study, we established study sites on the west coast of Okinawa Island to answer the following questions: (1) Are reactions toward humans different between species of anemonefish? (2) Do anemonefish behavioral responses differ between sexes? (3) How does anemonefish behavior change with distance to humans? (4) Can anemonefish acclimate to human approaches?

2. MATERIALS AND METHODS

2.1. Study sites and recordings

We selected study sites that are known to be popular with divers, referring to previous studies conducted for the community structure of anemonefish (Hayashi et al. 2018). Four species of anemonefish common at these study sites, *Amphiprion clarkii*, *A. frenatus*, *A. ocellaris* and *A. perideraion*, were selected for the present investigation (Hayashi et al. 2018). All 6 study sites are popular dive locations for tourists located on the west coast of Okinawa I. (S1: 26° 26' 29" N ~ 26° 19' 17" N, 127° 47' 22" E ~ 127° 44' 53" E; see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m615p219_supp.pdf).

One observer stayed in front of each host anemone and recorded the behavior of anemonefish by using a video camera (PT-055, Olympus). We selected host colonies that included a breeding adult pair based on their size and body color (Fautin & Allen 1992, 1997) as study targets. To avoid any unnecessary disturbance, we did not examine colonies in which adult pairs were caring for eggs. The ratio of total length in males and females was measured from still video images when individuals were lined up on the same plane. In each

colony, the size of the adult female was set to 1, and that of the adult male was quantified accordingly. These values were averaged for respective species and were compared among species. Video recording was conducted between 10:00 and 14:00 h.

2.2. Effect of approach distance

From May to June 2018, we recorded the behavior and position of anemonefish. The total recorded time was 195 min (3 min for each of 65 pairs). Initially, the observer stayed 2.0 m away from a host anemone and the behavior of the adult male and female in a colony was recorded for 60 s. Next, the observer slowly narrowed the distance to 1.0 m from the host, and again recorded fish behavior for 60 s. Finally, the distance from the host anemone was slowly reduced to 0.3 m, and the behavior was again recorded for 60 s.

The acquired video material was stopped every second and the behavior and the position of each individual fish was noted. Based on the ethogram of *Amphiprion ocellaris* (Wong et al. 2013), we defined 7 types of behavior in our study, 2 of which were designated as agonistic towards humans (Table 1). The duration of agonistic behaviors was counted as the time (s) during which the fish behaved in a lateral flickering or forward orientation manner within 60 s. The position of each fish was classified into 1 of 3 categories: inside, under, or outside the host anemone. When the anemonefish was hiding within or touching the tentacles of a host anemone, the position was assigned as 'inside.' When the anemonefish was swimming away from the tentacles, the position was 'outside.' Hiding under the tentacles or body wall of the anemone was classified as 'under' the host anemone. We calculated the time during which the fish remained at each position within 60 s. Recording was conducted once per survey for each colony.

Sexual differences in the duration of agonistic behavior and time spent outside the host anemone during 60 s were tested by Mann-Whitney *U*-test. The differences in duration of agonistic behavior and in the position of anemonefish at each distance from the observer were tested by Friedman test. In cases where differences were significant, we conducted Dunn-Bonferroni post hoc tests.

2.3. Effect of elapsed time

In July 2018, we recorded the behavior of anemonefish for 180 s at a distance of 0.3 m from the host

Table 1. Ethogram of behaviors exhibited by anemonefish

Category	Behavior	Description
Agonistic behavior toward observers	Lateral flicking	Crossing vigorously to the left or right in front of human observers
	Forward orientation	Swimming towards human observer
Maintenance	Leave	Leaves contact of host anemone
	Out of sight	Enters anemone and cannot be seen; or fish cannot be seen because it moves under or behind anemone
	Contact with host anemone	Enters host anemone
Other	Chase	Chasing anemonefish in same colony or other fish species
	Move	Move to another host anemone

anemone. The total recorded time was 105 min (3 min for each of 35 pairs). The recorded video was stopped every second, and the duration of agonistic behavior and the position of individuals (inside, under, or outside the host anemone) were counted. To assess whether the duration of agonistic behavior decreased over time, data were compared between 3 time periods: the first, second, and third (final) minute of observation. Definitions of agonistic behavior and positions of anemonefish were the same as mentioned above.

