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Seasonal Occurrence of Gastropterids (Gastropoda: Cephalaspidea) and Their Habitat Selection in a Subtropical Back-reef on Okinawajima Island (Ryukyu Archipelago, Japan)

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Daisuke Tanamura and Euichi Hirose (2017) Gastropterids are generally small sea slugs with vivid colors. In a shallow back-reef at Zanpa (Okinawajima Island, Ryukyu Archipelago), five gastropterid species were observed during route censuses (0-2.6 m in depth, 400 m in length) conducted from November 2011 to June 2014 (89 times, 32 months): *Sagaminopteron ornatum*, *Siphopteron brunneomarginatum*, *Si. citrinum*, *Si. flavum*, and *Si. tigrinum*. Among them, *Si. tigrinum* was observed only in 2013, but the other four species were observed every spring during the survey. When the route was divided into four zones based on depth and dominant substrata, each species was mainly found in particular zones. In laboratory experiments for substrate selection, *Si. flavum* significantly preferred natural sand and rubble to flat glass-bottoms, glass beads, dried sand, or bleached rubble, suggesting that substrate material is a potential key for habitat selection in this species. Although a larger number of individuals preferred natural sand from their principle habitat to natural sand from a deeper zone where this species was rarely observed, no significant difference was found in the preference by binomial test.

Key words: Coral reef, Gastropteridae, Route census, Sea slug, Seasonality, Substrate selection.

BACKGROUND

Many sea slugs occur seasonally and this occurrence is likely to be influenced by the seasonal fluctuation of food availability and the presence of predators in relation to water temperature (*e.g.* Ros 1978; Todd 1983; Aerts 1994; Domenech et al. 2002). On one of Okinawajima Island's subtropical coral reefs (Ryukyu Archipelago, Japan), sea slug species and individual number showed seasonal changes, attaining a peak in May in a shallow back-reef where yearly water temperature fluctuations were 17°C to 32°C (Tanamura and Hirose 2016a). While the sacoglossan sea slug *Plakobranchus ocellatus* van Hasselt, 1824 has been reported to appear on a sandy beach on Okinawajima Island year-round, the population density was observed to change depending on water temperature (Tanamura and Hirose 2016b).

Gastropteridae Swainson, 1840 is a family of cephalaspid sea slugs with 41 species in four genera (Bouchet 2010; Ong et al. 2017). Gastropterids have been recorded in warm waters in the Indo-Pacific and Atlantic Oceans (*e.g.* Gosliner 1989; Gosliner et al. 2008). They are generally small - only several millimeters in length - and sometimes swim in the water column with their parapodia (*e.g.* Gosliner 1989). While the reproductive behavior of *Siphopteron* spp. has been studied (*e.g.* Lange et al. 2014), many other parts of gastropterid life history remain unknown. Ono (2004) reported 12 known and five potentially undescribed gastropterid species

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from the Ryukyu Archipelago, Japan. In a shallow reef off Okinawajima Island, gastropterid species were typically found in the spring and in particular areas or habitats within the back-reef (Tanamura, personal observation). However, the seasonal occurrence of these sea slugs has never been verified by quantitative field surveys.

The present study aimed to add basic information on the small-scale, spatio-temporal distribution and abundance of gastropterids inhabiting shallow areas (< -2.6 m) as well as the habitat preference of species found in these areas. In this study, we surveyed the occurrence of gastropterids and their habitat in the back-reef at Zanpa (Okinawajima Island, Ryukyu Archipelago) monthly from November 2011 to June 2014. As a result, we quantified the gastropterid assemblage and habitat usage. Moreover, the substrate selection of *Siphopteron flavum* (Tokioka and Baba 1964) was tested in the laboratory to elucidate the factor(s) related to spatial distribution.

MATERIALS AND METHODS

Route censuses

We carried out field surveys in a shallow back-reef at Zanpa (Okinawajima Island, Ryukyu Archipelago, Japan: 26°26'16"N, 127°42'47"E) from November 2011 to June 2014 (Fig. 1A). The route for the censuses was two parallel lines perpendicular to the beach toward the reef crest, 175 m long and 50 m apart (Fig. 1B). The survey area was a gentle slope and ranged in depth from 0 to 2.5 m at low tide. Habitats on the route were categorized into four zones based on depth and dominant substrata: I (0-0.6 m, algae on sandy bottom), II (0.6-1.1 m, *Porites* corals on sandy bottom), III (1.1-1.7 m, sandy bottom), and IV (> 1.7 m, rocks).

