Population Dynamics of the Sea Slug *Plakobranchus ocellatus* (Opisthobranch: Sacoglossa: Elysioidea) on a Subtropical Coral Reef off Okinawa-jima Island, Ryukyu Archipelago, Japan

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Daisuke Tanamura and Euichi Hirose (2016) *Plakobranchus ocellatus* is a sacoglossan sea slug that can retain functional chloroplast from its algal food. This species feed on multiple species of siphonous green algae and can survive several months without food by utilizing retained chloroplasts in its digestive gland (kleptoplasty). While the population dynamics of opisthobranchs are often influenced by the seasonal fluctuation of the abundance of food resources, the fluctuation of food availability would not be a crucial factor to restrict the occurrence of *P. ocellatus*. We monitored the population density of *P. ocellatus* for 20 months on a subtropical coral reef where the water temperature fluctuated from 17°C to 32°C, in order to examine whether the population density, distribution pattern of individuals, and size distribution of *P. ocellatus* are stable or seasonally change. The present results showed that *P. ocellatus* appeared all year round in the study site, while the population density changed seasonally. The population density decreased in cold (≤ 21°C) and hot (≥ 27°C) periods, and densities in the months of intermediate temperature range (< 21°C, > 25°C) were significantly higher than the densities in other months (Student's *t*-test, *P* < 0.0001). Accordingly, population density is probably influenced by water temperature. Morisita's *I* indicated that the sea slugs were distributed in random patterns (13 months) or clumped patterns (7 months). Our field observations indicated that the sea slugs do not feed in daytime, and probably feed at night. Whereas *P. ocellatus* individuals of less than 10 mm were rarely recorded in the monitoring area, a decrease of the average body length and increase in population density in April – May suggest active recruitment of small individuals in this period.

**Key words:** Monthly monitoring, Population density, *I*, Water temperature, Kleptoplasty.

**BACKGROUND**

Many opisthobranch species occur in spring, while some occur all year round in subtropical coral reefs where water temperatures are over 30°C in summer and below 20°C in winter. For instance, in a route census conducted for 32 months on a back reef off Okinawa-jima Island (Ryukyu Archipelago, Japan), *Gymnodoris nigricolor* (Doridina: Gymnodoridiae) was recorded every May but never occurred in any other months, while *Phyllidiella granulates* (Doridina: Phyllidiidae) was recorded in 23 months (Tanamura and Hirose 2016). In opisthobranchs, population dynamics are often influenced by seasonal fluctuations of the abundance of food resources and predators (e.g., Ros 1978; Todd 1983).

*Plakobranchus ocellatus* van Hasselt, 1824 is a sacoglossan sea slug that is distributed from the Ryukyu Archipelago to tropical Australia, while its number of color varieties possibly include several distinct species and from a species group (e.g., Jensen 2007). This species is well known for kleptoplasty (or chloroplast retention) as seen in many other sacoglossans: it feeds on siphonous green algae and retains the algal chloroplasts in...
its digestive gland for photosynthesis (Kawaguti 1941; Greene 1970; Hirose 2005). This sea slug is estimated to retain functional chloroplasts for more than 11 months, the longest retention period among sacoglossans so far studied (Evertsen et al. 2007). Stable nitrogen isotopic composition of amino acids showed that the trophic position of wild *P. ocellatus* is that of a primary consumer (= algivore), while that of starved *P. ocellatus* is a producer (= phototropic organism) (Maeda et al. 2012). Moreover, Yamamoto et al. (2013) demonstrated the survival and relative weight were greater in the *P. ocellatus* individuals reared under light condition than those reared under complete darkness. These findings indicate that photosynthesis of the retained chloroplast is functional enough to maintain the host individual under starvation conditions. In many sacoglossan sea slugs, each species selectively feeds on only a few algal species (Jensen 1980; Williams and Walker 1999) but *P. ocellatus* has a broad spectrum of algal food including several genera of siphonous green algae (Jensen 1980; Adachi 1991; Maeda et al. 2012; Christa et al. 2013). Therefore, food resources are likely not a crucial factor to restrict occurrence, suggesting a year-round occurrence of *P. ocellatus*.