Sexual differences in the duration of agonistic behavior and time spent outside the host anemone during 60 s were tested by Mann-Whitney *U*-test. The changes over time in the duration of agonistic behavior and in the amount of time that the fish remained outside of an anemone were tested for each sex of each species by Friedman test. If there was a significant difference, we conducted Dunn-Bonferroni post hoc tests.

3. RESULTS

3.1. Effect of approach distance

In total, 17 pairs of *Amphiprion ocellaris*, 17 pairs of *A. clarkii*, 16 pairs of *A. perideraion*, and 15 pairs of *A. frenatus* were examined (Table S1 in the Supplement). When the female total length was standardized to 1.00, male length was (mean \pm SD) 0.75 ± 0.09 in *A. ocellaris*, 0.94 ± 0.07 in *A. clarkii*, 0.69 ± 0.11 in *A. perideraion*, and 0.55 ± 0.12 in *A. frenatus*.

When observers stayed at a distance of 2.0 m from host anemones, both sexes of all species of anemonefish showed no detectable agonistic behavior towards the divers (Fig. 1A). We found species and sexual differences in reactions when observers were 1.0 m from host anemones. When observers approached to

0.3 m from host anemones, agonistic behavior was recorded in both sexes of all species. In *A. frenatus* and *A. perideraion*, the duration of agonistic behavior toward humans was significantly longer in females than in males (Fig. 1A, Table 2A).

Statistical tests on the effect of distance indicated that duration of agonistic behavior increased with decreasing distance to humans in both sexes of *A. clarkii*, *A. perideraion*, and *A. frenatus*. However, both sexes of *A. ocellaris* did not change their behavior significantly. Multiple comparisons by Dunn-Bonferroni post hoc tests indicated that female *A. clarkii* and female *A. frenatus* increased their agonistic behavior at 1.0 m distance, and both sexes of *A. perideraion* increased their agonistic behavior at 0.3 m from host anemones (Fig. 1A).

The swimming positions of anemonefish over 60 s are indicated in Fig. 1B. *A. clarkii* swam outside of host anemones most frequently, while *A. ocellaris* was most commonly inside host anemones. In *A. frenatus*, females swam outside anemones for a longer time than males (Fig. 1B, Table 2B).

Statistical tests on the effects of distance indicated that the amount of time spent outside anemones increased with decreasing distance from human observers in both sexes of *A. frenatus* and in female *A. perideraion*. Multiple comparison tests indicated that female *A. frenatus* increased the time outside anemones at 1.0 m distance, but male *A. frenatus* and female *A. perideraion* only increased the time outside anemones at 0.3 m from observers (Fig. 1B).

3.2. Effect of elapsed time

In total, 10 pairs of *A. ocellaris*, 8 pairs of *A. clarkii*, 9 pairs of *A. perideraion*, and 8 pairs of *A. frenatus* were examined (Table S1). These pairs were a subset of the 65 pairs used in the previous experiment. The