The census was carried out one to four times a month during calm conditions - 89 times over 32 months in total. Each time, a snorkeler took about two hours to swim along the route and record gastropterid species and numbers on the route. We also recorded the zones (I-IV) at which each individual was found and their habitats (*i.e.* sand, rock, coral, algae, and rubble). Gastropterids were searched for only by visual inspection and the snorkeler did not search within macroalgae or on the underside of rocks to avoid disturbing the habitat. Gastropterids found on the route were collected for species identification following Nakano (2004) as well as the descriptions of the external morphology in Gosliner (1989). When species identification was difficult *in situ*, we collected animals and examined them under a stereomicroscope on the beach. They were subsequently released back to the same habitat within a few hours. Water temperature on the seafloor was measured with an alcohol thermometer at the same location in each zone during the survey. When we surveyed multiple times in a month, the average of temperature measurements was used.

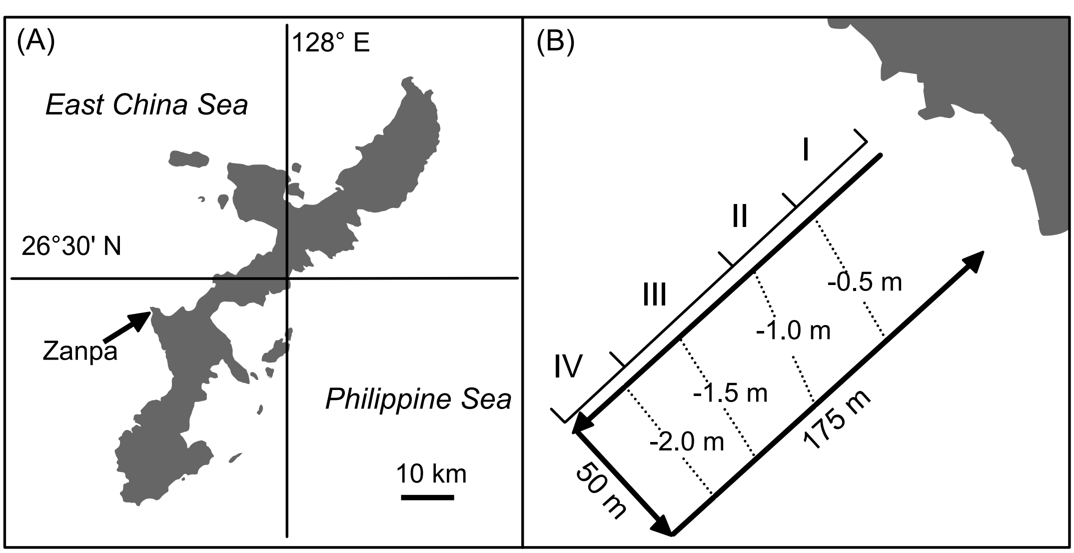

Fig. 1. (A) The map of Okinawajima Island indicating the study site. (B) The census route. Dotted lines indicate the approximate depths on the route. I-IV indicate the zones based on the depth and dominant substrata.

Laboratory experiment: substrate selection

We used *Si. flavum* in substrate selection experiments because this species was the most abundant gastropterid in the vicinity of our study site. We hypothesized that gastropterids prefer stable substrates and are able to recognize substrate material. Furthermore, we tested whether the subjects could discriminate between substrate from their principle habitat and substrata from other zones.

We collected *Si. flavum* individuals by hand from the back-reef at Zanpa outside the route census area from March to June in 2014. Animals were temporary held in a 5-L bucket containing seawater from the collection site with aeration at room temperature (about 28°C) for 1-2 weeks before the experiments. No food was supplied and approximately half of the seawater was changed every three days.

