3種の緑藻を給餌した場合のアマクサアメフラシAplysia julianaの摂取,成長,および産卵

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The Effect of Different Macrophytic Algae on Growth, Egg Production, and Food Consumption in *Aplysia juliana* Quoy and Gaimard

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Abstract

The use of different green seaweeds (*Ulva pertusa* Kjellman, *Ulva fasciata* Delile, and *Enteromorpha intestinalis* Link) for the culture of sea hare *Aplysia juliana* from immaturity to adult stage was examined during a period of 130 days under laboratory conditions. Specimens at the start of the experiment ranged in size from 0.07 to 0.08 g wet weight. All the seaweeds tested were suitable foods for the sea hare and promoted growth rates: for *E. intestinalis*, 0.14 g/day; for *U. fasciata*, 0.12 g/day; and for *U. pertusa*, 0.10 g/day. Spawning activity was first detected on the 15th, 18th and 29th days of culture when *Aplysia* were fed *E. intestinalis*, *U. fasciata* and *U. pertusa*, respectively. Total egg production was higher using *E. intestinalis* (25.1 g in dry weight per animal) when compared to *Ulva* (20.8 g for *U. fasciata* and 10.8 g for *U. pertusa*). A positive correlation was found between temperature and egg production after the first 10 days of egg-laying. Food consumption of *Ulva* by *Aplysia* was significantly ($p \leq 0.01$) higher than that of *Enteromorpha*. Consumption rate of *U. pertusa* by *Aplysia* was 22.4 % of its mean body weight per day, whereas it was 14.6 % for *U. fasciata* and 8.5 % for *E. intestinalis*. The following conclusions were obtained from the results: 1. food consumption followed the order *U. pertusa* > *U. fasciata* > *E. intestinalis*; 2. the gross assimilation efficiency values of *A. juliana* for each algal diet including the total weight of eggs produced in the measure of total growth were 31.6 % for *Enteromorpha*, 18.9 % for *U. fasciata* and 10.1 % for *U. pertusa*; 3. *A. juliana* has potential to control the plagues of macro algae such as *Ulva* and *Enteromorpha*, usually present in semi-intensive or extensive aquaculture facilities.

Introduction

The marine gastropod mollusc *Aplysia* has been used extensively in neurophysiological studies, and for investigating the neural basis of behaviour[1]. The chemical substances in the digestive glands, whole animal body and eggs are of great economical interest, with considerable pharmacological effects like antibacterial and antineoplastic activities[2,3].

Aplysia juliana is a cosmopolitan species and has the greatest distributional range among the sea hares. The present paper describes the laboratory culture of *A. juliana* found in Kagoshima bay, Southern Japan, from reproductively immature individuals to the adult stage fed on seaweeds such as *Enteromorpha intestinalis*, *Ulva fasciata* and *Ulva pertusa*. The study reveals the con-

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siderable growth characteristics and egg production potential of *Aplysia*, as well as the remarkable ability of the animal to consume large quantities of seaweeds.

Macro algae such as *Ulva*, used in fish and shrimp culture systems as biofilter to remove nutrients[4-6], if uncontrolled can become a menace, reducing dramatically the growth of aquatic animals. Dead rotten *Ulva* sp. led to heavy pollution of pond substrate, by increasing the volume of sludge and reducing shrimp growth due to stress and the negative oxidation-reduction potential of the pond bottom[7]. Kuruma shrimp farmers facing the problem of over-growth of a macro algae have to spend a lot of time removing it from the ponds, the operation thereby becoming costly. Juvenile shrimps may be trapped between thalli and food can be lost inside the algal mass. Algae accumulating over the sand substrate also disturbs the natural behaviour of shrimp by reducing the area for burrowing and stressing the culture organisms (M. F. Vazquez, personal communication, 1995). Very few studies have been conducted on the control of macrophyte in sea water aquaculture. Some workers, however, have suggested that the browsing nature of gastropod abalone would be effective in bio-control of algae in polyculture systems[8,9].

Through the present experiment we suggest that sea hares could be introduced into polyculture systems to control unwanted algal growth, considering their ability to consume large quantities of macrophytic algae; not only contributing to the homeostasis of the aquaculture systems, but also producing useful by-products for the pharmaceutical industry.

Materials and Methods

Juveniles of *Aplysia juliana* Quoy and Gaimard, were collected from the intertidal channel close to the Kamoike Marine Experimental Station of Kagoshima University, Kagoshima Prefecture. Collections were made during October 1994. The animals were found in the habitats convered by the green macrophytic alge *Ulva fasciata* Delile.

