アメフラシ（Aplysia dactylomela Rang）の摂餌と成長

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Food Consumption and Growth of *Aplysia dactylomela* Rang
(Gastropoda: Opisthobranchia)

Stefanos Palatzidis\(^1\), Shigehisa Yamasaki\(^1\), Hachiro Hirata\(^2\), and Takehiko Imai\(^1\)

\(^1\) Faculty of Fisheries, Kagoshima University, Shimoarata 4–50–20, Kagoshima 890, Japan
\(^2\) Faculty of Agriculture, Kinki University, 3327–204 Nakamachi, Nara 631, Japan

Abstract

The consumption of two macrophytic algae, namely *Ulva pertusa* and *Enteromorpha linza*, by the adult gastropoda *Aplysia dactylomela* was studied for a period of 36 days under laboratory conditions. Animals were fed three times a day to satiation. Both algae species were well consumed and promoted increase in the body weight, body length as well as the egg production of the gastropoda. The consumption of *Ulva* by *Aplysia* was 22.5% of mean body weight eaten per day, and was independent of temperature, but *Enteromorpha* consumption was 15.0% and increased with increasing temperatures. Nevertheless, animals fed on *Enteromorpha* had a better growth rate and egg production than those fed on *Ulva*. The gross assimilation efficiency was highest on the algae giving the better growth (29% for *Enteromorpha* and 14% for *Ulva*). The study found that the detrimental thalli of *Ulva pertusa* and also that of *Enteromorpha* may eventually be controlled by the introduction of gastropods in fish or shrimp ponds.

A common phenomenon in Japanese sea water aquaculture, during late spring and early summer is the rapid growth of macrophyte algae, like *Ulva* and *Enteromorpha*, when water quality parameters deteriorate. Initially the presence of the algae can improve the quality of water through an uptake of the toxic metabolites. Many researchers have shown the remarkable application of *Ulva* as a biofilter in fish and shrimp culture systems\(^1\)\(^-\)\(^5\). Nevertheless, uncontrolled algal growth, can lead to algal die-off, depleting D.O. levels, and releasing large amounts of toxic metabolites in to the culture pond depressing fish growth and/or causing death\(^6\). Shigeno observed that dead and rotten *Ulva sp.* led to heavy pollution, reducing dramatically the growth of shrimp, due to a negative oxidation–reduction potential of the pond bottoms\(^7\).

Several authors have demonstrated that polyculture, using herbivorous species to control algal blooms in commercial fish ponds, is an effective way to improve water quality\(^8\)\(^-\)\(^9\). Very few research have been conducted on the control of macrophytes in sea water aquaculture. Some workers, however, have shown that herbivorous gastropod abalone could be cultured using *Ulva lactuca* as the main source of food, to convert large quantities of rich algal biomass into a high value marketable commodity\(^10\)\(^-\)\(^11\).

The opisthobranch mollusc *Aplysia* has been reported as an efficient consumer of macrophytic algae, and grows much more rapidly than most marine animals\(^12\). Although various reports have shown the

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ability of *Aplysia* to consume *Ulva* and other macrophyte algae, few have studied the possibility of using this animal in polycultural systems. *Aplysia* is a species of potential economic value by itself, apart from being utilized as a model preparation for neurophysiological and neurobehavioral studies, because of its well identified cells with known interconnections and behavioral functions \(^{13,14}\). Furthermore, sea hares are of pharmacological interest and have attracted the attention of investigators working on chemical compounds of marine invertebrates. An interesting example is the characterization of the antibacterial and antineoplastic glycoproteins in *Aplysia juliana*, and *A. kurodai* \(^{15-16}\). Over and above this, in some countries eggs of sea hares are utilized as food.

The present work was conducted to study the potential use of the *Aplysia* in polyculture systems with *Ulva* and *Enteromorpha*. Therefore, one of the dominant species of sea hares *Aplysia dactylomela*, found in the Kagoshima bay, Southern Japan, was cultured in laboratory conditions with the two macrophyte species. Their ability to consume these two macrophytic algae, the improvement in body measurements, and egg production were observed over a period of 36 days.

**Materials and Methods**

*Field collections*

Reproductively mature individuals of *Aplysia dactylomela* Rang. body weights ranging between 170-180 g, were collected from the intertidal area close to the Kamoike Marine Experimental Station of Kagoshima University, Kagoshima Prefecture. Collections were made in mid-spring (March 1994) and represented the common body weights for *Aplysia dactylomela* in that season and area.

The particular biotope, formed by concrete breakwater extends 1-2 km parallel to the shore, creating a oblong, protected tide channel. Several conduits in the wall of breakwater allow the water circulation with the sea. Depth of the water body along the channel ranges up to 5 m. The animals were found in the habitat covered by the green alga *Enteromorpha linza*.