In a rectangular glass tray (18 × 14 × 5.5 cm) filled with seawater from the collection site, one material (substratum-A) was spread on half of the bottom and another material (substratum-B) was spread on the other half. Substrata used in this experiment were: none (glass bottom of the tray) as control, glass beads (1.5 mm diameter) as an artificial foothold, sand from Zones I-IV, dead coral rubble from Zones II and IV, and coralline algae from Zone II. Because the materials on the substrate surface may have affected substrate selection, glass beads and some rubble from Zone II were bleached to clean the surface; the material was immersed in 0.05% sodium hypochlorite for 1 hr, extensively washed with tap water, and then rinsed with seawater. To denature the materials on the substrate surface, some of the sand from Zone II was dried at 60°C for 1 week (dried sand) before use.

The experiments were carried out at approximately 28°C under ambient illumination in the laboratory. Seven to 19 individuals of *Si. flavum* were placed in the center of the tray. After 30 min, the numbers of individuals on substratum-A and B were counted to test the substrate preference. We excluded swimming individuals and those crawling around the border of the substrata or on the lateral wall of the glass tray from counts. The trial was repeated ten or eleven times for each combination of substrata. Significant preference for one substratum over the other was tested by binomial test using R (R Core Team, 2014).

RESULTS

Occurrence of gastropterids on the route

483 individuals were recorded during the survey period: *Sagaminopteron ornatum* Tokioka and Baba, 1964 (n = 13, 2.7%), *Siphopteron brunneomarginatum* (Carlson and Hoff, 1974) (n = 16, 3.3%), *Si. citrinum* (Carlson and Hoff 1974) (n = 57, 12%), *Si. flavum* (n = 392, 81%), and *Si. tigrinum* Gosliner, 1989 (n = 5, 1%) (Fig. 2). While *Si. tigrinum* occurred only in 2013, the other four species occurred every spring surveyed.

The water temperatures in the four zones were all similar, with minimum-maximum temperature (difference) during the survey period of 18.3-32.2°C (13.9°C) in Zone I, 17.3-31.8°C (14.5°C) in Zone II, 18.1-31.3°C (13.2°C) in Zone III, and 17.5-30.2°C (12.7°C) in Zone IV (Fig. 3A). The occurrence of gastropterid species fluctuated seasonally (Fig. 3B). The monthly average occurrence per census was 3.5 individuals (ind.) per census (76% of total) in Spring (March-May), 1.5 ind./census (11%) in Summer (June-August), 0.1 ind./census (1%) in Autumn (September-November), and 1.8 ind./census (13%) in Winter (December-February). *Siphopteron citrinum* was found from February to June every year. *Siphopteron flavum* also usually occurred from February to June, but did not occur in February 2012 and did in January 2013. Additionally, small numbers of *Si. flavum* were irregularly recorded in August and November of 2012 (0.5 ind./census in each month) and July 2013 (0.25 ind./census). The other three species were recorded within a shorter period in the present survey period (Fig. 3B). *Siphopteron flavum* occurred abundantly in spring; the maximum was 16 individuals in the census on 9 April 2013 and 28 March 2014. In contrast with *Si. flavum*, the other gastropterids were rare even at their peak; the maximum numbers were only 4 for *Si. citrinum* and 2 for *Sa. ornatum*, *Si. brunneomarginatum*, and *Si. tigrinum*.

The zones in which the gastropterids were found varied between species (Fig. 4, left panel); *Sa. ornatum* was only found in Zone IV, five individuals (31%) of *Si. brunneomarginatum* each occurred in Zones I, II and III, 34 individuals (59.7%) of *Si. citrinum* and 230 individuals (58.7%) of *Si. flavum* occurred in Zone II, and three individuals (60%) of *Si. tigrinum* occurred in Zone III. Moreover, *Si. citrinum* and *Si. tigrinum* never occurred in Zone IV. The gastropterids also appeared to have species-specific preferences for substrates and none were found on live corals (Fig. 4, right panel); *Sa. ornatum* mostly occurred on rock and more than 50% of *Si. brunneomarginatum*, *Si. citrinum*, and *Si. flavum* occurred on sand. Although only five *Si. tigrinum* were observed in total during the survey, they were found on a variety of substrata.

