Effect of Food Availability on Reproduction and Growth in the Intertidal Sea Hare *Aplysia juliana*

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Abstract: The purpose of this study was to investigate the effect of a change in food availability on the growth and reproduction of the hermaphrodite sea hare *Aplysia juliana*. All groups of *A. juliana* were fed algae for five days (conditioning period), and then we stopped feeding half of each group for the subsequent five days (experimental period) while the other half of each group were fed algae. There was a negative relationship between the initial body weight of *A. juliana* and the increment rate in body weight under the fed condition, and positive relationships were found between the weight of *A. juliana* and fecundity: i.e., the number of egg masses and the weight of them. When we stopped feeding, small individuals had more copulations than their counterparts which were under the food-rich condition although there was no increase in the number of copulations with large individuals. However, both the number of egg masses and the egg mass weights decreased when we stopped feeding. This implies that the degree to which small individuals divide energy into reproductive activity as males, though not as females, might increase when food availability decreases.

Key words: allocation, *Aplysia*, life history, mating, resource

INTRODUCTION

Food resources show spatial and temporal variations for most organisms. Spatial distribution and abundance of foods can vary even within a season, and response to changes in food availability has a critical role in maintaining the fitness of an organism. Since organisms need to allocate energy to competing functional demands that can be categorized into survival, growth and reproduction (Roff 1992; Stearns 1992), the degree to which organisms allocate energy into each category should vary with food availability.

General life history theory predicts that reduced life expectancy should change resource allocation decisions in favor of increased reproductive effort (Clutton-Brock 1984; Roff 1992; Stearns 1992). The appropriate responses in invertebrates have been traced to the presence of predators (Riessen 1999), a harsh abiotic environment (Roitberg et al. 1993), and physical injuries (Gellar 1990). On the other hand, a species of moth, *Scototeryx chenopodiata*, deprived of food as adults, increased oviposition activity (Javois & Tammaru 2004), while two species of fruit flies ignored food deprivation and another fly species even decreased oviposition rate in response to these cues of reduced life expectancy (Prokopy et al. 1993). Thus, the effect of food deprivation on reproductive effort is a controversial topic in life history evolution. This paper aims to examine the effect of food deprivation as a factor of reduced life expectancy in the sea hare, *Aplysia juliana*.

*Aplysia juliana* is a common annual sea hare in our study site, Uranouchi Inlet, southern Shikoku, Japan; it is widely distributed around the coasts of Japan and over extensive parts of the Pacific, Indian and Atlantic Oceans (Hamatani 2000). The adults mainly occur and reproduce from early spring to early summer. In spring, the green algae *Ulva* spp. (mainly *U. pertusa*) is the most common along the coast of Uranouchi Inlet, existing as patches on ropes, piers, floating docks and on other natural and artificial structures. *Aplysia juliana* actively eats *Ulva* under rearing conditions and shows a high growth rate (Carefoot 1970), suggesting that *Ulva* is a main food of *A. ju-
liana in the field. However, the distribution and abundance of *U. pertusa* rapidly change (Choi et al. 2001; Yoshida et al. 2002), leading to a situation in which *A. juliana* faces a rapid change in food availability during its reproductive season. Temporal dynamics of food availability are known to affect the growth and reproduction in opisthobranchs (Chester 1996; Ito et al. 1996). Herbivorous sea hares in temperate areas might have a high plasticity in energy allocation to growth and reproduction to cope with changes in the abundance of algae in order to maximize fitness.

The objective of this study is to determine how the growth in body weight and reproductive effort, in terms of mating behavior and the number and wet weight of egg masses, were affected by a change in the availability of algae in *A. juliana*. Since responses to food availability may vary with body size, we also examined whether the effects of algal availability on growth and reproduction change with body weight of *A. juliana*.

**MATERIALS AND METHODS**

Individuals of *A. juliana* were collected around the coast of Uranouchi Inlet (33°26’N, 133°26’E), Tosa Bay, southern Shikoku, Japan, on 25 March 2004, and we weighed them using an electronic balance (Mettler, PC440) to the nearest 0.001 g. We categorized the 152 sea hares into 19 groups. Each group consisted of eight sea hares with similar weights, and the eight individuals of each group were further divided into two subgroups of four individuals each. We placed the subgroups in 38 plastic aquariums (20×12×14 (height) cm) with a throughflow of natural seawater (Fig. 1). Two plastic aquariums for the subgroups of each group were placed in the same container (48×31×12 (height) cm). Seawater overflowing the two aquariums collected in the container, and cascaded into the next two aquariums on the following step. The number of steps for each experimental setup was from two to four. The following data for growth and reproduction of *A. juliana* were analyzed for each subgroup.

