Behavioral observation of a facultatively symbiotic goby at a shrimp burrow entrance

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Abstract: Ecological studies of the facultatively symbiotic goby Acentrogobius sp. 2 (sensu AKIBITO et al., 2013) are important because there is limited knowledge on the facultative relationship in goby-shrimp symbiosis in the Pacific. The present study surveyed the surface activity of Acentrogobius sp. 2 around the burrows of snapping shrimp (Alpheus brevicrissa) by quantitative observation on a tidal flat during high tides in southern Japan. Acentrogobius sp. 2 used the area in front of the burrow entrance for approximately 30% of the 10-min observation period only. Acentrogobius sp. 2 sometimes went farther than 10 cm from the burrow entrance, but most gobies returned to the burrow entrance. Surveys conducted at low tides confirmed that the goby showed surface activity in tidepools, but with a reduced time than that at high tides. The burrow-retreating bouts by the goby were triggered by approaching omnivorous and carnivorous fish and crab species. Future studies on shrimp burrow use by closely related Acentrogobius species may elucidate the evolutionary process of the facultative relationship of this genus.

Keywords: goby-shrimp symbiosis, facultative relationship, Alpheus brevicrissa, tidal flat

1. Introduction

A variety of fish can construct a burrow in sediment, which is used for predator avoidance, survival, feeding, reproduction, and egg incubation (ATKINSON and TAYLOR, 1991; GONZALES et al., 2008; Dinh et al., 2014). Other fishes (mainly gobies) use the burrows created by invertebrates (mainly crustaceans) for the same reasons as burrowing fishes (ATKINSON and TAYLOR, 1991; KARPLUS, 2014). The relationship between gobies and crustacean burrows is diverse; several gobies are known as commensals (Henmi et al., 2018; INUI et al., 2018; Henmi et al., 2020b) and the others are mutualists (KARPLUS and THOMPSON, 2011; Hou et al., 2013; Thompson et al., 2013; Kohda et al., 2017; Cropp and Norbury, 2018).

The relationship between gobies and alpheid shrimps is one of the best-studied cases of marine mutualism (KARPLUS, 2014). Over 120 goby-shrimp interactions are thought to be obligate, where the goby and the shrimp are contingent upon each other and are never found without their partners (Thompson, 2004, 2005; Karplus, 2014).
and Thompson, 2011; Karplus, 2014). In goby-shrimp mutualism, the alpheid shrimp of the genus Alpheus constructs and maintains the burrow that the goby also lives in. The goby benefits from the use of the burrow as a shelter from predators, and the shrimp benefits from warning signals of approaching predators by the goby through tactile communications (Karplus and Thompson, 2011; Karplus, 2014). Additionally, the goby’s feces have been suggested as an important food item for shrimp in certain cases (Kohda et al., 2017). It is also known that gobies mate and incubate eggs in shrimp burrows (Yanagisawa, 1982; Karplus, 2014).

Facultative relationships between gobies and alpheid shrimps are reported in five cases, where they may gain advantages from their partner, but they can survive without them (Karplus, 2014; Lyons, 2013). Facultative relationships have been studied in the Atlantic where the A. florianus burrow was used by the obligate goby, Nes longus, and facultative gobies, Ctenogobius saepellence, and Bathygobius curacao (Karplus, 1992; Randall et al., 2005; Kramer et al., 2009; Lyons, 2013, 2014a, b). However, there is limited knowledge of the facultative relationship in goby-shrimp symbiosis in the Pacific. The present study focused on the relationship between the goby Acentrogobius sp. 2 (sensu Akhito et al., 2013) and the snapping shrimp (A. brevicristatus) living in a tidal flat in Japan. The goby Acentrogobius sp. 2 was formerly recognized as ‘A. pflaumii’ and as a facultative goby by Yanagisawa (1978). Senou et al. (2004) recognized three morphs for this species, namely Acentrogobius sp. A, Acentrogobius sp. B, and Acentrogobius sp. C, which were subsequently named as Acentrogobius sp. 2, A. virgatulus, and A. pflaumii, respectively, in the revision reported by Akhito et al. (2013). These species are differentiated genetically (Matsui et al., 2012b), and their habitats are also different (Senou et al., 2004; Horinouchi, 2008; Matsui et al., 2012a). Acentrogobius sp. 2 prefers a shallow muddy bottom from the intertidal zone to a depth of approximately 2 m with a wide salinity range (Horinouchi, 2008; Matsui et al., 2012a; Koyama et al., 2017). In contrast, A. pflaumii inhabits deeper areas (5-30 m) with high salinity and A. virgatulus inhabits intermediate areas between Acentrogobius sp. 2 and A. pflaumii (Horinouchi, 2008; Matsui et al., 2012a).