Laboratory experiments: selection of substrata

We tested 13 combinations of substrates and the binomial test supported that *Si. flavum* significantly selected a particular substrate in six combinations (Table 1). This gastropterid species significantly selected glass beads, sand, rubble, and bleached rubble over the control. However, there was no significant preference found between coralline algae and the control or dried sand and the control. The gastropterids significantly preferred intact sand and rubble to dried sand and bleached rubble. Although 39 individual selected intact sand from Zone II, the principle habitat of the species, and 24 individuals selected intact sand from Zone IV, where the species was rarely found, there was no significant difference in preference between the intact sand from Zones II and IV (P = 0.077).

DISCUSSION

Some sea slug species occur in particular seasons, while others occur year-round (*e.g.* Aerts 1994; Domenech et al. 2002; Betti et al. 2017, Nimbs and Smith 2017, Smith and Nimbs 2017). For instance, Tanamura and Hirose (2016a) reported that *Gymnodoris nigricolor* Baba, 1960 occurred every May but never in any other month, whereas *Phyllidiella granulata* Brunckhorst, 1993 was recorded 23 months of a 32-month survey on the same census route at Zampa as this study. In the present study, five gastropterid species occurred on the route census, all of which have previously been recorded in southern Japan and the Ryukyus (*e.g.* Ono 2004; Nakano 2004). We consider these animals to be seasonal, as all five species predominantly occurred in spring (March-May). In contrast, some species, including - *Si. citrinum* - were found throughout the year off of Guam (Carlson and Hoff 1973, 1974). The seasonal occurrence of gastropterids in the present study could be caused by the large variation of water temperature in the subtropical coral reef, which ranged from 17°C to 32°C.

Based on the present study, we conclude that four gastropterid species - *Sa. ornatum*, *Si. brunneomarginatum*, *Si. citrinum*, and *Si. flavum* - occur at the study site every spring,

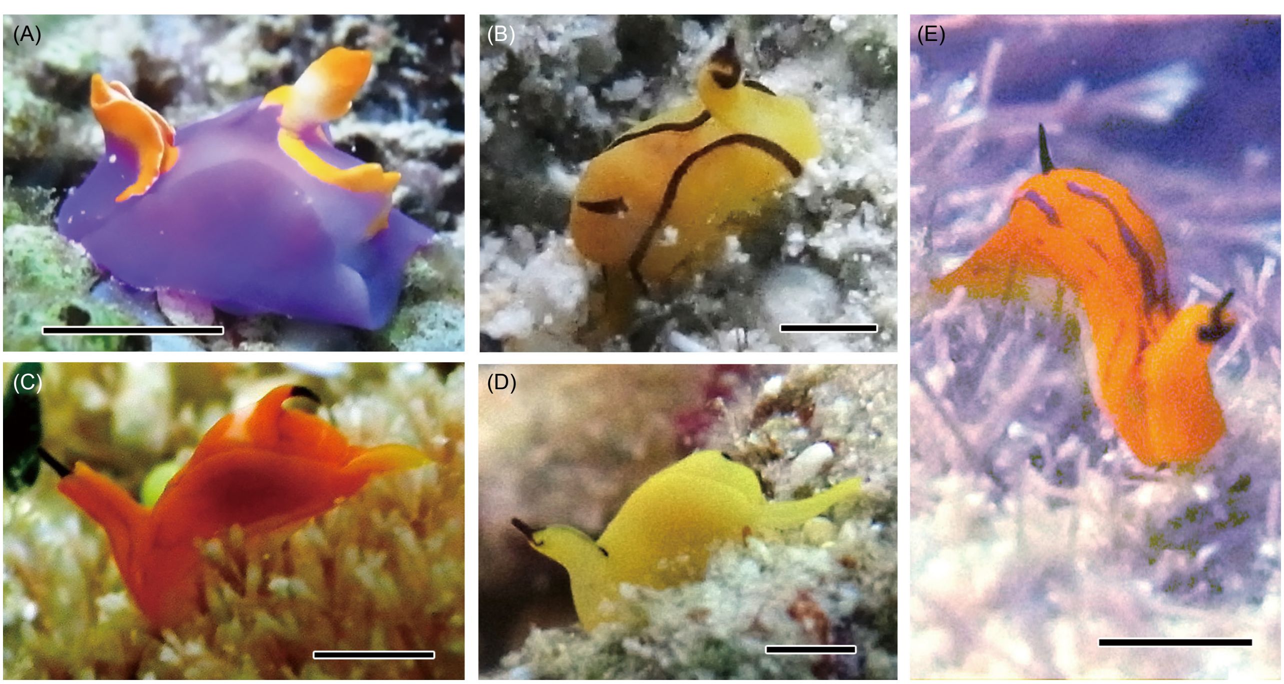

Fig. 2. *In situ* photographs of gastropterids at the study site with approximate scales. (A) *Sagaminopteron ornatum*. (B) *Siphopteron brunneomarginatum*. (C) *Si. citrinum*. (D) *Si. flavum*. (E) *Si. tigrinum*. Scale bars: A = 10 mm, B-E = 1 mm.