Using 1 × 1 m quadrats randomly placed on the sea bottom, we monthly monitored the population density of *P. ocellatus* for 20 consecutive months on a back reef off Toguchi Beach, Okinawa-jima Island to examine whether the population density, distribution pattern of individuals, and size distribution of *P. ocellatus* are stable or seasonally change in a subtropical coral reef where water temperatures fluctuate approximately 15°C over the course of a year.

**MATERIALS AND METHODS**

The population density of *Plakobranchus ocellatus* was monthly monitored on a back reef off Toguchi Beach (Okinawa-jima Island, Ryukyu Archipelago, Japan) from April 2012 to November 2013 (20 months) (Fig. 1). Although *Plakobranchus* has been a monotypic genus with *P. ocellatus*, this species is currently regarded as a species group based on molecular phylogeny of mtDNA (Krug et al. 2013) as well as color patterns and reproductive traits (Adachi 1991). In our sampling site, the *P. ocellatus* individuals has black spots ringed with white on the ventral surface of the foot and corresponds to ‘type 1’ *sensu* Adachi (1991) or ‘black-type’ *sensu* Krug et al. (2013). We cannot exclude the possibility that the sea slugs included small number of ‘type 3’ or ‘white-type’ individuals that have smaller black spots with white ring on the foot.

The monitoring was carried out around noon on or near the one of the spring tides in each month, and the surface water temperature was recorded at each monitoring. The monitoring site located in a subtidal zone less than 0.5 m in

![Fig. 1. Map of Okinawa-jima Island (A) and the monitoring site off Toguchi Beach (B). Quadrats were randomly placed within the rectangular area (ca. 50 × 200 m). *Plakobranchus ocellatus* sea slugs were collected for measurement of total body length within the circular area.](image-url)
depth at low tide. A frame (1 × 1 m) made of PVC (polyvinyl chloride) pipes was randomly placed on the bottom of the back reef within an area of approximately 50 × 200 m area along the beach (rectangle in Fig. 1B). The number of Plakobranchus ocellatus individuals was recorded within each quadrat. Fifteen to 45 quadrats (15 – 45 m²) were examined during each monitoring. The number of quadrats depended on the weather and wave conditions.

The pattern of distribution of individuals was evaluated using Morisita’s $I_D$-index (1962). This is an index for intraspecific aggregation, such as uniform random, or clumped dispersions. $I_D$ falls between 0 and 1 in uniform patterns, and is larger than 1 in clumped patterns, and $I_D$ equals 1 in random distribution following a Poisson frequency distribution. The significant deviation from random distribution was tested using critical values of the Chi-squared distribution.

The population densities in a particular temperature range were compared with those of other temperature ranges using Student’s $t$-test, following a Kolmogorov – Smirnov test and Bartlett’s test for normality of the dataset and equality of the variances. In the same way, water temperatures and population densities were respectively compared between the months of random distribution and the months of clumped distribution. Correlations were tested by Pearson’s correlation coefficient test between water temperature and population density, between $I_D$ and temperature, and between $I_D$ and population density, respectively. We performed these statistical analyses using InStat software (v. 3.1a, Graphpad Software, 2004).

The habitat of each individual was also recorded during the survey from October 2012 to November 2013. The substrata in the monitoring area were categorized as sand, rubble without algae, rocks without algae, or rubble and rocks bearing algae.

In addition, seven to nineteen individuals were monthly collected by hand to measure the total body lengths in the area neighboring the monitoring area (circle in Fig. 1B). The collected sea slugs were narcotized in a mixture of equal volume of seawater and 0.37 M MgCl₂. Subsequently, the body length was measured with a ruler to the nearest 1 mm. The body length data of September 2012 was excluded from the analysis, because some individuals died during anesthesia. The all narcotized individuals in the other months came out from anesthesia in normal seawater. Afterward, the sea slugs were released back to the back reef to avoid depletion of the population.

RESULTS

Population density

Table 1 summarized the monthly survey; we examined 495 quadrats and found 319 individuals in total. Plakobranchus ocellatus was found every month between April 2012 to November 2013 (20 months) on the back reef off Toguchi Beach, during which time the water temperature varied from 17°C in March 2013 to 32°C in August 2013 (Fig. 2A). The average population density was 0.64 individuals per 1 m², and the monthly density values fluctuated from 0.43/m² to 1.0/m² (Fig. 2B). Correlation between water temperature and population density was not significant (Pearson’s correlation coefficient: $P = 0.312$). However, population density tended to be lower when the water temperature was cold ($≤ 21°C$) and hot ($≥ 27°C$) (Fig. 2B). The difference of the densities in intermediate temperatures ($< 21°C$, $> 25°C$: 9 months) compared to other temperatures ($≤ 21°C$ or $≥ 25°C$: 11 months) was extremely significant (Student’s $t$-test, $P < 0.0001$). Chronologically, the density increased in April and May, sharply dropped in June, and remained low in July and August. Then, it recovered from September to December, and again became lower in winter (January – March).

