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The Importance of Some Marine Red Algae Inhabiting Fishing-port Waterbreak Vertical Surface as Natural Food for Juvenile Horned Turban *Turbo* (*Batillus*) *cornutus*

Daisuke FUJITA*[1], Hideo OKADA*[2] and Kanzo SAKATA*[2]

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The vertical surface of Ozakai fishing-port waterbreak in Himi has been utilized as a nursery ground of horned turban, Turbo (Batillus) cornutus. There inhabited 52 species of marine algae, among which *Gelidium amansii* was the most dominant. As the result of feeding experiment, *Hypnea charoides*, *Centroceras clavulatum*, *G. amansii* and *Pterocladia capillacea* were foraged well in turn, but *Plocamium leptophyllum* was not. Feeding activity evaluated by Avicel Plate Method showed that the shells grazed Avicel zones of the palatable algal MeOH extracts, but not those of *P. leptophyllum*, though TLC analysis of the extracts showed the presence of potent feeding-stimulant glycerolipids for all species. The results suggest the existence of feeding inhibitors for chemical defense. Among the red algae, *G. amansii* seems to be the most important algal food for horned turban in the fishing-port waterbreak vertical surface because of all-year-round abundance and absence of feeding-inhibitor.

Key words; feeding stimulant, *Gelidium amansii*, glycerolipids, Hypnea charoides, marine algal flora, marine red algae, *Turbo* (*Batillus*) *cornutus*.

Horned turban or top shell, *Turbo* (*Batillus*) *cornutus* ("Sazae") is one of the most important marine archaeogastropods in Toyama Prefecture. The meat of this horned turban is often eaten raw as "Sashimi" or grilled as "Tsuboyaki" . Recently, the catch and import from neighboring Noto district (Ishikawa Prefecture) have been rapidly increasing in Himi district (Fig.1) , where the horned turban is consumed in many tourist guest houses. As seeding production of horned turban has recently been introduced at the Toyama Prefectural Fish Farming Center (TPFFC) (Kotaniguchi and Fujita 1989, 1990) , the first author has been trying the experimental marine ranching. As a result of the trial conducted over a year time, two-year-old horned turban grew well when released on

*[1] Toyama Prefectural Fisheries Experiment Station, Namerikawa, Toyama 936,Japan.

*[2] Research Laboratory of Marine Biological Science, Faculty of Agriculture, Shizuoka University, Mochimune, Shizuoka 421-01, Japan.

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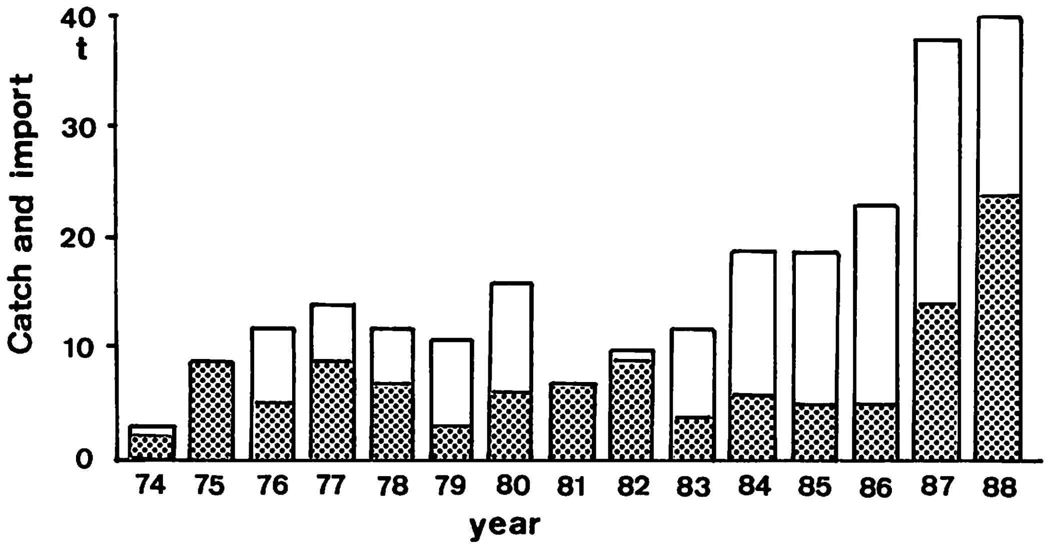