Thalli of *Enteromorpha linza* J. Agardh, were collected from the same biotope habituated by *Aplysia dactylomela* and that seemed to be the preferred food. Stock of *Ulva pertusa* Kjellman was obtained from Hirakawa Beach, Kagoshima Prefecture. Collections were made once a week, and the stock was renewed at the same time. Algae were maintained outdoors, under the natural varying lighting and temperature conditions in four (4) 100-liter circular Plexiglas tanks, filled with natural seawater. The algae were grown unattached and kept suspended in the water column by air diffusers situated at the bottom of each tank.

*Animal culture system*

Animals were acclimated for upto 1 week before being used for the experiment in two (2) 100 liter's outdoor Plexiglas tanks containing filtered and aerated natural seawater, that was replaced daily. The thalli of the green algae *Ulva pertusa* and *Enteromorpha linza* was offered as food and was available in great abundance at all times.

Three days prior to the experiment, the animals were starved, for better determination and selection of the initial weights. Animals with average $211.1 \pm 5.5$ g (range $199.7-219.8$ g) live body weights, were divided into 6 groups of 3 individuals for each algae. The approximately equal aggregate live weight groups of sea hares, were transferred into six (6) 45 l glass-walled aquaria. Moreover they were divided into two dietary groups. The aquaria were housed in a room, and covered with opaque material to minimize the influence of light and assure favorable culture conditions for the predominantly nocturnal sea hares. The sea water in the aquaria was renewed every day, filtered through a $5 \mu m$ mesh and stored for one day in 200 l circular tanks before use.

Every 6 days the animals were weighed, after being placed on a paper towel for a few seconds to drain off excess water. Body length was measured at full extension every 12 days. For the measurements, the animals were allowed to crawl on a transparent plastic material, forming a cylindrical tube forcing the animals to move forwards. The tube diameter was adjusted to suit the size of each animal. This tube, with the animal inside was submerged in the water with one extreme leaning downwards. Measurements were taken with a ruler inside the water, once the animal had extended the entire body length. Eggs were collected daily from
the holding aquaria, drained of excess water and weighed. A known amount of food was provided to saturation three times a day to each of the sea haries aquarium. The thalli of the algae given as food or uneaten remnants each time was washed with tap water and weighed after centrifugation for 3 min in a converted washing machine to remove excess water. Dry weight equivalents of body weights, egg masses and seaweeds were obtained by correcting for moisture content. Moisture content was obtained by drying 18 or 30 separate samples of animals, egg masses and seaweeds at 110°C and averaging the results.

The water temperature during the 36 day period ranged from about 17 to 21°C. This range of temperature, depending on season, reflects the natural conditions for Aplysia dactylomela. Water temperature was monitored twice daily in each aquarium. Dissolved oxygen (D.O.) was maintained close to air-saturation level with aeration. The salinity ranged from 36-37 ppt. The 36-day experimental period was long enough to double the average body weights in both treatments.

Analysis of data

Treatments were compared using a one-way classification of ANOVA (F-test) and the Scheffe test was used to detect the differences between the means. The level of statistical significance was accepted as $p < 0.05$.

Results

Growth and food consumption

The growth of Aplysia dactylomela, measured as dry weight increase, achieved with the two species of seaweeds is shown in Fig. 1. The growth data, including dry weights of food consumed and eggs produced by A. dactylomela fed on each alga, are summarized for the entire study period in Table 1. Of the two seaweeds studied, significantly higher ($p < 0.01$) rate of growth was attained on Enteromorpha linza. The green alga Ulva pertusa, also supported comparatively good growth. Similarly, biometrics for the length, in Aplysia feeding on Enteromorpha was significantly higher ($p < 0.05$), than on a diet of Ulva. The values of length growth rate were found to be 0.25 cm when food was Enteromorpha, and 0.15 cm for Ulva.

Fig. 1. Growth and egg production of Aplysia dactylomela fed on Enteromorpha linza and Ulva pertusa diets under laboratory conditions. Each point in the curve represents the mean ± S.D. of 9 animals. Each column represents the average weight of the egg masses deposited every day in each aquarium.

