クロアワビに対する摂餌刺激評価法

Journal of National Fisheries University 52 (2) 63−66 (2004)

A Rapid and Simple Method for Evaluating Feeding Stimulants for Black Abalone *Haliotis discus*[*1]

Katsuhiko HARADA[*2] and Taiko MIYASAKI[*2]

Large numbers of black abalone *Haliotis discus* are cultured in Japan. The juveniles are eventually released as a means of restocking natural populations. Stimulating young black abalone to eat in culture tanks is thus important. To do this, methods are needed to evaluate feeding stimulants. Many studies on feeding stimulants for diverse gastropods have been reported, and various methods for identifying stimulants have been devised based on their specific behaviors.[1-14] Many methods for measuring the response to stimulants are based on the amounts of food eaten.[1,2,6,10,14] However, these methods take generally long time. Faster (although less quantitative) tests have been developed based on apparent feeding behavior; aggregation on spirogyra,[3] aroused behavior in response to green laver,[4] proboscis extension to shrimp,[5] eating movement cycle performed by the buccal-radula system to sugars,[8] approach to seaweed,[9] changing position in response to amino acids,[11] both proboscis extension and biting in response to amino acids.[12] However these behavioral-based methods are not standardized because the feeding behaviors generally vary among different gastropods, and none was specific to young black abalone. In this paper, we describe a new behavioral-based method for evaluating feeding stimulants for young black abalone that is more rapid, simple and quantitative than ones used previously. As a means of testing this method, we used green laver *Ulva pertusa* as a test stimulant.

Materials and Methods

Test animals

Juvenile black abalone *Haliotis discus* (average shell length 1.0cm) were supplied by Yamaguchi Gaikai Sea Farming Center and placed in a opaque polyvinyl chloride test tank (62 x 30 x 15cm deep). The numbers of test animals used in each experiment ranged from 30 to 50. The abalones were reared in the test tank with running seawater and aeration at room temperature (20 to 25°C) and fed freely at 4-day intervals with whole green laver *Ulva pertusa* exclusively. The laver was placed in the tank in the evening and removed the following morning. Behavioral tests were conducted only on the fourth day of starvation.

Test materials

Fresh thalli of the green laver *Ulva pertusa* obtained around the local seashore were exclusively used. The thalli were mixed with an equal volume of seawater (v/w) for 60 min at room temperature, and the seawater was filtered through No. 2 filter paper (Advantec Co.). The filtrate was used as the thallus test solution. The test solution (25 μl) was gently applied to the head region of an abalone with a microsyringe. The behaviors described below were observed within about 3 sec after the application.

Observation of feeding behaviors using whole thallus

In general, the behaviors observed in the abalones before the behavioral test could be divided into static behavior (without extending bilateral tentacles) and dynamic behavior (extending and varying bilateral tentacles or constantly wandering without even extending tentacles). The dynamic abalones behaved irregularly when exposed to a stimulant, whereas the static ones showed a series of uniform feeding behaviors: exploratory and searching behavior with tentacles, protruding proboscis and finally head lifting or mushroom behavior. Therefore, static abalones were exclusively used for the behavioral test.

2003年8月21日受付. Received August. 21, 2003.

＊1 Studies on the Feeding Stimulants for Fishes and Shellfishes-XLII.

＊2 Department of Food Science and technology, National Fisheries University (原田勝彦・宮崎泰幸：水産大学校食品化学科).