Pattern of distribution

Morisita’s $I_D$-index fluctuated from 0.71 to 3.08 (Fig. 2C). The highest $I_D$ (3.08) and the lowest population density (0.43/m²) were recorded in February 2013, and the lowest $I_D$ (0.71) and the second lowest population density (0.47/m²) were recorded in June 2012. Pearson’s correlation coefficient test did not support the significant correlations between $I_D$ and temperature ($P = 0.665$) and between $I_D$ and population density ($P = 0.102$), respectively. Chi-square values indicated clumped distributions in seven months (October and December in 2012 and February, March, July, September, and November in 2013) and random distributions in the other 13 months. Uniform distribution was not supported in any months. Between the months of random distribution and the months of clumped distribution, Student’s $t$-test did not support significant difference in water
temperature ($P = 0.287$) and population density ($P = 0.774$), respectively.

**Habitat**

The major part in the monitored area consisted of a sandy bottom, and the other parts of the area consisted of rubble or rocks with or without seaweeds. The sea slugs often were covered in sand grains entirely or partially, and some were embedded in the sand bottom (Fig. 3A). Sea slugs rarely crawled on the bottom, and their parapodia were always closed. From October 2012 to November 2013 (14 month), we examined 405 quadrats and recorded the habitats of 259 individuals. Sea slugs were found on sand, rubble without algae, and rocks without algae, and 151 of 259 individuals (58%) were found on sandy substrata (Fig. 3B). Whereas eighteen individuals were recorded on the rubbles or rocks bearing algae, none of them were found directly on algae. Therefore, it is uncertain whether the algae on these rubbles and rocks were food for *P. ocellatus* or not.

**Total body length**

The body length of 193 individuals in total was examined, excluding the data for September 2012 when the specimens died during anesthesia. The average body length was 32 mm, maximum was 64 mm recorded in April 2013, and the minimum was 7 mm recorded in February 2013. The monthly average gradually decreased in April and May, and reached a minimum value in June in both 2012 and 2013 (Fig. 4). Individuals less than 10 mm in length only numbered three so far examined in the present survey: one individual each was respectively recorded in February, May, and June 2013.

**DISCUSSION**

The seasonal fluctuation of food availability is supposedly one of the key factors influencing the seasonal occurrence in opisthobranchs (e.g., Ros 1978; Todd 1983). However, *P. ocellatus* feeds on multiple genera of siphonous green algae (Jensen 1980; Adachi 1991; Maeda et al. 2012; Christa et al. 2013), and thus, its food resources are relatively stably available in the field compared with other sacoglossans that have a narrow spectrum of algal species as food.

### Table 1. Summary of the monthly survey from April 2012 to November 2013

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<th>Month</th>
<th>Total number of quadrats</th>
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(Jensen 1980; Williams and Walker 1999). As well, even if algae temporally disappear in the field, *P. ocellatus* can survive a considerable period utilizing kleptoplasts. The wide spectrum of food algae and kleptoplasty may allow the year-round occurrence of *P. ocellatus* in the monitoring site. In another sacoglossan, *Elysia chlorotica*, that can retain functional chloroplasts for 8–9 months, all adults in the population synchronously die in spring after egg-laying, possibly due to an annual viral outbreak that coincides with the mortality event (Pierce et al. 1999). A similar event never occurred in the present study, and we also did not observed the egg-laying of *P. ocellatus*.