To standardize the energetic condition of individuals before the experimental period, we divided the rearing period into two parts; the conditioning period in which all aquariums were supplied with algae *ad libitum*, and a subsequent experimental period in which we manipulated the food availability (Fig. 2). All subgroups were fed every day abundant algae that were composed of green algae, mainly *U. pertusa*, and some red and brown algae during the conditioning period, from 25 to 30 March...
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2004. Then, we weighed them after the conditioning period of 5 days. We stopped feeding a subgroup (N-subgroup; no food) of each group and kept them for the experimental period, from 30 March to 4 April 2004 (Fig. 1). The other subgroup was continuously fed algae ad libitum for the experimental period (F-subgroup; fed). We weighed them again after the experimental period. Before comparing the two subgroups that differed in food availability from each other, we divided the groups into two size classes based on the weights at the end of the conditioning period; small (wet weight of a subgroup <100 g) and large (>100 g). Both subgroups in the same group were categorized into the same size class.

To compare the relative frequency of mating activity among the subgroups, we observed the mating behavior of sea hares two or three times per day and recorded the number of copulations throughout the rearing period. *Aplysia juliana* is a hermaphroditic species with fully functional male and female reproductive organs, and sometimes forms mating chains of three or more animals, the one at the front acting solely as a female and the one at the rear solely as a male. Other individuals in between act as both males and females. We scored each mating pair consisting of two individuals as a copulation, and when *A. juliana* formed a mating chain of three or four individuals we valued them as two or three copulations, respectively. When the mating pair or mating chain formed as a circle, one was added to the number of copulation for a mating pair (i.e., 2) or mating chain (i.e., 3 or 4, respectively). We also counted the number of egg masses in each subgroup and weighed the egg masses every day. Although several egg masses were usually spawned at the same place, we could distinguish the egg masses from each other, based on the color of each egg mass. We returned the sea hares to the field after the experiments.

We examined with Wilcoxon’s signed-ranks test and Kendall’s coefficient of rank correlation whether the food availability affected the growth and reproduction in *A. juliana*.

RESULTS

Body weights of individuals were between 3.86 g and 37.86 g at the beginning of the conditioning period and between 9.19 g and 54.19 g in the end of the period. All subgroups increased in wet weight during the conditioning period. On the whole, wet weights of subgroups at the beginning of the period significantly correlated with those at the end of the period (Kendall’s coefficient of rank correlation, $z=6.68, p<0.0001$) (Fig. 3a). Increment rate that was calculated as the wet weight of a subgroup after 5 days divided by that at the beginning of the period ranged from 1.33 to 2.53. Smaller subgroups had a higher increment rate (Kendall’s coefficient of rank correlation, $z=-2.23, p<0.05$) (Fig. 3b). We detected no difference in the increment rates of wet weight between subgroups that were going to be fed algae and no algae in the subsequent 5 days of experimental period (Wilcoxon’s signed-ranks test, $T=71, p>0.05$). Significant positive relationships were found between the wet weights of subgroups at the beginning of the conditioning period and the number of copulations, the numbers of egg masses and the wet weights of egg masses of the subgroup that were summed during the 5 days of the conditioning period (Kendall’s coefficient of rank correlation, $z=3.46, 3.24$ and $5.18$, respectively, $p<0.01$) (Fig. 4).

In the experimental period, F-subgroups showed growth and reproduction in a similar pattern as those during the conditioning period. Smaller subgroups achieved a higher increment rate in wet weight during the experimental period (Kendall’s coefficient of rank correlation, $z=-3.88, p<0.001$) (Fig. 5a). Larger subgroups more frequently copulated (Kendall’s coefficient of rank correlation, $z=2.40, p<0.05$) and bred more (Kendall’s coeffi-
Fig. 4. Reproduction of A. juliana during the conditioning period: the relationship between the initial wet weight of subgroups at the beginning of the conditioning period and the number of copulations (a), the number of egg masses (b) and wet weight of egg masses (c).

cient of rank correlation, $z=2.56, p<0.05$) and had heavier egg masses (Kendall’s coefficient of rank correlation, $z=4.79, p<0.0001$) (Fig. 5b, c, d). On the other hand, the N-subgroups partly differed from those during the conditioning period in the performance of growth and reproduction (Fig. 6). There was a significant positive relationship between the wet weight of the N-subgroups at the beginning of the experimental period and the wet weight of egg masses that were bred during the experimental period (Kendall’s coefficient of rank correlation, $z=3.04, p<0.01$). However, no significant relationships were detected between wet weights of subgroup and increment rates, the number of copulations and the number of egg masses (Kendall’s coefficient of rank correlation, $z=0.75, 0.65$ and $1.65$, respectively, $p>0.05$).