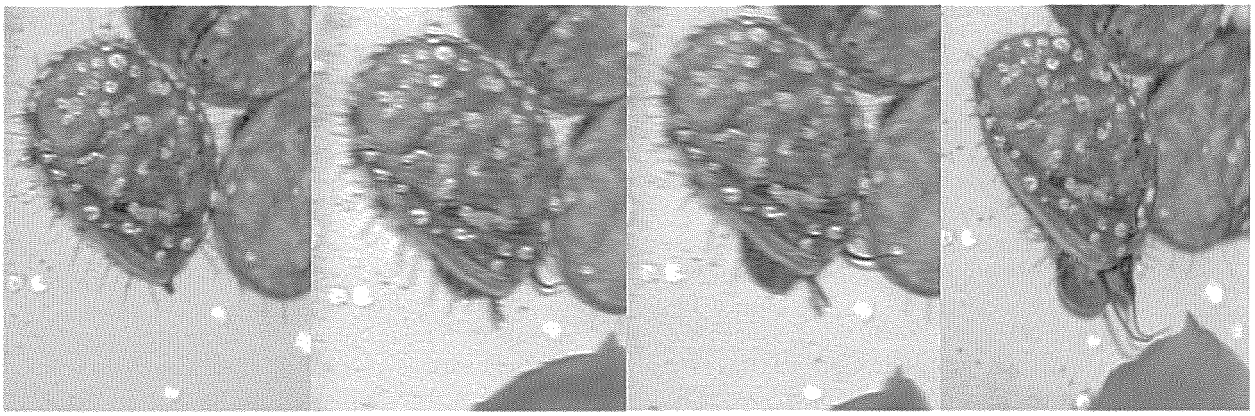
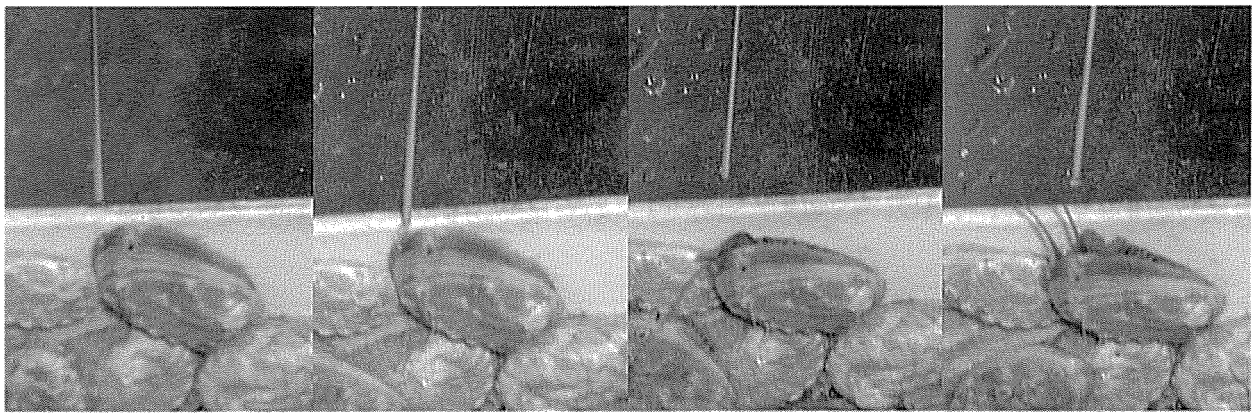


Fig. 1. Representative feeding behavior patterns of static abalone to green laver.



Seawater 1/10-diluted 1/2-diluted non-diluted

Fig. 2. Representative feeding behavior patterns of static abalone to diluted test solution.

Observation of apparent feeding behavior

We first tried to develop an evaluation method for stimulants using the whole thallus of green laver. Photographs of a series of feeding behaviors observed within about 10 sec after the application of the laver are shown in Fig. 1. The observed behaviors in response to exposure to fresh whole thallus were scored as follows. Abalone that did not extend bilateral cephalic tentacles (static abalone) were scored as 0; abalone that extended the tentacles were scored as 1; abalone that extended the tentacles and protruded the proboscis were scored as 2; abalone that lifted the head or elevated the body (showing specific mushroom behavior) were scored as 3. Similar behaviors were observed in snail *Busycon carica*[1], common sea hare *Aplysia juliana*[4], mud snail *Nassarius obsoletus*[5], and sea hare *Aplysia californica*[6]. These behaviors may be shared by most gastropods. The tests were then repeated using test solutions exposed to different treatments to confirm the validity of the results.

Results and Discussion

Dilution of thallus test solution

To ascertain the validity of this evaluation method, the stimulant activity score was recorded for different dilutions of the thallus test solution. The results are shown in Table 1. Photographs are shown in Fig. 2. For each dilution of the test solution, the individual scores tended to be similar. As expected, the scores increased with increasing concentration of the test solution.

Properties of thallus test solution

Test solution prepared with an immersion time of 60 min had the highest stimulation activity (Table 1).

