Laboratory quantification of burrow utilization by the symbiotic varunid crab *Sestrostoma toriumii*

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Received 4 April 2014; Accepted 10 October 2014

**Abstract:** Use by a symbiotic crab *Sestrostoma toriumii* of burrows of a mud shrimp *Upogebia yokoyai* was investigated in laboratory experiments. Crabs used shrimp burrows in the presence of the host when the mud surface was covered with water. Crabs entered shrimp burrows soon after introduction to the tank (taking a mean of 656 s to enter), where they spent 61–81% of their total time in the experimental tanks. Half the crabs left the burrow at least once on the first day, whereas only a few crabs left on the second day. Although the crabs were expelled by the host shrimps in some cases, it is not known whether exiting is always due to host aggressive behavior. Two individuals that did not enter a shrimp burrow buried themselves in the sediment most of the time. Burying in the surface sediment may also be an adaptive behavior of *S. toriumii* when the crab has left the burrow for some reason. By living in the shrimp burrow, *S. toriumii* would benefit through predator avoidance.

**Key words:** burrow association, commensalism, *Sestrostoma*, symbiosis, *Upogebia*

The burrows or tubes constructed by marine benthos are often occupied or utilized by symbiotic animals. Particularly appropriate examples are the burrows of echiuran worms where a variety of animals such as bivalves, polychaetes, brachyuran crabs, alpheid shrimps, copepods and gobid fishes live commensally (MacGinitie 1935, Morton 1988, Anker et al. 2005). The burrows or tubes of sipunculids, polychaetes and holothurians are similarly used (e.g. Morton 1988, Japanese Association of Benthology 2012). Crustacean burrows also offer habitats for symbiotic animals (MacGinitie 1935, Dworschak et al. 2012, Japanese Association of Benthology 2012), although crustacean hosts might be able to expel symbiotic animals from the burrows by aggressive behavior or by using their chelipeds (Itani 2002).

*Sestrostoma toriumii* (Takeda) is one of three species of the varunid genus *Sestrostoma* which were removed from genus *Acmaeopileura* by Davie & Ng (2007). Davie (1992) and Sakai (2000) have implied that *S. toriumii* was associated with upogebid shrimps or echinarians, but were unable to determine where this species lives. At a mud flat in Yamaguchi Prefecture, Japan, where *Upogebia yokoyai* (De Haan) was abundant, *S. toriumii* was often collected from mud shrimp burrows, but was never found in the surface sediment (Itani 2002). Itani (2004b) further collected *S. toriumii* from the burrows of several species of upogebid mud shrimps and callianassid ghost shrimps by careful sampling of tidal flats in western Japan. However, it is not known whether individual *S. toriumii* live in a burrow all the time or use it only temporarily.

In order to elucidate the association between *S. toriumii* and the host crustacean, laboratory observation is necessary, because ex-situ observation is difficult. In the present study, burrow utilization in *S. toriumii* was quantified in aquaria where *Upogebia yokoyai* Makarov burrows were constructed. *Upogebia yokoyai* inhabits a Y-shaped burrow up to 50 cm in depth in sandy mud bottom sediment in estuarine environments (Itani 2004a, Kinoshita et al. 2010). Compared with *U. major*, burrowing more than 2.5 m deep (Kinoshita 2002), *U. yokoyai* is suitable for studying burrow associations in aquaria. The objectives were to clarify whether or not *S. toriumii* uses mud shrimp burrows, and to quantify symbiotic burrow occupation bout duration, frequency, and total time by *S. toriumii*.

Experiments were performed during May to August 2013 at the laboratory of Kochi University, where room temperature was maintained at 20–25°C. Day length and light intensity were not controlled. *Sestrostoma toriumii* and host mud shrimps *U. yokoyai* used in the laboratory experiments were collected from the intertidal mud flat at

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Uranouchi Inlet (33°25′50″N, 133°25′53″E), Kochi Prefecture, Japan. Both crabs and shrimps were collected by digging with a shovel or using a yabby pump. In the laboratory, the two species were kept separately in aquaria filled with sandy mud prior to introduction into experimental tanks. Crabs were fed frozen krill or commercial flake food (TetraMin®). The mud shrimp were fed Chaetoceros diatoms or mashed krill.