Fig.1 Recent catch (dotted) of horned turned *Turbo* (*Batillus*) *cornutus* in Himi, Toyama Prefecture, and import (white) from Noto District, Ishikawa Prefecture.

the inner vertical surface of a fishing-port waterbreak, which served as a nursery ground. These surfaces are not only protected from waves, also provided for suitable microhabitats (e.g., slits between concrete blocks, spaces behind sessile animals) and abundant marine algal food. However, no biological or chemical studies have been made on the interrelation between released juvenile horned turbans and their marine algal food. In this paper, marine algal flora and seasonal changes of dominant red algal species were investigated on the vertical surface of a fishing-port waterbreak in Himi. Feeding experiment of the shell using dominant red alga, and bioassay to determine the response of the shell to methanol extracts from these algae were also carried out.

Materials and Methods

Study Site Both the investigation of algal flora and collections for experiments were conducted at the inner vertical surface of the east waterbreak of Ozakai fishing-port, Himi, along the west coast of Toyama Bay. The location and overview of the east waterbreak is shown in Figure.2.

Algal collections and weighing For the investigation of algal flora, two points (ca. 2m depth) of vertical surface were selected and two marine algal samples within $0.2m^2$ (5 quadrat meshes of $0.2\times0.2m$) were harvested six times from August, 1988 to July, 1989 by hand from each point; one sample from the shallower and the other from the deeper square meter. The algae were put into cloth bags and kept in icebox. Identification and weighing were carried out at

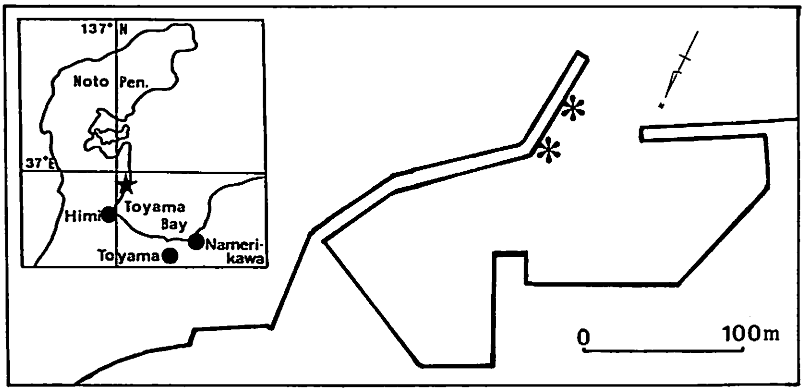

Fig.2 Location(★)and overview of Ozakai fishing-port in Himi. Algae for experiments were collected these sites(✻).

Toyama Prefectural Fisheries Experiment Station (TPFES) at Namerikawa. The wet weight of the collected algae was measured after wiping off the attatched water with paper towels.

The algae for feeding experiment and chemical analysis were collected around the waterbreak in September, 1989, and brought to TPFES with the same way as described above. The algae for feeding experiment were kept alive in an outdoor aerated concrete tank (395×95×65cm height, 56cm depth) at TPFES until used. The algae for bioassay were dried for a few days, and then sent to the Research Laboratory of Marine Biological Science (RLMBS) , Faculty of Agriculture, Shizuoka University in Shizuoka.

Feeding experiment Feeding experiment was conducted in September, 1989. Of all marine algae on the waterbreak, five dominant species of red algae;*Gelidium amansii*, *Pterocladia capillacea*, *Hypnea charoides*, *Plocamium leptophyllum* and *Centroceras clavulatum* were selected for the experiment. One-year-old juvenile horned turbans of 15mm shell height, which were hatched and cultured at TPFFC in Himi were used for the experiment. Twenty juveniles were placed with 5g of one algal species in an aerated plastic bottle (1 ℓ) , with a perforated screw-cap for an aeration tube. Five bottles were set in a concrete tank of the same size as above, to keep water temperature in the bottle about 22℃ with running water. The leftover algae (i.e., intact algae and broken pieces of branch) were taken out for weighing after 2, 4, and 12 days. These leftover algal samples were wiped with paper towel, weighed and then returned to the same bottle with new seawater. In the preliminary bottle culture of these algae alone, no growth or deteriolation was observed for two weeks probably because of low light intensity and the short period of time.

Bioassay method for the feeding stimulant activity of algal MeOH extracts.

Each air-dried alga (100g) was extracted with MeOH (ca. 300mℓ×3) at room temperature. The combined extracts were concentrated under reduced pressure to give an algal MeOH extract (ca.100㎖) .