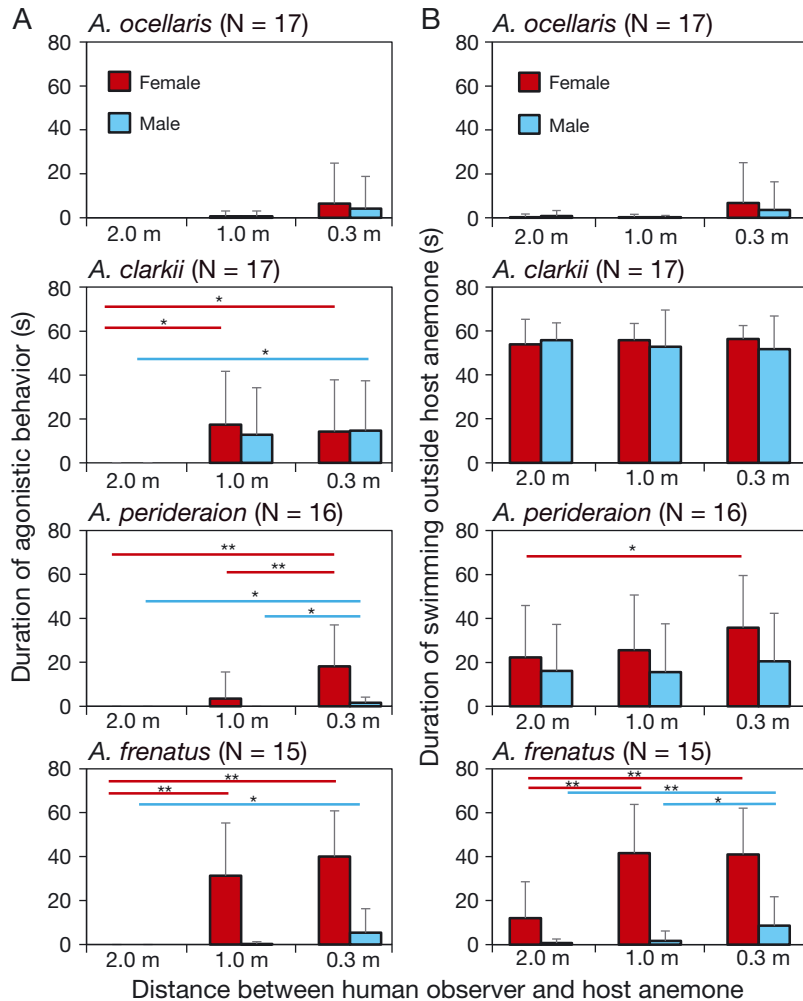


Fig. 1. Differences in observed behavior and positional changes of anemonefish within 60 s during the approach of a diver at 2.0, 1.0, and 0.3 m from host anemones. Bars indicate means and SD. Significant differences by Dunn-Bonferroni post hoc test are indicated by asterisks; * $p < 0.05$, ** $p < 0.01$

Table 2. Mann-Whitney U -test statistics for sexual differences in the effect of distance between a human observer and the host anemone on anemonefish behavior. There was no detectable agonistic behavior at 2.0 m from the diver. Significant differences between the sexes are indicated by asterisks (** $p < 0.01$)

Species	2.0 m		1.0 m		0.3 m	
	U	p	U	p	U	p
(A) Agonistic behavior						
<i>Amphiprion ocellaris</i>	–		144.50	1.00	144.00	0.98
<i>A. clarkii</i>	–		127.00	0.50	148.50	0.87
<i>A. perideraion</i>	–		112.00	0.15	56.00	<0.01**
<i>A. frenatus</i>	–		32.00	<0.01**	18.50	<0.01**
(B) Swimming position						
<i>A. ocellaris</i>	153.00	0.55	144.50	1.00	142.00	0.90
<i>A. clarkii</i>	140.00	0.86	138.50	0.80	122.50	0.37
<i>A. perideraion</i>	109.50	0.47	92.50	0.17	84.50	0.10
<i>A. frenatus</i>	54.00	<0.01**	12.00	<0.01**	22.00	<0.01**

duration of agonistic behavior differed significantly between sexes in *A. perideraion* and *A. frenatus* (Table 3A). Durations of agonistic behavior were longer in females than males during 3 min for *A. frenatus* and during 2 min for *A. perideraion* (Fig. 2A, Table 3A).

The durations of agonistic behavior for both sexes of *A. clarkii* and *A. ocellaris* and male *A. frenatus* did not differ significantly over time. According to multiple comparison analyses, agonistic behavior gradually decreased over 3 min in female *A. frenatus*, while it decreased after the first minute for both sexes of *A. perideraion* (Fig. 2A).