Acentrogobius sp. 2 and A. virgatulus are known to use Alpheus shrimp burrows (Senou et al., 2004; Yoshigou, 2009), whereas no information is available on the symbiotic relationship between A. pflaumii and alpheid shrimps. Behavioral observations of these gobies around the shrimp burrows are scarce. Yanagisawa (1978) reported that ‘A. pflaumii’ had a facultative relationship with alpheid shrimps; moreover, its association with the shrimp burrow seems rather weak and the goby often swim away from the approaching diver without retreating into the shrimp burrow. However, it is not known which of the three species of ‘A. pflaumii’ Yanagisawa (1978) studied. In the case of A. virgatulus, the results of field manipulative experiments in the subtidal area suggest that the goby-shrimp relationship may be weak (Horinouchi, 2007). To date, two studies have quantitatively reported Acentrogobius sp. 2 and A. brevicristatus relationships. Koyama et al. (2017) have suggested that Acentrogobius sp. 2 is facultatively associated with A. brevicristatus and A. dolichodactylus, based on generalized linear models of distributional data in an estuary in southern Japan. Henmi et al. (2020a) have confirmed that the A. brevicristatus burrow is used by Acentrogobius sp. 2 in mesocosm experiments; however, they have suggested that the goby may have a possible negative effect on the burrowing activity of
the shrimp. In contrast to the obligate goby, which spawn eggs in shrimp burrows, *Acentrogobius* sp. 2 and *A. virgatulus* spawn eggs under shell fragments or stones (Inui et al., 2011).

The present study surveyed the surface activity of *Acentrogobius* sp. 2 around the burrows of *A. brevicristatus* via quantitative observation on a tidal flat during high tide and low tide in southern Japan. In this paper, we describe and compare the pattern of shrimp burrow use by *Acentrogobius* sp. 2 between high and low tides. The aim was to bridge the information gap of facultatively symbiotic goby between the Atlantic and the Pacific. Another aim of this study was to widen the knowledge on the behavior of goby living in soft-substrate tidepools. Recent studies collectively show the importance of tidal flats and tidepools as nursery ground and/or permanent habitat for gobies in Japan (Okazaki et al. 2012; Kanou et al., 2018; Kunishima and Tachihara, 2020). However, to the best of our knowledge, this is the first quantitative study of the surface activity of goby-shrimp symbiosis in an intertidal area. Analyses of behavioral patterns of the shrimp are beyond the scope of this study and will be published elsewhere.