The effect of food availability varied with the wet weight of the subgroup. Small N-subgroups more frequently copulated than the F-subgroups consisting of small individuals (Wilcoxon’s signed-ranks test, $n=6, T=0, p<0.05$) (Fig. 7a). There was no significant difference in the number of egg masses between the N- and F-subgroups consisting of small individuals (Wilcoxon’s signed-ranks test, $n=6, T=4, p>0.05$) (Fig. 8a). On the other hand, there was not a significant difference in the number of copulations between the N- and F-subgroups consisting of large individuals (Wilcoxon’s signed-ranks test, $n=13, T=41, p>0.05$) (Fig. 7b). In large individuals, the F-subgroups bred more egg masses than the N-subgroups (Wilcoxon’s signed-ranks test, $n=11, T=4, p<0.01$) (Fig. 8b), and the wet weights of egg masses of the F-subgroups were heavier than the N-subgroups (Wilcoxon’s signed-ranks test, $n=13, T=0, p<0.01$). A significant difference in the wet weight of egg masses between the N- and F-subgroups was also detected in small size class (Wilcoxon’s signed-ranks test, $n=6, T=0, p<0.05$). Increment rates of the F-subgroups were higher than those of the N-subgroups in both size classes (Wilcoxon’s signed-ranks test, (small) $n=6, T=0, p<0.05$, (large) $n=13, T=0, p<0.01$).

Fig. 5. Growth and reproduction of A. juliana under the food-rich condition during the experimental period: the relationship between the initial wet weight at the beginning of the experimental period and the increment rate during the experimental period (a), the number of copulations (b), the number of egg masses (c) and wet weight of egg masses (d).
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Fig.6. Growth and reproduction of A. juliana under no food conditions during the experimental period: the relationship between wet weight at the beginning of the experimental period and increment rate during the experimental period (a), the number of copulations (b), the number of egg masses (c) and wet weight of egg masses (d).

DISCUSSION

In our study, growth and reproduction of A. juliana varied with body size due to the change of food availability. Under conditions of sufficient food during the conditioning and experimental periods, the subgroups consisting of small individuals had higher increment rates of wet weight than those of large individuals, and positive relationships were found between the weight of A. juliana and the number of egg masses and the weight of egg masses. On the other hand, after feeding was stopped, the increment rate did not show a size dependency and neither the number of matings nor the number of egg masses had a positive relationship with the body size.

Reproduction and growth have been reported to vary in response to different levels of food availability (e.g. Chester 1996; Cheung & Lam 1999). It is often assumed that organisms allocate resources differentially with changing environmental conditions. In some marine invertebrates, reproductive effort is reduced with environmental stress or food depletion (Menge 1974; Harvey & Vincent 1989; Nakaoka 1994). However, Susswein (1984) demonstrated that A. fasciata under food deple-
tion spend more time mating than do individuals under sufficient food. In our experiment, both the number of egg masses and the egg mass weight of the F-subgroups were higher than the N-subgroups. However, small individuals of the N-subgroups had more copulations than their counterparts of the F-subgroups although large individuals did not. This implies that the mating activity in small individuals, but not large individuals, was higher under the no food conditions than under the food-rich conditions in A. juliana.

At the beginning of mating behavior of A. juliana, an individual acting as a male climbs on the back of another individual acting as a female, and no resistance of the female is observed. The increase in the number of copulations in small individuals might result from the increase of activity as males, not as females. Yusa (1996) found that small individuals of A. kurodai tended to mate as males in the field while large individuals did so as females. Since small individuals have a lower fecundity as females than large individuals, in order to maximize their reproductive success they should act as males. In our experiment, all individuals of the N-subgroups had a low fecundity as females because of food depletion. Not only body size but also nutrient conditions might affect the sexual role in sea hares. Although there was not a significant difference in the number of copulations between the N- and F-subgroups of large individuals, they might allocate time and energy into reproductive activity as much as they can irrespective of the food condition.

In conclusion, this study has clarified that food availability changes the energy allocation between growth and reproduction and the effects depend on body size in the sea hare A. juliana.

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