The duration of swimming outside host anemones differed between sexes in *A. frenatus* for the first 2 min (Fig. 2B, Table 3B). It significantly decreased with time in female *A. perideraion* and female *A. frenatus*. According to multiple comparison analyses, in female *A. perideraion*, the amount of time spent swimming outside anemones significantly decreased after the first minute, while it decreased gradually over 3 min in female *A. frenatus* (Fig. 2B).

4. DISCUSSION

Our study indicates that approaches by SCUBA divers induced territorial defense behavior in 4 species of anemonefish. Territorial behavior towards heterospecific intruders is common in reef fish where many species co-exist in a local area (Myrberg & Thresher 1974). For example, jewel damselfish are more aggressive to species that are abundant and to repeated territorial infringements (Di Paola et al. 2012). In Okinawa I., divers often invade the territory of anemonefish, and it seems that anemonefish therefore showed agonistic behavior toward observers. The responses of anemonefish to humans may differ in places where the frequencies of human contact are different. In the future, local differences in the agonistic behaviors toward humans must be investigated to better understand the mechanisms for decision making in territory defense.

Table 3. Mann-Whitney *U*-test statistics for sexual differences in the effect on anemonefish behavior of the duration of a human observer's presence (3 min in total) for (A) duration of agonistic behavior and (B) duration of swimming outside of the host anemone during 60 s. Significant differences between the sexes are indicated by asterisks (**p* < 0.05, ***p* < 0.01)

Species	1 min		2 min		3 min	
	<i>U</i>	<i>p</i>	<i>U</i>	<i>p</i>	<i>U</i>	<i>p</i>
(A) Agonistic behavior						
<i>Amphiprion ocellaris</i>	50.00	1.00	44.50	0.50	40.00	0.15
<i>A. clarkii</i>	26.00	0.52	27.50	0.61	25.50	0.41
<i>A. perideraion</i>	8.00	<0.01**	22.50	<0.05*	31.50	0.15
<i>A. frenatus</i>	5.00	<0.01**	6.00	<0.01**	9.50	<0.01**
(B) Swimming position						
<i>A. ocellaris</i>	50.00	1.00	44.50	0.50	40.00	0.15
<i>A. clarkii</i>	20.50	0.21	25.00	0.45	29.00	0.74
<i>A. perideraion</i>	21.50	0.09	27.50	0.24	26.50	0.20
<i>A. frenatus</i>	2.00	<0.01**	7.00	<0.01**	15.00	0.06