2. Materials and Methods

Study site

This study was conducted on a tidal flat in the Uranouchi Inlet (33°25’37.4" N, 133°25’58.4" E), Kochi Prefecture, southern Pacific side of Japan. Behavioral observations of *Acentrogobius* sp. 2 around the shrimp burrow were analyzed at high tides from September to October 2017 (4 cases), October to November 2018 (9 cases), and September 2019 (4 cases). Behavioral observations at low tides were conducted in tidepools of the same tidal flat from September to October 2016 (16 cases) and September 2017 (2 cases). Although the study extended for as long as four years, no evident environmental changes were observed at the study site. This study was conducted during non-reproductive periods of the goby as reported by Inui et al. (2011) and Matsui et al. (2014) because the goby may have different behavioral tendencies in the reproductive season, such as using shell fragments for spawning nests. The surface water temperature off the fishery station of the Kochi Prefecture near the observation site (at a distance of 1.5 km) in 2016 to 2019 was lowest in February (average 13.3 °C) and highest in August (average 30.1 °C) with a salinity usually of 28–34, except for several months as low as 7 in salinity after a typhoon or heavy rain (Kochi Prefecture, 2020). During the observation periods, the surface water temperature ranged from 20–29 °C with a salinity of 20–33 (Kochi Prefecture, 2020). The burrow of *A. brevicristatus* is long but shallow, with several funnel-shaped openings and short cul-de-sac branches (Hemmi et al., 2017). The burrow openings used by *Acentrogobius* sp. 2 were randomly selected to observe the goby surface activity (Fig. 1a). Care was taken not to observe the burrow more than once by mapping the place of the observed burrow every year. The observation area was 40 × 40 cm² with a burrow opening at the center. The recording was performed for 15 min using a video camera (RICOH WG-M1 or GoPro Hero5 Black) set near the observation area with a tripod (approximately 50 cm high; Fig. 1b) and the first 5 min were excluded as domestication time. The water depth was approximately 60–80 cm at high tides and approximately 3–5 cm at low tides.

Surface activity of the goby

The observation area was separated into nine positions similar to, but smaller than, that reported by Karplus (1992) and Lyons (2014a; Fig. 1c). The area on the inner ring (positions A to D)
Fig. 1 (a) *Acentrogobius* sp. 2 in front of the burrow of *Alpheus brevicristatus*. The shrimp is throwing sediment from inside the burrow. (b) Observation of an *A. brevicristatus* burrow (circle) at high tide. The vertices of the observation square are marked with ribbons. (c) Positions used to quantify goby location and burrow use in goby-shrimp association. Position A represents the area into which shrimp emerge from the burrow. The center (black) represents the burrow hole. The arrow indicates direction of the burrow opening. The black fan-shape area represents the entrance to the burrow.

was within 10 cm of the burrow opening. Position A is known as the main surface activity zone for the obligate goby and shrimp (Karplus, 1992; Lyons, 2014a), and a trench of approximately 2 cm depth was observed owing to the shrimps’ bulldozing behavior (Yanagisawa, 1984; Kohda et al., 2017). Positions E to H indicated the area between 10 and 20 cm from the burrow opening. Position I comprised the area over 20 cm from the burrow entrance. The time spent by the goby (seconds) was determined on a monitor (Dell Inc. U2720QM) to which a clear sheet drawing nine positions was attached. We counted the number of retreats to the shrimp burrows by the gobies and the intruding fishes and invertebrates to the observation area. Owing to the limitation of video camera resolution, a behavioral association between the goby and the shrimp (such as shrimp antennal contact or goby tail flicks) was not observed. Time spent inside the burrow, on positions A (the activity zone), B to D (within 10 cm of the burrow opening, except for A), E to H (the area between 10 and 20 cm), and I (the area over 20 cm), and the number of retreats were compared between high and low tides (n = 17 at high tide, n = 18 at low tide), using t-tests after log (x + 1) transformation (JMP 14.3).

3. **Results**

At both tides, all the goby (n = 17 at high tides, n = 18 at low tides) went out from the shrimp burrow and showed surface activity. At high tides, seven gobies stayed within 10 cm from the burrow (positions A to D) during the observation period. Among the ten gobies that went farther than 10 cm (positions E to I), nine returned to position A within the 10-min observation period. At low tides, eight gobies stayed within 10 cm from the burrow (positions A to D) during the observation period. Among ten gobies that went farther than 10 cm (positions E to I), six gobies returned to position A within the observation period.