Over the period of 36 days Ulva is consumed more than Enteromorpha but overall growth on this alga is only 73% of that on Enteromorpha (Table 1). Feeding rates for each diet on a dry weight basis, were found to be 4.46 g/animal/day for Ulva, and 2.96 g/animal/day for Enteromorpha. For comparison, values including the dry weight of eggs produced as a measure of total growth, are presented in Table 1. The growth rate increased 0.27 g/animal/day, for the animals feeding on Enteromorpha and 0.15 g/animal/day for those feeding on Ulva, based on eggs produced. The gross assimilation efficiency over the total study period, calculated on a dry weight basis, is given for A. dactylomela on each algal diet in Table 1. Enteromorpha appears to give the higher growth efficiency of 28.5%. However A. dactylomela feeding on Ulva appear to convert the food into body tissues at an efficiency of 13.7%. On including the dry weight of eggs produced in the measure of total growth, the growth efficiency was adjusted to 37.6% for Aplysia eating Enteromorpha and 17.0% for Aplysia eating Ulva (Table 1).

Egg production

The amount of eggs produced by A. dactylomela feeding on each alga is given in Table 1. The dry weight of eggs produced per individual per day was 0.27 g for those eating Enteromorpha and 0.15 g for animals fed
Table 1. Growth characteristics, egg production and food consumption of *Aplysia dactylomela* fed on *Enteromorpha linza* and *Ulva pertusa* diets in laboratory conditions under a temperature regime 17-21 °C. Data expressed as mean ± SD

<table>
<thead>
<tr>
<th>Algal Diet Fed</th>
<th>Enteromorpha linza</th>
<th>Ulva pertusa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days kept</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Body weight dry, g/animal</td>
<td>19.8 ± 0.4**</td>
<td>19.8 ± 0.6</td>
</tr>
<tr>
<td>Initial</td>
<td>50.1 ± 1.7**</td>
<td>41.9 ± 1.5</td>
</tr>
<tr>
<td>Final (including egg production)</td>
<td>(59.8 ± 1.3)**</td>
<td>(47.1 ± 0.4)</td>
</tr>
<tr>
<td>Growth rate of body weight</td>
<td>0.84 ± 0.04**</td>
<td>0.61 ± 0.03</td>
</tr>
<tr>
<td>dry, g/animal/day</td>
<td>(1.11 ± 0.04)**</td>
<td>(0.76 ± 0.02)</td>
</tr>
<tr>
<td>Body length cm/animal</td>
<td>19.7 ± 1.0*</td>
<td>19.9 ± 1.1</td>
</tr>
<tr>
<td>Initial</td>
<td>28.6 ± 1.0*</td>
<td>25.4 ± 0.7</td>
</tr>
<tr>
<td>Rate of length growth cm/animal/day</td>
<td>0.25 ± 0.03*</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Amount of food consumed</td>
<td>106.6 ± 4.1**</td>
<td>160.4 ± 5.5</td>
</tr>
<tr>
<td>dry, g/animal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding rate dry, g/animal/day</td>
<td>2.96 ± 0.11**</td>
<td>4.46 ± 0.15</td>
</tr>
<tr>
<td>% of mean body weight eaten/day</td>
<td>15.0 ± 0.7*</td>
<td>22.5 ± 1.0</td>
</tr>
<tr>
<td>Gross assimilation efficiency (%)</td>
<td>28.5 ± 1.3**</td>
<td>13.7 ± 0.7</td>
</tr>
<tr>
<td>(including egg production)</td>
<td>(37.6 ± 1.2)**</td>
<td>(17.0 ± 0.3)</td>
</tr>
<tr>
<td>Total weight of eggs produced</td>
<td>9.69 ± 0.83*</td>
<td>5.26 ± 0.99</td>
</tr>
<tr>
<td>dry, g/animal</td>
<td>0.27 ± 0.02*</td>
<td>0.15 ± 0.03</td>
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Differences were, *significant at p < 0.05; **significant at p < 0.01.

1. Gross assimilation efficiency = growth (dry wt) ×100/ingested food (dry wt).

on *Ulva* (Table 1). The daily egg production of *Aplysia* fed on *Enteromorpha* and *Ulva* is shown in Fig. 1. Some periodicity that occurred in the egg laying behavior during the experimental period, depending on exogenous or endogenous factors, is also presented in Fig. 1.

Effect of temperature on consumption, and egg production

There was a strong positive correlation between the temperature and the consumption of *Enteromorpha* (R = 0.7; p < 0.05); unlike in the case of *Ulva*. The trend was the same as noted previously in the values of correlation between food consumption and egg production; the highest correlation was for *Aplysia* eating *Enteromorpha* (R = 0.742; p < 0.01). The correlation between the temperature and egg production was R = 0.768; p < 0.01 for the animals eating *Enteromorpha* and R = 0.565; p < 0.01 for those eating *Ulva*.

Discussion

The growth performance of the *Aplysia* in terms of body weight gain, length increase, egg production, and the consumption of the algae was significantly affected by the type of algae. While *Enteromorpha linza* promoted a better biological performance of the gastropoda, *Ulva pertusa* consumption (dry and wet weight basis) was significantly higher. Superior growth and egg production among the animals fed on *Enteromorpha* may indicate its higher nutritional value

17. and explain the
preference for this alga observed in animals grazing under natural conditions. Laboratory-grown *A. dactylomela* fed with *Enteromorpha sp.* had better growth rate, egg production, and higher food consumption, when compared to the *Ulva fasciata*\(^{17}\).

