

海産藻食性巻貝,サザエおよびバテイラの摂餌刺激物質検索 用生物試験法

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A Simple Feeding Stimulant Assay for Marine Herbivorous Gastropods, the Turban Shell *Turbo cornutus* and the Top Shell *Omphalius pfeifferi*^{*1,2}

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We have already established a simple and reliable feeding stimulant assay method for the young abalone *Haliotis discus* using Avicel (crystalline cellulose) plates. We recently found this simple assay procedure applicable to other marine herbivorous gastropods, the turban shell *Turbo cornutus* and the top shell *Omphalius pfeifferi*. The methanol extracts of several species of algae were subjected to the assay. Both test animals showed their feeding preference to the extracts of most species of algae tested. The extract of the green alga *U. pertusa* was the most active.

Gastropods are known to have olfactory and gustatory receptors and are considered to find their foods by chemoreception.¹⁻⁴⁾ We have been interested in chemical studies on feeding behaviors of marine herbivorous gastropods and have already established bioassay methods for phagostimulants for the young abalone *Haliotis discus*⁵⁾ and the sea hare *Aplysia juliana*.⁶⁾ We have clarified that such complex glycerolipids as digalactosyldiacylglycerol (DGDG) and phosphatidylcholine (PC) from the brown alga *Undaria pinnatifida* were phagostimulants for the abalone^{7,8)} and 1,2-diacylglyceryl-4'-O-(N,N,N-trimethyl)-homoserine (DGTH) from the green alga *Ulva pertusa* for the sea hare *Aplysia juliana*.⁹⁾

The turban shell ("sazae" in Japanese) *Turbo cornutus* and the top shell ("bateira" in Japanese) *Omphalius pfeifferi* are both marine herbivorous gastropods whose delicate tastes are loved by Japanese. Annual production of the turban shell has been decreased as a result of over-capturing and some trials for its seedling production have recently started in Japan.

Some biological studies on the turban shell clarified their feeding behaviors: i) they are nocturnal and begin food-searching after sunset;

ii) they feed on brown algae such as *Eisenia bicyclis* and *U. pinnatifida* and red algae *Gelidium* spp. and well grow on the brown alga *E. bicyclis*.¹⁰⁾

No report is found on feeding behavior of the top shell *O. pfeifferi*. Furthermore there is no report on the chemical studies on the feeding behaviors of these herbivorous gastropods. In this paper we describe the details of the bioassay procedure for their feeding stimulants.

Materials and Methods

Test Animals and Experimental Aquaria

The turban shell *T. cornutus* and the top shell *O. pfeifferi* were collected at Shimoda, Shizuoka, Japan or purchased from the city market. Ten to fifteen specimens of small turban shell (diameter of the opercula, 15-25 mm) were kept in each test aquarium (ca. 32×61×18 cm, a commercially available plastic container colored skyblue) into which the seawater filtered through sand was introduced keeping the depth (5 cm) of the water with a drain.

In the case of *Omphalius*, ten individuals (diameter of the opercula, 5-8 mm) were kept in each test aquarium (ca. 15×15×60 cm, a commercially

*1 Chemical Studies on Phagostimulants for Marine Gastropods. Part VII. For Part VI, see Reference 11.

*2 A part (on *Turbo*) of this report was presented at the autumn meeting of the Japanese Society of Scientific Fisheries, Kagoshima, October, 1985.