The experiment was to test if S. toriumii would utilize a mud shrimp burrow in the presence of the host when the mud surface was covered with water. Experimental acrylic tanks (50×200×300 mm high), covered with opaque vinyl chloride sheets to exclude light, were filled with sandy mud to around 200 mm in height. Sandy mud sediment used in the experiments was sampled from the shrimp collection sites, purged of macrobenthos, and dried at room temperature for at least 3 weeks. A single U. yokoyai was introduced into the experimental tank and allowed to construct its burrow. Twenty shrimps (10 males and 10 females) were used (8.1–15.6 mm in carapace length; mean, 10.2 mm). The tanks were placed in large aquaria with circulating seawater for at least 4 days until the upper U-shaped part of the burrow was completed. Burrow conditions and survival of U. yokoyai were confirmed by removing the vinyl chloride sheet. The tank was removed from the large aquarium when used for experiments. A single crab was placed inside the tank and the sandy mud surface was immediately recorded using a video camera (Canon ivis HF M51 or HFR21). The crab’s behavior was recorded for two successive days during the day under artificial illumination (ca. 2.8 k lux) with each session lasting 2 h. The camera was set approximately 30 cm over the tank using tripods. The tank was not aerated during recording because air bubbles disrupt the water surface, but was aerated between recording sessions, when not used in the experiment. Twenty-eight crabs (15 males, 11 females and 2 unknown) were used in this experiment, ranging from 1.6 to 5.9 mm in carapace width (mean, 4.2 mm). Although crabs were randomly assigned to experimental tanks, the burrows were large enough for the crabs to enter.

Behavioral data of crabs entering burrows were obtained from video recordings. The frequencies of burrow entering bouts, bout duration, and total time spent in the shrimp burrow were summarized. Although the crab’s behavior was recorded just after the introduction to the tank, the first 20 min were excluded as domestication time; thus, behavioral observation was for 100 min per session. Bout duration was underestimated when the crab was in the burrow at the beginning or at the end of the recording. Because the data were not normally distributed, nonparametric Wilcoxon paired-sample testing was used to assay the effects of habituation (first and second days) to analyze the frequency of burrow entering bouts and total time spent in the burrow. The Mann-Whitney U test was also applied in analyzing bout durations from all the experimental individuals in each session. Some crabs were observed to bury into the sediment surface. This burying behavior was too rare to be analyzed statistically.

Twenty-six individuals of S. toriumii utilized the mud shrimp U. yokoyai burrows (Fig. 1). Twenty-two individuals entered the shrimp burrows during the domestication period; and four individuals entered afterwards (mean 656 s after introduction to the tank; range, 0–5,175 s). Duration of burrow entering bouts varied widely 17 to 6,000 s. Eight individuals remained in the mud shrimp burrow continuously (6,000 s) on the first day; 18 individuals on the second day, with a significant habituation effect (Fisher’s exact test, p=0.015; Fig. 1). Fifteen individuals exited once or more than once on the first day, whereas only four individuals exited on the second day (Fisher’s exact test, p=0.003; Fig. 1). In three instances on the first day, the chelipeds of the host shrimps were detected at the burrow opening when the crab exited. The crab usually emerged from the burrow using the same opening that it entered (69 of 72 instances). Mean (±standard error) duration of burrow entering bouts was 1,787±294 s (n=61) on the first day and 2,669±385 s (n=51) on the second day, with no significant habituation effect (Mann-Whitney U test, p=0.793; Fig. 2).

Frequency of burrow entering per 100 min was 2.1±0.5 (n=28; range, 0–11) on the first day and 1.8±0.7 (n=28; range, 0–19) on the second day, with no significant habituation effect (Wilcoxon paired-sample test, p>0.05; Fig. 3). Mean (±standard error) total time spent in the mud shrimp burrow by S. toriumii per 100 min was 3,680±432 s (n=28; range, 0–6,000 s) on the first day and 4,862±379 s (n=28; range, 0–6,000 s) on the second day. A single crab used (8.1–15.6 mm in carapace length; mean, 10.2 mm). Although crabs were randomly assigned to experimental tanks, the burrows were large enough for the crabs to enter.

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In total, five individuals buried in the sediment surface. Four and seven burying bouts were observed on the first and second day, respectively (Fig. 3). Two individuals that did not enter a shrimp burrow on the second day remained buried in the sediment most of the time. The duration of burying bouts ranged from 136 to 5,420 s (Fig. 2). The mean total time spent burying in the surface sediment per 100 min was 182±148 s (n=28; range 0–4,069 s) on the first day and 550±288 s (n=28; range 0–5,563 s) on the second day (Fig. 4).