Stock of the *U. fasciata* was collected from the same biotop where *A. juliana* was found. The other seaweeds used in the study were *Enteromorpha intestinalis* Link, and *Ulva pertusa* Kjellman. These were collected intertidally, from different biotopes where the animals were found, namely Kamoike region for *Enteromorpha* and Kokubu Beach for *U. pertusa*. Collections were made once a week, and the stock was renewed at that time. Algae were maintained outdoors, under the varying natural light and temperature conditions in five (5) 100-liter circular Plexiglas tanks, filled with natural seawater. The algae were grown unattached and kept suspended in the water column by air diffusers located at the bottom of each tank.

All observations on growth, egg production and food consumption were made at ambient seawater temperature (range 13-26 ℃), in the laboratory for a period of 130 days. This range of temperature, reflects the natural conditions for *A. juliana* depending on the season. Animals were starved for three days prior to the determination of the initial weights and selection of the experimental animals. Small specimens of *Aplysia* in the size range from 0.07 to 0.08 g wet weight were divided into 9 groups of 5 individuals each and were transferred into nine (9) 45 liter glass-walled aquaria. Moreover, they were divided into three dietary groups. The aquaria were placed away from direct sunlight, under varying natural light conditions. The sea water in the aquaria was replaced every day by fresh seawater, filtered through a 20 μm mesh and the salinity ranged from 35-36 ppt. Dissolved oxygen was maintained close to air-saturation level through aeration.

Every 10 days the animals were weighed, after drying them on a paper towel for a few seconds. The body length was also measured at full extension, by allowing the animals to crawl and extend forwards inside a transparent cylindrical plastic tube. The tube diameter was adjusted to fit the size of the animal. Eggs were collected daily from holding aquaria, drained of excess water and weighed. A known amount of algae was presented twice a day in each aquarium and the sea hares were allowed to feed until satiation. Thalli of the algae were offered as food and uneaten remnants were washed with tap water and weighed after centrifugation for 3 minutes in a modified washing machine to remove excess water. The wet weight values for growth, egg production, and food consumed were converted to their dry weight equivalents by applying appropriate correction factors obtained by drying known amounts to constant weight at 110 ℃.

In the present study, the effect of diet on growth was expressed as gross assimilation efficiency, which is the ratio of the amount of assimilation or growth (dry wt) to the amount of food ingested (dry wt).

Results

The growth of *A. juliana*, measured as dry body weight increase, when fed on the three seaweed diets is shown in Fig. 1. The growth data, including dry weights of food consumed and eggs produced by *Aplysia* fed the different alga are given in Table 1. The best growth rate was obtained on a diet of *Enteromorpha* (0.14 g/animal/day), closely matched by that on diets of *U. fasciata* (0.12 g/animal/day), and *U. pertusa* (0.10 g/animal/day). When the total dry weight of eggs produced was included in the measure of total growth, the growth rates were found to be 0.34 g/animal/day for *Enteromorpha*, 0.28 g/animal/day for *U. fasciata*, and 0.18 g/animal/day for *U. pertusa*. Similarly, when growth rate was expressed in terms of body length, it was highest for the *Enteromorpha* diet group (0.15 cm/animal/day) and slightly lower for the diets of *U. fasciata* (0.14 cm/animal/day) and *U. pertusa* (0.12 cm/animal/day).

The trend for food consumption of the three groups was inverse to that of the growth rates (Table 1). Feeding rates for each diet, on a dry weight basis, were found to be 1.77 g/animal/day for *U. pertusa*,

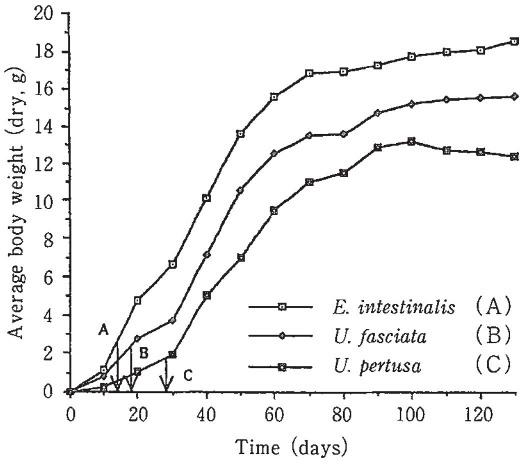

Fig. 1. Increase in dry weight of *Aplysia juliana*, fed on selected algal diets for 130 days in laboratory conditions. Each point represents the mean of 15 animals. The arrows A, B, C in horizontal axis represents the chronic recordings of the first egg laying activity in each algal diet.