![Fig. 2. Surface water temperature in the monitoring site (A) and sequential change of *Plakobranchus ocellatus* population density (B) and Morisita’s $I_\delta$-index (C) form April 2012 to November 2013. Black letters in B and C indicate the month in 2012 and gray letters indicate the month in 2013. Asterisks in C indicate significant departure from random distribution ($I_\delta = 1$) based on chi-square values (*, $P < 0.05$; **, $P < 0.01$).](image1)

![Fig. 3. *Plakobranchus ocellatus* covered with sand grains (A), and habitats where *P. ocellatus* individuals were recorded from October 2012 to November 2013 (B).](image2)
Whereas *P. ocellatus* always occurred throughout the year, population density seasonally fluctuated in the study site. The population density tended to decrease in cold (≤ 21°C) and hot (≥ 27°C) periods, and the densities in the months in the intermediate temperature range (< 21°C, > 25°C) were significantly higher than the densities in the other months. This may indicate that summer is too hot and winter is too cold for *P. ocellatus* to maintain a stable population density in subtropical coral reefs where the water temperature ranging from 17°C to 32°C. Although food availability appears to be less crucial for *P. ocellatus* than other opisthobranchs, high/low temperature may have considerable effects on its metabolic activity. In the hot period (June – September), several typhoons with heavy rains strike or come close to Okinawa-jima Island every year, and disturbances due to stormy weather and rapid changes in salinity in back reefs may also decrease the population density. The population density of *P. ocellatus* may be more stable in tropical coral reefs where the annual change of water temperature is much smaller (e.g., Glynn et al. 1991; Srinivasan and Jones 2006), as well as being less impacted by typhoons.

The pattern of distribution of individuals was random in 13 months and clumped in 7 months based on the chi-square values of the Morisita’s *I*δ-indices, while neither water temperature nor population density was significantly different between the months of random distribution and clumped distribution. Tendency of the seasonal fluctuation of *I*δ-indices was unclear, and both temperature and population density did not have significant correlation with *I*δ. This may indicate that the sea slugs do not aggregate seasonally, and the distribution pattern would be affected by short-term events, such as disturbance of stormy weather.

More than half of the sea slugs were found on sandy substrata, probably because sandy bottoms dominated the monitoring area and *P. ocellatus* was often distributed at random. In the present survey, no individuals were found directly on the algae, and thus we had no observations of the sea slugs feeding. Although some of algal species have been identified based on DNA-barcodes of chloroplasts retained in the digestive gland of *P. ocellatus* (Maeda et al. 2012; Christa et al. 2013), there have been no observations of *P. ocellatus* individuals feeding in the field (see Jensen 1980). However, some were found on the rubble or rocks bearing algae that include siphonous green algae, such as *Boodlea*- or *Cladophoropsis*-like species. Therefore, it is possible that these algae included the food species for *P. ocellatus*. We supposed that the sea slugs rarely feed in daytime, and they photosynthesize utilizing the kleptoplasts in this period. Since *P. ocellatus* individuals often had sand grains on their bodies in our survey, the solar radiation in daytime may be too strong and the sea slugs may shade the kleptoplasts from radiation with its parapodia and via this ‘sand-coating’. *P. ocellatus* probably feeds at night, as stable nitrogen isotopic composition has shown that *P. ocellatus* is a primary consumer (= algivore) in the field (Maeda et al. 2012). The nocturnal behavior and distribution pattern of *P. ocellatus* remain to be investigated in future.

The monthly average of total body length seasonally changed, reaching a minimum value in...
June. While the average of body length decreased in April and May, the population density increased at the same time. Many small individuals are probably recruited to the back reef in this period. As we found only a few individuals of less than 10 mm in the present study, juveniles and young individuals probably have a different habitat. However, the number of individuals examined in our survey was not enough to discuss the duration of the life cycle and growth of *P. ocellatus*, and their life in younger stages is poorly understood to date.

**CONCLUSIONS**

Whereas *P. ocellatus* has been studied to disclose the mechanism of kleptoplasty, there has not been much field research that aims to understand kleptoplasty from the viewpoints of ecology and evolutionary adaptation. The present study aimed to shed light on the life of *P. ocellatus* living in subtropical coral reef, and showed that seasonal population dynamics are probably influenced by water temperature. The *I*δ*-indices indicated that the sea slugs were distributed in both random patterns or clumped patterns in different months, while uniform patterns were not found in any months. Based on field observations, *P. ocellatus* does not feed in the daytime, and probably instead feeds at night. The present results also suggested the active recruitment of small individuals to the study site (shallow back reef) in April – May. The differences of ranges of temperature fluctuations may cause selection and/or speciation within the *P. ocellatus* species group inhabiting tropical and subtropical environments, and it is important to promote comparative field studies in various sites in both the tropics and subtropics.

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