These results clearly showed that *S. toriumii* uses *U. yokoyai* burrows in the presence of the host when the mud surface was covered with water. Most of the crabs observed entered the burrows promptly and spent 61% and 81% of the observed time in the burrows on the first and second day, respectively. Half the crabs exited at least once on the first day, whereas only four crabs exited the burrow on the second day, probably due to a habituation effect. Although the crabs were expelled by the host shrimps in some cases, it is not known whether exiting is always due to host aggressive behavior. Possible explanations are that the crab was surveying the shrimp burrow and/or the experimental tank environment more frequently; and/or the host shrimp detected the crab and tried to expel the crab more frequently on the first day. Similar declines in the frequency and duration of wandering, surveying, and burrowing have been reported also for alpheid shrimp from a laboratory study extending 5 consecutive days (Palomar et al. 2005).

Burying behavior in smaller crab species is thought to be an anti-predator mechanism (Bellwood 2002). In the present study, some crabs buried in the surface sediment for a long period, instead of entering shrimp burrows. Itani (2002) found that *S. toriumii* individuals buried themselves in the sediment when placed on a mud surface where no burrows were available. Burying into the surface sediment may also be an adaptive behavior of *S. toriumii* when the crabs leave a burrow for some reason.

When placed into an artificial vinyl tube containing *U. major*, *S. toriumii* was soon detected by the shrimp and ejected from the tube (Itani 2002). Although the crab probably moves more quickly inside a shrimp burrow than in a slippery vinyl tube, the circumstances under which the host shrimp tolerates the presence of *S. toriumii* inside its burrow in nature are not known. The symbiotic pinnotherid crabs *Austinixa gorei* (Manning & Felder) and *A. aidae* (Righi) inhabit an upper narrower part (10–40 cm deep) of the burrow system of callianassid ghost shrimps, while the host shrimp inhabits a deeper part (McDermott 2006, Peiró et al. 2012). Further studies must examine in which part of the burrow *S. toriumii* lives in order to coexist with the host shrimp.

It is widely believed that the most important function for burrow symbioses is predator avoidance as well as food acquisition and taking refuge from desiccation (MacGinitie 1935, Morton 1988, Anker et al. 2005). Itôh and Nishida (2013) experimentally deduced that the copepod *Hemicyclops gomsoensis* avoided fish predation by inhabiting the burrows of *U. major*. By living in the shrimp burrow, *S. toriumii* would benefit through predator avoidance. It is thought to filter-feed in the burrow using its long setal 3rd maxillipeds as well as to feed on organic materials that might enter the burrow with water currents created by the host shrimp (Itani 2002, Itani et al. 2002, Davie & Ng 2007). Further studies are required to investigate the possible effects of the crab on the host shrimp through competition for plankton and other organic materials. Griffen et al. (2004) estimated that *Cryptomya* clams associated with mud shrimp burrows removed up to 39% of suspended particulate matter.

The experimental observation methods reported here were first used to study the behavior of the estuarine goby *Eutaeniichthys gilli* (Jordan & Snyder), where the goby was observed to frequently enter and exit shrimp burrows, with bout durations of several seconds to several minutes (Henmi & Itani 2014). Although behavioral data under laboratory conditions impose limitations on the extrapolation of results, the information derived is still valuable (Palomar et al. 2005). Further studies on symbiotic crustaceans

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**Fig. 3.** Frequency of burrow entry and sediment burying within a 100 min period in *Sestrostoma toriumii* (mean±standard error).

**Fig. 4.** Time spent in shrimp burrow and buried in the sediment per 6,000 seconds in *Sestrostoma toriumii* (mean±standard error). Only significant differences between the first and second days are shown.
and gobies using this observation method may elucidate patterns and diversity of burrow symbioses in shrimp burrows. Host specificity of symbiotic animals can also be tested using this method. Host burrow morphology and/or feeding mode may have an influence on the association between the symbiotic benthos and the hosts.

Acknowledgements

We thank D. Matsuyama, A. Shimizu, A. Mackawa, and M. Morimoto for assistance with sampling and Dr. E. Kornienko for providing Russian literature on Sestrostoma.

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