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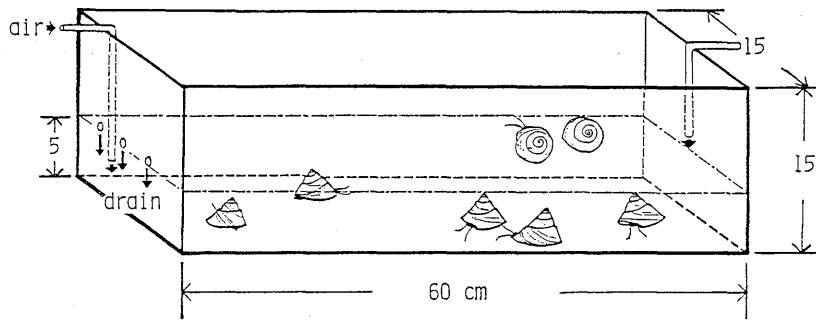


Fig. 1. A diagrammatic view of an experimental aquarium used for *O. pfeifferi*.

available plastic container colored dark blue, Fig. 1) with the running seawater of 5 cm depth. A few pieces of dried brown alga *E. bicyclis* were fed the both test animals once every a few days as a maintenance food.

Assay Method for the Feeding Stimulant Activity

The test animals were starved for at least a day before the assay. Methanol extracts of each alga were obtained by the same way as previously reported.⁵⁾ A part of each methanol extract was concentrated to give a test sample solution. The test sample solutions (50–100 μ l) were applied on the sample zone (25–35 mm in diameter) made by a compass on an Avicel plate, which was made in the conventional way [coating Avicel SF (crystalline cellulose, Asahi Kasei Co. Ltd.) on glass plates (5 or 10 \times 20 cm) in 0.25 mm thickness followed by drying at 120°C for 1 h].⁵⁾ The sample zone treated with only the solvent was assayed as a reference. The test plates were set at the bottom of the test aquarium after sunset. The seawater supply was stopped during the assay.

Next morning, the test plates were taken out for judgment of the activity. In the case of *Turbo*, however, the test plates were usually taken out at around 10.00 p.m. (about 3–4 h after beginning of the assay), because *Turbo* often bit off almost all the Avicel on the test plate. When the feeding traces on the plate were not enough to judge the activity, they were kept in the test aquaria till next morning. The assay was repeated at least twice. Assay results were more reproducible in the case of *Omphalius* than *Turbo*.

Fig. 2 shows typical assay results obtained by using *T. cornutus* (upper) and *O. pfeifferi* (lower). Typical feeding traces were found on the plate. Judgment of the activity of each sample was

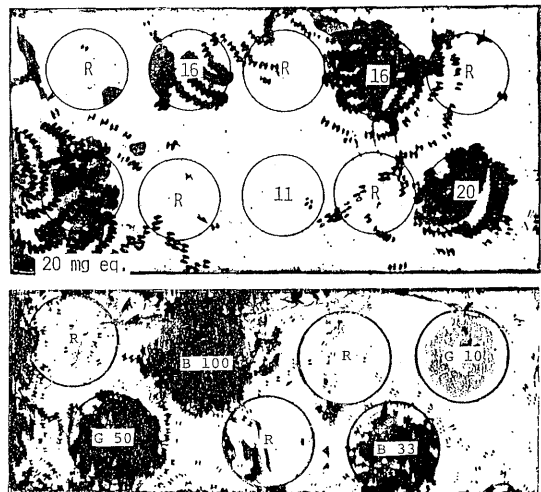


Fig. 2. Typical assay results obtained by using *T. cornutus* (upper) and *O. pfeifferi* (lower).

The upper: By *T. cornutus*. R, reference, without any sample; the MeOH extracts equivalent to 11, 16, and 20 mg of air dried brown alga *U. pinnatifida* were absorbed in each sample zone.

The lower: By *O. pfeifferi*. R, reference; G, green alga *Ulva pertusa*; B, brown alga *Undaria pinnatifida*; sample amount equivalent to the fresh weight (mg) of alga giving each extract. Details of the assay were shown in the text.

performed as in the case of the abalone: ++, the characteristic biting traces similar to those of abalones are observed almost all over the sample zone, and the activity can be judged clearly; +, much more biting traces are found in the sample zone than those outside in a unit area (the maximum area containing only the sample zone in question), but the judgment is not very clear; \pm , a few more biting traces are observed in the sample zone than outside; and -, none

or nearly the same number of the biting traces are left inside of the sample zone as those outside.⁵⁾

Results and Discussion

Based on the experience of establishing the simple and reliable bioassay ("Avicel plate method") for feeding stimulants for *Haliotis*,⁵⁾ we attempted to establish a feeding stimulant assay for the other marine herbivorous gastropods such as *T. cornutus* and *O. pfeifferi*. The Avicel plate method also proved to be applicable to these gastropods.