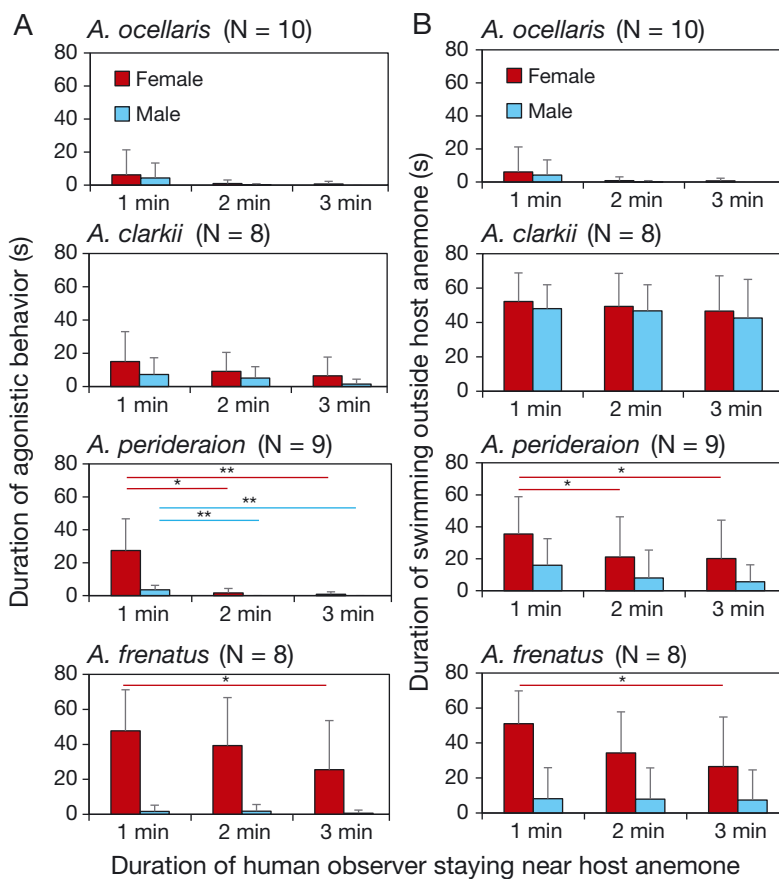


Fig. 2. Differences in observed behavior and positional changes of anemonefish every 60 s during the 3 min approach of a diver at 0.3 m from host anemones. Other details as in Fig. 1

Our study also indicates that approaching divers may have variable effects on different species of anemonefish, even when they inhabit the same area. Among the 4 investigated species, the duration of

agonistic behavior in female *Amphiprion frenatus* was highest. *A. frenatus* is a specialist which settles only in 1 species of host anemone, *Entacmaea quadricolor*, although this host can be used by all species of anemonefish. In Okinawa I., *E. quadricolor* is used by *A. clarkii*, the most abundant anemonefish species (Hayashi et al. 2018). *A. frenatus* may be able to monopolize *E. quadricolor* hosts via high levels of inter-specific aggression, which may in turn reflect high levels of aggressiveness towards divers.

There were differences between sexes in the duration of agonistic behavior in *A. frenatus* and *A. perideraion*. Among the 4 species, sexual differences in total length were largest in *A. frenatus* and second largest in *A. perideraion*. In anemonefish pairs, males spend more time on egg care than females (e.g. Ross 1978). Labor division in breeding anemonefish may be related to sex differences in body length and to the frequency of agonistic behavior, although sexual differences in breeding behavior among different species have not been fully investigated.

In female *A. frenatus* and *A. clarkii*, durations of agonistic behavior increased when the distance from observers decreased to 1.0 m. *A. perideraion*, especially females, showed agonistic behavior when the distance from observers was reduced to 0.3 m. Duration of agonistic behavior did not change in either sex of *A. ocellaris* with distance but was always relatively short. The maximum lengths of *A. clarkii* and *A. frenatus* were larger than those of *A. perideraion* and *A. ocellaris* (Fautin & Allen 1992). Feeney et al. (2019) conducted phylogenetic analyses of the evolutionary history of fish-anemone mutualisms and speculated that the small body size of fish promotes obligate mutualism. Body length in fish has also been related to the frequency of agonistic behavior towards predators (Goiran & Shine 2015). We hypothesize that the distance

and frequency of agonistic reactions toward divers may be related to anemonefish body sizes.

None of the species showed agonistic behavior when divers stayed at 2.0 m from host anemones. Our

study indicates that recreational divers should be permitted to approach no closer than 2.0 m from host anemones in order to maintain a balance between conservation and utilization. As suggested by Roche et al. (2016), the role of dive guides is necessary to ensure the compliance of leisure divers. In addition, limiting diver numbers should also be considered.

When human observers remained close (0.3 m) to host anemones, the duration of agonistic behavior did not change, except in female *A. frenatus* and in both sexes of *A. perideraion*. Observers stayed only for 3 min, and anemonefish behavior did not return to fully pre-observation behavior during this time span. In a previous study examining *A. chrysopterus*, no apparent acclimation occurred even when observers were present for 25 min (Nanninga et al. 2017). Unlike mobile fish species, site-attached species cannot leave an area disturbed by humans, and thus the continued presence of human observers might affect the fitness of anemonefish, for example through suppression of feeding, egg care, or courtship behaviors (Nanninga et al. 2017). Long-term monitoring is necessary to understand how agonistic behaviors towards humans affect the survival and breeding of each species of anemonefish.

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