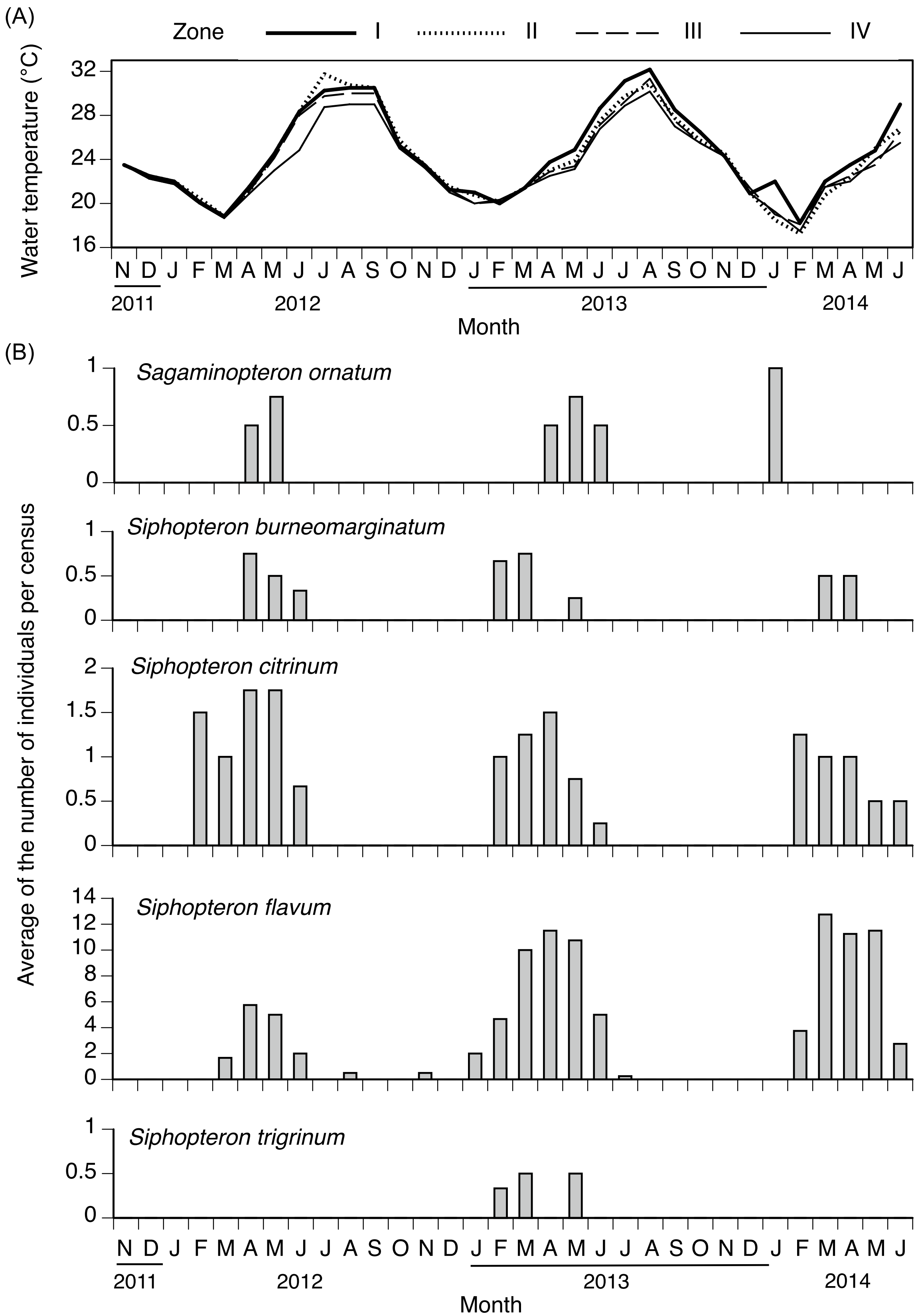

Fig. 3. (A) Water temperature in the four zones (I-IV) on the census route from November 2011 to June 2014. (B) Monthly average of the number of individuals per census of the five gastropterid species.