Table 1. Results of the culture experiment of *Aplysia juliana* fed on *Enteromorpha intestinalis*, *Ulva fasciata* and *U. pertusa* diets in laboratory conditions at temperature regime (13-26 ℃). The data is expressed per individual as mean ± S.D.

Seaweed Diets	*E. intestinalis*	*U. fasciata*	*U. pertusa*
Experimental period (days)	130	130	130
Body weight gain (dry, g)	18.5 ± 1.1 [a]	15.6 ± 1.3 [b]	12.4 ± 1.1 [c]
Including egg production[1]	43.7 ± 1.8 [a]	36.4 ± 3.4 [b]	23.2 ± 1.9 [c]
Growth rate of body weight (dry, g/day)	0.14± 0.01 [a]	0.12± 0.01 [b]	0.10± 0.01 [c]
Including egg production[1]	0.34± 0.01 [a]	0.28± 0.03 [b]	0.18± 0.02 [c]
Initial body length (cm)	0.92± 0.03	0.92± 0.04	0.91± 0.03
Final body length (cm)	20.3 ± 0.7 [a]	18.8 ± 0.7 [b]	17.1 ± 0.7 [c]
Growth rate of body length (cm/day)	0.15± 0.01 [a]	0.14± 0.01 [b]	0.12± 0.01 [c]
Total food consumption (dry weight, g)	138.9 ±15.0 [a]	192.1 ±17.5 [b]	230.6 ± 9.5 [c]
Feeding rate (dry weight, g/day)	1.07± 0.12 [a]	1.48± 0.13 [b]	1.77± 0.07 [c]
(%) of mean body weight eaten/day	8.5 ± 0.7 [a]	14.6 ± 1.0 [b]	22.4 ± 0.5 [c]
Gross assimilation efficiency (%)	13.4 ± 1.2 [a]	8.2 ± 0.6 [bc]	5.4 ± 0.1 [c]
Including egg production[1]	31.6 ± 2.2 [a]	18.9 ± 0.7 [b]	10.1 ± 0.4 [c]
Total egg production (dry weight, g)	25.1 ± 1.4 [a]	20.8 ± 3.3 [ab]	10.8 ± 1.5 [c]

[a,b,c] Treatments with the same superscript are not significantly different at $p \leq 0.01$.

[1] The dry weight of eggs produced, included as part of total growth.

1.48 g/animal/day for *U. fasciata* and 1.07 g/animal/day for *Enteromorpha*. The consumption of *U. pertusa* by *Aplysia*, when expressed as percentage of mean body weight eaten per day, was 22.4, 14.6 for *U. fasciata* and 8.5 for *Enteromorpha*.

The gross assimilation efficiency values of *A. juliana* for each algal diet, calculated on a dry weight basis, are presented in Table 1. The highest value associated with the diet giving the best growth was obtained for *Enteromorpha* (13.4 %). The corresponding figures for *U. fasciata* was 8.2 % and 5.4 % for *U. pertusa*. When the total dry weight of eggs produced was included in the measure of total growth, the growth efficiency was found to be 31.6 % for *Enteromorpha*, 18.9 % for *U. fasciata*, and 10.1 % for *U. pertusa*.

The occurrence of the first egg laying activity of *A. juliana* fed on three seaweed diets during the 130-day culture period, is shown in Fig. 1. The first batch of eggs came on the 15th day for animals fed on *Enteromorpha*, 18th day for animals fed on *U. fasciata* and 29th day for animals fed on *U. pertusa*. The total amount of eggs produced by the different groups of *A. juliana* (dry weight, g/animal), is given in Table 1. Specifically *Aplysia* fed on *Enteromorpha* and *U. fasciata* were found to be more productive (25.1 g and 20.8 g dry weight/animal, respectively) than animals on the diet of *U. pertusa* (10.8 g dry weight/animal).

There was a strong positive correlation between the temperature and egg production after a 10 day period of the first egg laying activity of *Aplysia* in each of the treatments (Fig. 2). The coefficient of correlation for *Aplysia* fed *U. pertusa* and *U. fasciata* was ($R \geq 0.928$; $p \leq 0.01$) and ($R \geq 0.875$; $p \leq 0.01$) respectively and for those fed *Enteromorpha* the value was ($R \geq 0.819$; $p \leq 0.01$). However, no significant correlation was obtained for these factors in none of the treatments during the first 10-day period of the egg laying activity.