Typical assay results were shown in Fig. 2. Estimation of the activity was generally made in the same manner as in the case of *Haliotis*.⁵⁾ We found this time two critical points which give a big influence on the results of this assay: starvation degree and assay duration.

The test animals were usually starved for a day before the assay. It gave good results in the case of *Haliotis*⁵⁾ and *Omphalius*. In the case of *Turbo*, it was very important how much the test animals were starved before the assay. When the test plates were kept in the test aquarium till next morning, they often ate almost all the Avicel on the plates because of hungeriness, we guess, and judgment of the activity was impossible. It never happened in the case of *Haliotis* and

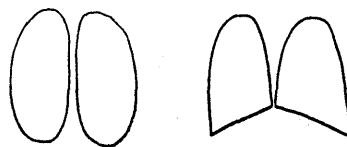


Fig. 3. A diagrammatic drawing of the feeding traces made by *T. cornutus* (left) and *O. pfeifferi* (right).

Omphalius. Shortening the assay duration from sunset to around 10.00 p.m., however, overcame this problem and gave good results.

Interestingly the feeding traces left by *Turbo* have the same shape as those made by *Haliotis*, but are clearly different from those by *Omphalius* as shown in Fig. 3.

Several algal methanol extracts were subjected to the assay (Table 1). Feeding behavior of both test animals were generally strongly stimulated their feeding by most of the extracts tested so far. The extract of the green alga *Ulva pertusa* was one of the most active among them for not only the both test animals but also *Haliotis* and *Aplysia*. Accordingly, *U. pertusa* was the one source of algal material used in the isolation of the phagostimulants contained in the alga to isolate several glycerolipids like digalactosyldiacylglycerol (DGDG), 1,2-diacylglycerol-4'-O-(*N,N,N*-tri-

Table 1. Feeding stimulant activity of the algal methanol extracts for herbivorous gastropods, *Turbo cornutus* and *Omphalius pfeifferi*

Name of Algae		MED Value*	
Scientific	Japanese	<i>T. cornutus</i>	<i>O. pfeifferi</i>
[Chlorophyceae]			
Ulvaceae			
<i>Ulva pertusa</i>	"Anaaosa"	<50	<50
<i>Ulva conglobata</i>	"Botan aosa"	<50	30
[Phaeophyceae]			
Dictyotaceae			
<i>Padina arborescens</i>	"Umiuchiwa"	50	<50
Laminariaceae			
<i>Eisenia bicyclis</i>	"Arame"	50	25
Alariaceae			
<i>Undaria pinnatifida</i>	"Wakame"	(16)	(8)
[Rhodophyceae]			
Chaetangiaceae			
<i>Pseudogloiophloea okamurai</i>	"Nisefusanori"	50	25
Cryptonemiaceae			
<i>Gratelopuia okamurai</i>	"Kyonohimo"	100	50
<i>Pachymeniopsis lanceolata</i>	"Fudaraku"	50	25

* Minimum effective dose (mg), the minimum amount of the methanol extract equivalent to each fresh alga to show "+" activity. The signs (< and >) mean that the minimum effective dose has not been determined and was clearly judged less (<) or more (>) than the value indicated, respectively, from the assay results. The values in parentheses are expressed as the amount of the methanol extracts equivalent to the airdried alga (weighing about one fourth of fresh one).

methyl)-homoserine (DGTH), 6-sulfoquinovosyl-diacylglycerol (SQDG), and so on.¹¹⁾

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