Test solution stored at -80°C was found to retain its activity for more than one month (Table 1). Test solutions

Table. 1. Effects of dilution, immersion time, freezing and heating on the ability of a test solution prepared from *Ulva pertusa* to stimulate feeding of Juvenile black abalone *Haliotis discus*

Treatment		Raw Scores*	Score (mean ± S.D.)
Dilution of test solution	0 (Seawater)	0, 0, 0, 0, 0	0 ± 0
	1/10	1, 0, 1, 1, 1	0.8 ± 0.4
	1/2	2, 1, 2, 2, 2	1.8 ± 0.4
	1	3, 2, 3, 2, 3	2.6 ± 0.5
Immersion time (min)	0	0, 0, 0, 0, 0	0 ± 0
	20	1, 2, 1, 2, 1	1.4 ± 0.5[a]**
	40	2, 2, 1, 2, 2	1.8 ± 0.4[a]
	60	2, 3, 2, 3, 3	2.6 ± 0.5
Frozen time (day)	0	3, 2, 3, 3, 3	2.8 ± 0.4[b]
	16	3, 2, 3, 2, 3	2.6 ± 0.5[b]
	33	3, 3, 3, 3, 3	3.0 ± 0[b]
	43	3, 3, 3, 2, 2	2.6 ± 0.5[b]
Heating at 100°C (min)	0	2, 3, 3, 3, 2	2.6 ± 0.5[c]
	5	2, 2, 3, 3, 2	2.4 ± 0.5[c]
	10	3, 3, 3, 2, 3	2.8 ± 0.4[c]

* , n= 5 individuals for each test. Meanings of scores are described in Methods.
**, Values among each treatment not sharing a common superscript letter are significantly ($p<0.05$) different when tested by t-test.

heated to 94°C for 5 or 10 min were found to retain some activity (Table 1), indicating that some components are heat-stable and non-volatile.

Algae produce many extracellular products at various concentrations.[15] Among these substances, some carbohydrates and nitrogenous compounds were found to stimulate feeding activity in various gastropods.[16] Because the metabolism of algae is different from that of animals, algae may produce some novel substances that act as stimuli for the black abalone.

References

1) M. R. Carriker: *Ecology*, **32**, 73-83 (1951).
2) R. T. Paine: *Veliger*, **6**, 1-9 (1963).
3) R. V. Bovjerg: *Malacogogia*, **2**, 199-207 (1965).
4) H. Frings and C. Frings: *Biol. Bull.*, **128**, 211-217 (1965).
5) W. E. S. Carr: *Biol. Bull.*, **132**, 90-105 (1967).
6) S. Kikuchi, Y. Sakurai, M. Sasaki, and T. Ito: *Bull. Tohoku Reg. Fish. Res. Lab.*, No.27, 93-100 (1967).
7) M. Crisp: *J. mar.biol. Ass. UK*, **51**, 865-890 (1971).
8) J. C. Jager: *Nether. J. Zool.*, **21**, 1-59 (1971).
9) R. J. Prestone and R. M. Lee: *J. Comp. Physiol. Psychol.*, **82**, 368-381 (1973).
10) T. H. Carefoot: *J. exp. mar. Biol. Ecol.*, **42**, 241-252 (1980).
11) J. D. Thomas, B. Assefa, C. Cowley, and J. Ofosu-Barko: *Comp. Biochem. Physiol.*, **66C**, 17-27 (1980).
12) G. Bicker, W. J. Davis, E. M. Matera, M. P. Kovac, and D. J. Stormo Gipson: *J. Comp. Physiol.*, **149**, 221-234 (1982).
13) D. Rittschof, L. G. Williams, B. Brown, and M. R. Carriker: *Biol. Bull.*, **164**, 493-505 (1983).
14) K. Sakata, T. Itoh, and K. Ina: *Agric. Biol. Chem.*, **48**, 425-429 (1984).
15) J. A. Hellebust: in "Algal Physiology and Biochemistry" (ed by W. D. Stewart), Blackwell Scientific, Oxford, 1974, pp.838-863.
16) K. Harada and Y. Akishima: *Bull. Jap. Soc. Sci. Fish.*, **51**, 2051-2058 (1985).

クロアワビに対する摂餌刺激評価法

原田勝彦・宮崎泰幸

クロアワビのアナアオサに対する摂餌刺激行動から，迅速な簡易な刺激評価法を確立した。触角を出していない静止貝の頭部に，アナアオサを浸漬した試験液をマイクロシリンジで噴射した。そのときの行動を四種類に分けて数値化した。静止の状態，触角を出す，その上吻端を出す，さらに頭部あるいは体を持ち上げる行動を，それぞれ０，１，２，３と評価した。つまり数値が高いほど摂餌効果が強いことを意味する。一連の試験からアナアオサ浸漬液は高い評価が得られ，その有効物質は耐熱性並びに対凍性であることが示唆された。