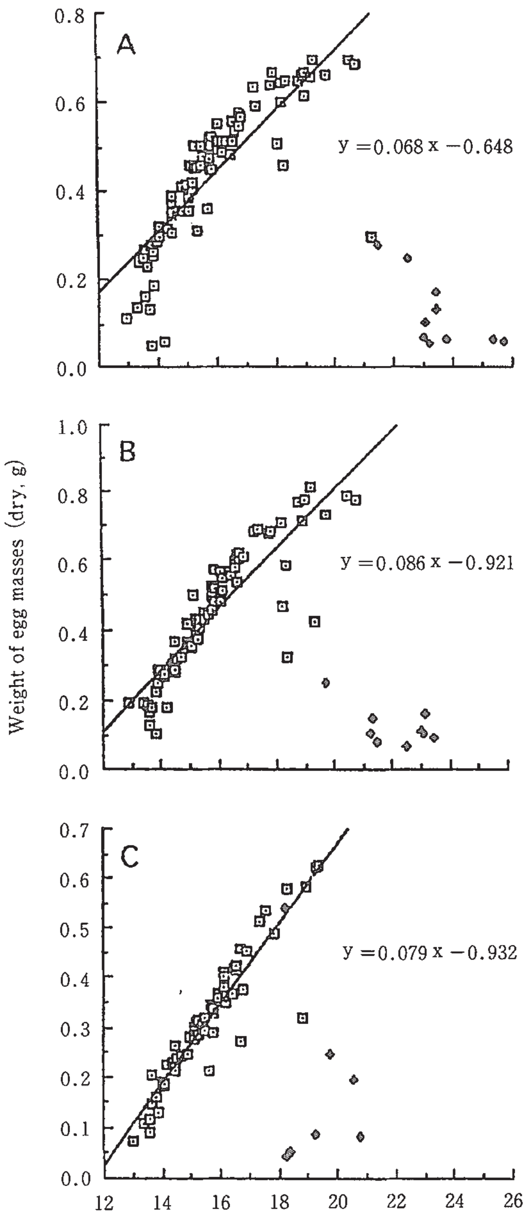

Fig. 2. Regression curves obtained between egg production and temperature in *Aplysia juliana* fed on *Enteromorpha intestinalis* (A), *Ulva fasciata* (B) and *Ulva pertusa* (C) diets. Only data after 10 days from the beginning of spawning were used in the analysis (white symbols). Dark symbols represent the first 10 days of spawning that didn't fit the curves and were not used for the regression, (look the text for correlation coefficients).

Discussion

There was a significant difference in results between the three types of algae used as food for the *Aplysia juliana*. The biological performance of the animals based on growth and egg production, indicates that *Enteromorpha intestinalis* has a dietary superiority over *U. fasciata* and *U. pertusa*.

The pattern of growth, a continuous increase in average animal weight for the three diet groups, was an asymptotic sigmoid curve[10] as found for other species. The growth observed was approximated by exponential functions in the beginning of the experiment, followed by linear and asymptotic functions.

The superior effect of the *Enteromorpha* diet on the growth of sea hares has also been documented in studies on adult *Aplysia dactylomela* (Palatzidis et al., unpubl.)[11]. In contrast, laboratory-grown *Aplysia juliana* fed with *Ulva fasciata* had a better growth rate, when compared to the *Enteromorpha* sp.[11]. It has been noted that different populations had their own distinct preferences, which varied depending on the seaweeds present in a given area[12]. Moreover, the feeding behaviour and inductive growth parameters were affected by their past dietary history, associated with season and age, especially in the stereotyped behaviour of mature individuals.

The *Ulva* also support good growth and are generally preferred by *A. juliana* in the field[11,13,14], but current observation on growth rates of the animals fed *U. fasciata* and *U. pertusa* were 14 % and 29 % lower respectively, when compared to the *Enteromorpha* diet. A slightly different picture emerged when we included the weight of eggs produced in the measure of total growth. The differences between growth rates increased according to egg production but the same hierarchy remained.