with abundances varying among species. Since *Si. tigrinum* occurred only in 2013 (*n* = 5), this species is probably rare to our study site, and it is uncertain whether *Si. tigrinuum* occurs every year or its occurrence in 2013 was an anomaly. As mentioned above, we did not survey places in algal bushes or on the undersides of rocks in order to avoid disturbing potential habitat. Therefore, any concealed gastropterids were overlooked in this survey, so the number of reported individuals is very likely an underestimate, particularly because Carlson and Hoff (1974) reported gastropterid species in Guam were commonly found under or on the sides of rocks and coral rubble.

All thirteen recorded *Sa. ornatum* individuals were found in Zone IV (> 1.7 m, rocks),

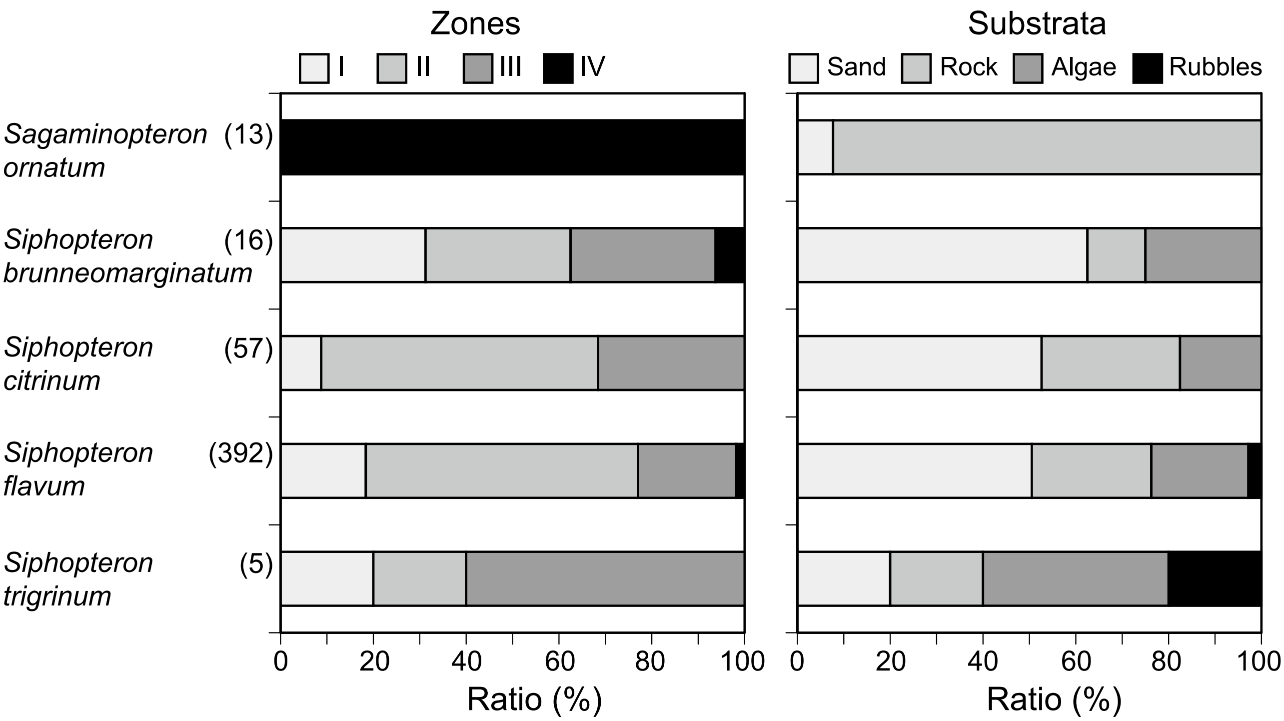

Fig. 4. Ratio of habitat usage *in situ* in each gastropterid species: zones (left) and substrata (right). No gastropterids were recorded on corals. Numbers in parentheses indicate the total number of individuals.