The goby was outside the burrow for 86% and 57% of the 10-min observation period at high tides (n = 17) and low tides (n = 18), respectively. The mean time (± standard error) spent by the goby at each position is presented in Fig. 2. At high tides, the goby stayed for a long time at position A (31%) and positions B to D (38%), followed by residence inside the burrow (14%),
Behavioral observation of *Acentrogobius* sp. 2

Fig. 2  Duration of goby remained in each position around the burrow (see Fig. 1c) and inside burrow at high and low tides. Data are presented as mean ± SE. The asterisks * and ** indicate significant differences between the tides at 0.05 and 0.01 significance levels, respectively.

and at position I (10%), and positions E to H (7%). At low tides, the goby stayed for a long time inside the burrow (43%) and at position A (27%), followed by positions E to H (13%), position I (11%), and positions B to D (6%). The time spent by the goby inside the burrow was significantly shorter (*t* = 2.16, *p* = 0.004) and significantly longer at positions B to D (*t* = 5.03, *p* < 0.001) at high tides than that at low tides. Time spent in the other areas was not significantly different between tides (position A, *t* = 1.40, *p* = 0.174; positions E to H, *t* = 0.39, *p* = 0.696; position I, *t* = 0.01, *p* = 0.993).

Seven and ten gobies retreated into the burrow at high tides and low tides, respectively, with insignificant mean frequencies of 0.4 and 1.1 (*t* = 1.78, *p* = 0.09). At high tides, seven gobies retreated once; at low tides, five gobies retreated thrice and five gobies retreated once. Four species of fish, namely *Gerres equulus*, *Acanthopagurus shlegelii*, *Terapon jarbua*, and *Takifugu niphobles* intruded the observation area at high tides. In contrast, the mudskipper (*Periophthalmus modestus*) and four species of crabs, namely *Philyra pisum*, *Macrothelphalmus banzai*, *Hemigrapsus takanoi*, and *Gaetice depressus*, appeared in the area at low tides. Three burrow-retreating bouts by the goby were triggered by *G. equulus* and *T. jarbua* approaching the goby at high tides, whereas three bouts were triggered by *P. modestus*, *M. banzai*, and *H. takanoi* at low tides.

4. **Discussion**

The benefit of the goby on the goby-shrimp association is that the goby can use the burrow of shrimps as a shelter to avoid predators (Karpplus and Thompson, 2011; Karpplus, 2014). At high tides, *Acentrogobius* sp. 2 used shrimp burrows when approached by *G. equulus* and *T. jarbua*, which are known omnivores or carnivores (Horinouchi and Sano, 2000; Nanjo et al., 2008; Yoknoi et al., 2019). Yanagisawa (1984) also described that *T. jarbua* triggered the retrieval of *Amblyeleotris japonica*, the obligate goby symbiotic with the *A. bellulus* burrow. Most
Acentrogobius sp. 2 individuals returned to position A, which was in front of the shrimp burrow opening, after going farther than 10 cm, suggesting that the goby frequently used the shrimp burrow as a shelter. In the Atlantic, the facultatively symbiotic gobies C. saeppellence and B. curacao used the A. floridanus burrow, but C. saeppellence also used empty burrows, shells, and other structures for predator avoidance (Karpus, 1992; Randall et al., 2005; Kramer et al., 2009; Lyons, 2013). The ability to use other structures for shelter by Acentrogobius sp. 2 is a prospect for future research. The behavior of this goby using shells and other structures as a reproduction site (Inui et al., 2011) may suggest its ability to use a wide variety of shelters.