Our results showed a similar trend except for the fact, that the consumption of *Ulva pertusa* was significantly higher than that of the *Enteromorpha linza*. The lower consumption of *Enteromorpha* compared to *Ulva*, can be attributed to a dramatic increase in egg production, when the *Aplysia* was fed on this alga. While the body weight gain of animals fed on *Enteromorpha* were 38% higher than those fed on *Ulva*, egg production increased by 84%. Feed consumption is inhibited by the bag cell hormones that induce egg laying\(^{10}\). Furthermore, the animals feeding on *Enteromorpha*, spends a larger proportion of the time in laying and encapsulating the eggs and further packing the capsules into an egg string, diminishing the time available for food consumption\(^{13}\). On the other hand, since *Aplysia* rapidly consumes the *Ulva* diet, digestion and absorption of this alga may be lower\(^{20}\).

Consumption was positively correlated with temperature when the food was *Enteromorpha*, but no correlation was found between temperature and the consumption of *Ulva*. Egg production in both groups was affected by rise in temperature. This is in agreement with the report of other authors who found that temperature affected the oogenesis of some *Aplysia* species due to increased activity of the bag cell which release the egg laying hormone\(^{21,22}\). In this way the natural variations in temperature during the experiment may account for some synchronism in egg laying activity. Additionally, there is evidence that a pheromone released by egg laying animals induces others to lay eggs, affecting this synchronism\(^{23,24}\). The periodicity in egg laying process can be attributed to the time spent by the animals, for the biological preparation of eggs.

Mechanisms of food consumption by *Aplysia* have been thoroughly studied by various authors, and are related to food factors, like palatability and phagostimulants\(^{25-28}\) and bulk properties of the food\(^{29}\), as well as some behavioral factors related to mating and egg production\(^{24,30}\). Furthermore, food consumption or egg laying in *Aplysia* species and also in *A. dactylomela*, occur during the night\(^{29,31-33}\). Being a nocturnal species, *Aplysia* remains inactive during the day light, and normally feeds only during hours of darkness. In our experiment, the animals were kept under dark conditions; the continuous regime enabled constant feeding and produced greater growth and egg production. This explains why we could obtain an increase in body weight combined with egg production, contrary to the earlier findings where a stagnation or loss of body weight was reported during the periods when the adult animals were laying their eggs\(^{12,34,36}\).

The consumption of macrophytic algae by the gastropoda has been clearly demonstrated here and in other works\(^{17,29}\), indicating that it could be utilized to control the macrophytic swarm, especially that of the *Ulva pertusa*. Additional studies may consider employing other gastropoda species, and observe the improvement of water quality parameters. These factors, combined with the market for the animal either for neurophysiological examinations or for the pharmacological industry, will determine the feasibility of its introduction in polyculture systems.

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**アメフラシ（*Aplysia dactylomela* Rang）の摂餌と成長**

Stefanos Palatzidis¹・山崎繁久²・平田八郎³・今井健彦²

(¹鹿児島大学大学院農学研究科，²鹿児島大学水産学部，³近畿大学農学部)

養殖漁場等の栄養塩類の豊富な場所では、大型藻類のアナアオサ（*Ulva pertusa*）の成長が良く、水質浄化に大きく貢献する可能性が示唆されている。しかし、現在、アナアオサの利用はその検討が始まったばかりで有効な利用法の開発が待たれている。そこで、本実験では、食物連鎖に基づいたアナアオサの利用によりアナアオサの繁殖の制御を行う基礎研究として、大型藻類食性のアメフラシの摂餌率と成長率の関係を調べた。なお、アナアオサと比較するために、アナアオサと同様に魚介類の飼育槽に繁殖しやすいウスパアオノリ（*Enteromorpha linza*）も餌として用いた。アメフラシンによるアナアオサの摂餌率は、飼および動物の乾燥重量換算で22.5%/day、ウスパアオノリで15.0%/dayとなり、アナアオサで特に高い値を示した。その摂餌率は、アナアオサの場合は温度にあまり左右されなかったが、ウスパアオノリの場合は温度の上昇に伴って増加した。また、飼料転換効率は、アナアオサでは14%，ウスパアオノリでは20%となった。これらの値から明らかにアメフラシンの成長が極めて速いことを考慮すると、アナアオサに限らずウスパアオノリに関してもアメフラシンによるそれからの藻類の繁殖の、効率の良い制御が期待される。