While the *Enteromorpha* promoted a better biological performance of the gastropoda, *U. pertusa* consumption (dry and wet weight basis) was significantly higher. The same trend is seen when we compare *U. fasciata* and *U. pertusa*. The food consumption by *Aplysia* has been studied by various authors and appears to be related to internal or external factors like bulk properties of the food[15], phagostimulants[16-18] and reproductive behaviour[19]. Certain studies on the phagostimulatory function of methanol extracts from different seaweeds for *A. juliana* proved *U. pertusa* to be the most active[18]. This may explain why the *U. pertusa* consumption was high in our study. Moreover, the increase in egg production when *Aplysia* was fed on *Enteromorpha* and *U. fasciata* was 132 % and 93 % higher when compared to that of the *U. pertusa*. It is also known that bag cell hormone produced during egg laying inhibits feeding in *Aplysia*[19]. Perhaps the animals in this study fed with *Enteromorpha* and *U. fasciata* algae had less opportunity to feed compared to those fed *U. pertusa*. Since food quality and absorption from diets are reflected in growth parameters and productivity of the sea hares[11,20]. The restricted growth obtained with *Ulva* in this laboratory experiment may indicate its inferior quality for *Aplysia*. Of the different *Ulva* diets tested here and in previous studies, *U. fasciata* proved to be the better food for *A. juliana*[21]. The consumption of *U. fasciata* by *Aplysia* was 14.6 % of mean body weight eaten per day; 8.5 % for *Enteromorpha* and the corresponding figures being 22.4 % for *U. pertusa*. The highest growth efficiency (32 %) was attained on a diet of *Enteromorpha*, the alga that gave the best growth. Values of growth efficiency for other seaweed diets decreased in relation to their growth supporting value to *Aplysia*, from 19 % for the *U. fasciata* to 10 % for the *U. pertusa*.

The reproductive potential of *A. juliana* is utilized from an early age and recruitment occurs throughout the year[21]. Among the several factors affecting reproduction of *Aplysia*[22-24], availability of food has a significant influence in the process. The occurrence of the first spawning in *Aplysia* differed among the different diet groups in our experiment, suggesting that algal quality can be an important determinant of the life cycle. The chronological order of the first spawning corresponded with the descending order for growth in the present study. The animals started to spawn earlier in the case of *Enteromorpha* diet, and later in the *U. fasciata* and *U. pertusa* diets. Egg production was positively correlated with temperature, when the data after the initial 10 days of the first egg laying activity was evaluated. This correlation is in agreement with other reports where the temperature effects on the oogenesis of some *Aplysia* species[22] has been described, linking it to increased activity of the neuroendocrine bag cell which releases the egg laying hormone (ELH)[25,26]. The absence of such a relationship during the initial 10 days, may be due to some inexplicable physiological process in the recently matured animals.

The results demonstrate the potential value of *Aply-*

sia as a biological control agent for macrophyte algae blooms which cause serious problems in commercial aquaculture ponds. Their rapid growth, high egg production, and voluminous food consumption make them ideal for introduction in aquaculture systems plagued with algae. These qualities, as well as the expanding market for *Aplysia* in the medical and pharmaceutical industries, will be an economical incentive for their use in polyculture systems.

This research is part of a study on possible applications of sea hares for biological control of macrophytic algae in aquaculture. These results have to be tried out in field conditions.

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3種の緑藻を給餌した場合のアマクサアメフラシ *Aplysia juliana* の摂餌，成長，および産卵

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ボウアオノリ，リボンアオサ，およびアナアオサに対するアマクサアメフラシの摂餌生態や餌料効果を比較するために，それら3種の緑藻を給餌した場合のアマクサアメフラシの摂餌率，生長，および産卵量等を調べた。飼育は湿重量で0.07～0.08 gのものを用いて開始し，室温下で成体まで130日間継続した。全飼育期間中の摂餌率は，ボウアオノリ，リボンアオサ，およびアナアオサの順に乾重量換算の体重比でそれぞれ8.5，14.6，および22.4%/dayと高い値を示し，アマクサアメフラシの摂餌量が極めて多いこと，特に，アナアオサが多く，次いでリボンアオサ，ボウアオノリの順であった。一方，日間生長率はボウアオノリ，リボンアオサ，アナアオサの順にそれぞれ0.14，0.12，0.10 dry-g/day，飼育期間中に生産された卵塊は積算乾重量としてそれぞれ25.1，20.8，10.8 dry-g/indiv.であった。なお，最初の産卵はボウアオノリでは飼育開始後15日目に，リボンアオサでは18日目に，アナアオサでは29日目に行われた。以上の結果から次のような事項が明らかになった。①アマクサアメフラシの摂餌量は極めて多い。なかでもアナアオサが多く，次いでリボンアオサ，ボウアオノリの順であった。②アマクサアメフラシの飼料転換効率は，体重増加に産卵量を加えたものを生長量とみなして算定した場合，ボウアオノリ，リボンアオサ，アナアオサの順に，それぞれ31.6，18.9，10.1%を示した。③アマクサアメフラシはこのように，良く食べ，速く成長するので，養殖水槽中に繁茂しやすい上記3種の緑藻の制御に利用できる可能性が伺えた。