Table 1. Substrates selected by *Siphopteron flavum* in laboratory experiments

Number of individuals: Substrata				Uncounted individuals[1]	Probability (binomial test)
A		B			
106	: Sand [II]	28	: None	15	*P* < 0.001
109	: Rubble [II]	16	: None	24	*P* < 0.001
42	: Coralline algae	33	: None	13	-
102	: Bleached beads	53	: None	53	*P* < 0.001
48	: Dried sand [II]	47	: None	17	-
64	: Bleached rubble [II]	27	: None	37	*P* < 0.001
82	: Sand [II]	31	: Dried sand [II]	35	*P* < 0.001
70	: Rubble [II]	47	: Bleached rubble [II]	5	*P* < 0.05
80	: Bleached sand [II]	70	: Beached rubble [II]	34	-
27	: Sand [II]	28	: Sand [I]	23	-
34	: Sand [II]	33	: Sand [III]	20	-
39	: Sand [II]	24	: Sand [IV]	8	-
48	: Rubble [II]	50	: Rubble [IV]	2	-

[1]Individuals around the border of the substrata or on the lateral wall of glass tray and swimming individuals.

and it is possible that the species' main habitat is in deeper water. To illustrate this, photographs of *Sa. ornatum* in Ono (2004) were taken at 5 m and 8 m in depth off Gahijima Island (Kerama Islands, Ryukyu Archipelago, Japan). In *Si. brunneomarginatum*, only one of 16 individuals occurred in Zone IV, with the others evenly occurring in Zones I-III. Therefore, the census route probably included the species' main habitat. Ten of the 16 individuals occurred on sand, which may indicate a substrate preference of this species, while sandy bottom is a common substrate in Zones I-III.

In both *Si. citrinum* (n = 57) and *Si. flavum* (n = 392), nearly 60% of individuals occurred in Zone II and more than 50% were found on sand. It is suggested that the census route included these species' the main habitat and the species prefer sand. In laboratory experiments using *Si. flavum* (Table 1), the gastropterids significantly preferred some footholds (*i.e.*, glass beads, sand, rubble, and bleached rubble) to flat bottom (bare glass of the tray) ($P < 0.001$). The materials on the substrate surface are probably keys for selection, as the gastropterids significantly preferred intact sand and rubble to dried sand and bleached rubble ($P < 0.001$ and $P < 0.05$, respectively). Although it is uncertain how gastropterids discriminate and select habitats, chemical signals associated with the substrates may play a key role in the habitat selection. The signals may be related to their diets, which remain unknown. Coralline algae and dried sand could be suitable substrata for *Si. flavum*, but the species did not significantly prefer them over no substrate (control). The materials on the coralline algae and the denatured materials on the sand possibly contain negative signals for selection. Binomial test analyses did not support a preference for sand and rubble from their habitat to substrates from other habitats. Therefore, the laboratory experiment did not verify whether gastropods can discriminate their principle habitat's sand/rubble from that of different sites. Moreira et al. (2011) reported on the abundance of some cephalaspid species, and concluded that variation in abundance depends on prey abundance but not on granulometric preferences. The sponge-associating gastropterids *Sagaminopteron nigropunctatum* Carlson and Hoff, 1973 and *Sagaminopteron psychedelicum* Carlson and Hoff, 1973 feed on the host sponge *Dysidea granulosa* Bergquist, 1965 and concentrate compounds produced in the sponge for chemical defense (Becerro et al. 2006). During our field survey, we did not find any specific association between gastropterids and other organisms and, hence, the diets of these species may be microscopic organisms occurring on the substrates. In sea slugs, population dynamics are often influenced by seasonal fluctuations in the abundances of food resources and predators (*e.g.* Ros 1978; Todd 1983). Seasonal occurrence in gastropterid species may be caused by food availability associated with temperature; this would mean that clarifying diet is an important key to understanding the biology of gastropterid species.

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Competing interests: DT and EH declare that they have no conflict of interest.

Availability of data and materials: The supporting data will be provided by the corresponding author on request.

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Ethics approval consent to participate: Not applicable.

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