The activity area of Acentrogobius sp. 2 was wider than the known range of the obligate goby N. longus. Acentrogobius sp. 2 used position A for approximately 30% of the observation period and went farther than 10 cm from the burrow entrance for approximately 20%, whereas N. longus stayed at position A for approximately 85% of the observation period (Lyons, 2014a). The facultative symbiotic goby C. saeppellens stayed at position A for approximately 30%, similar to the result of this study, but the goby switched the shrimp partner often and used alternative shelters (Lyons, 2014a). Frequent switching of the partner by C. saeppellens was also described by Randall et al. (2005) and Kramer et al. (2009). In particular, Kramer et al. (2009) revealed that the average distance of the goby to the burrow entrance was 44 cm, whereas it was only 8 cm in the case of N. longus. Although the use of alternative shelters and partner fidelity of Acentrogobius sp. 2 were not studied, Acentrogobius sp. 2 stayed closer to the shrimp burrow than C. saeppellens. The time budgets of the surface activity in another facultative goby, B. curacao, was similar to Acentrogobius sp. 2 at high tides (Karpus, 1992).

The cost to the obligately symbiotic gobies has been suggested to be a result of limited food source and limited opportunities for reproduction (Karpus, 2014; Lyons, 2013; Randall et al., 2005). Conversely, the benefit of the facultative goby is the availability of a wide range of food items or ease of finding mates. It is known that by ranging over a broader area for feeding, facultative symbiotic goby has greater selectivity of prey than obligate symbiotic goby (Randall et al., 2005). The main food items of Acentrogobius sp. 2 were detritus, polychaetes, bivalves, and harpacticoid copepods (Horinouchi, 2008). This goby may gain nutritious food items (invertebrates) by using a wide feeding area.

Several studies have elucidated the fish fauna in tidal flat tidepools (Meager et al., 2005; Okazaki et al. 2012; Kanou et al., 2018; Kunishima and Tachihara, 2020); however, studies on the differences in behavior of these fish between high and low tides are scarce. In this study, Acentrogobius sp. 2 showed surface activity in tidepools at low tides, but the patterns were different from that at high tides. We observed a reduced time of surface activity (57% at low tides compared with 86% at high tides) and a lower frequency at positions B to D (6% at low tides compared with 38% at high tides). At low tides, many intertidal crabs showed continuous activity in and near the tidal flat, and P. modestus triggered the retreating behavior of Acentrogobius sp. 2. The mudskipper is known as a carnivore (Liao et al., 2020) and several intertidal crabs are omnivores (Moron et al., 2020). In low tides, shorebirds also act as predators (Calle et al., 2016; Chan et al., 2019). Further, tidepools are known to be subject to extremes of high and low water temperature and salinity (Meager et al., 2005). Such differences in predatory animals and/or physical and chemical conditions may
have affected goby surface activity between tides. As the intertidal environment changes dramatically over the year, we cannot discuss beyond the autumn observation. Surveys, especially in winter, when P. modestus and intertidal crabs are inactive, are required.

In conclusion, the present study confirmed that Acentrogobius sp. 2 used shrimp burrows as a refuge, and that the area of goby activity was wider than the known range of obligate gobies. This study also found that the goby performed surface activity in tidepools at low tides but in a reduced time period compared with that at high tides. Owing to the limited observation area due to the video camera, the fidelity of the goby to a shrimp burrow was not elucidated. Observation by divers and laboratory experiments may further our knowledge on the facultative relationship in goby-shrimp symbiosis in the Pacific. Future studies should also confirm whether the goby warns the shrimp of approaching predators. Several ecological comparisons have already been made among closely related Acentrogobius species (Horinouchi, 2008; Inui et al., 2011; Matsui et al., 2012a; 2014); thus, further studies on shrimp burrow use by A. virgatulus and A. pflaumii may elucidate the evolutionary process of the symbiotic relationship in this genus. To the best of our knowledge, ours is the first quantitative study of the surface activity of goby-shrimp symbiosis in the intertidal area. Obligate goby-shrimp symbioses are also known in tropical intertidal environment (Yanagisawa, 1978; Karplus, 2014), and so, behavioral comparison of the present study with future surveys using obligate gobies in low tides may be interesting.

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