The test Avicel plates (5×20㎝ with 0.25㎜ thickness) were made in the same way as previously reported (Sakata et al., 1984) . Each algal extract (20-40 μ ℓ equivalent to ca.10-50㎎ of the air-dried alga) was applied with a microsyringe as evenly as possible on a sample zone (23㎜ in diameter) made on the test plate. Horned turban juveniles of 10-40㎜SH (=shell height) were transferred from TPFFC to RLMBS. Fifteen to twenty shells were kept in a compartment of a polyvinyl-choloride aquarium (36×35×15㎝) seperated into three compartments (12×35×15㎝) and was set in a box of styrene foam (Sakata et al., 1990). Water depth was maintained at 5㎝. Seawater temperature was maintained at 22℃ by circulation through an about 2㎜ diameter hole in the water circulation tube at about 250㎖/min. The air-dried brown alga, *Laminaria angustata* var. *longissima* as maintenance food were given to the shells once every a few days, and starved for a day before the assay.

The test plate for the assay was set in a compartment of the aquarium after sunset. The next morning, grazing patterns on the test plates were checked for feeding activity.

Fractionation and thin-layer chromatography (TLC) of algal MeOH extracts

The MeOH extracts of *H. charoides*, *G. amansii* and *P. leptophyllum* were concentrated and subjected to partition between ethyl acetate and water. Each ethyl acetate layer was chromatographed on silica gel (Wakogel C−300, $CHCl_3$−MeOH; MeOH−H_2O) to separate into several fractions. Each fraction was concentrated under reduced pressure and then subjected to the bioassay (mentioned above) and TLC analysis. The condition of TLC analysis is as follows: silica gel GF_{254} (Merck) ; $CHCl_3$−MeOH−AcOH−H_2O=100:25:12:5;BuOH−AcOH−H_12O=4:1:2.

Results

Algal Flora Marine algal species collected from the inner vertical surface of Ozakai fishing-port waterbreak are listed in Table 1. Fifty-two species were collected there, among which *Amphiroa misakiensis*, *Jania arborescens*, *Hypoglossum japonicum*, *Laurencia venusta* and *Lophocladia japonica* were new to algal flora of Toyama Bay (Fujita and Izumi 1989) . The seasonal changes of total algal standing crop (average of 2 samples) were shown in Figure 3. Algae covered most of the substrata (i.e., surface of waterbreak and sessile animals' shells) all year round. Total algal standing crop showed maxima in September and May. Among these algae, *G. amansii* was the most dominant nearly all the year through. In another aspect, the average algal standing crop of shallower samples (0-1mdepth, N=2) was larger than that of deeper samples (1-2mdepth,

Table 1. List of marine algae inhabiting the inner vertical face of Ozakai fishing-port waterbreak.

Chlorophyceae(7)	Rhodophyceae(30)
Ulva pertusa	*Goniotrichum alsidii*
Chaetomorpha aerea	*Erythrocladia irregularis*
Chaetomorpha crassa	*Gelidium amansii*
Cladophora sakaii	*Pterocladia capillacea*
Caulerpa okamurae	*Peyssonellia conchicola*
Codium adhaerens	*Amphiroa beauvoisii*
Codium fragile	*Amphiroa misakiensis*
	Fosliella farinosa
	Jania arborescens,
Phaeophyceae(15)	Pneophyllum lejolisii
Ectocarpus sp.	*Lithophyllum tumidulume*
Sphacelaria sp.	*Grateloupia imbricata*
Leathesia difformis	*Plocamium leptophyllum*
Colpomenia sinuosa	*Plocamium telfairiae*
Undaria pinnatifida	*Hypnea charoides*
Ecklonia kurome	*Hypnea flagelliformis*
Dictyopteris prolifera	*Gracilaria bursa—pastoris*
Dictyopteris undulata	*Gracilaria verrucosa*
Dictyota dichotoma	*Gracilaria textorii*
Dictyota linearis	*Gymnogongurus flabelliformis*
Dilophus okamurae	*Champia parvula*
Pachydictyon coriaceum	*Antithamnion nipponicum*
Sargassum macrocarpum	*Callithamnion corymbosum*
Sargassum micracanthum	*Centroceras clavulatum*
Sargassum ringgoldianum	*Ceramium boydenii*
	Spyridia filamentosa
	Dasya sessilis
	Hypoglossum nipponicum
	Laurencia intermedia
	Lophocladia japonica

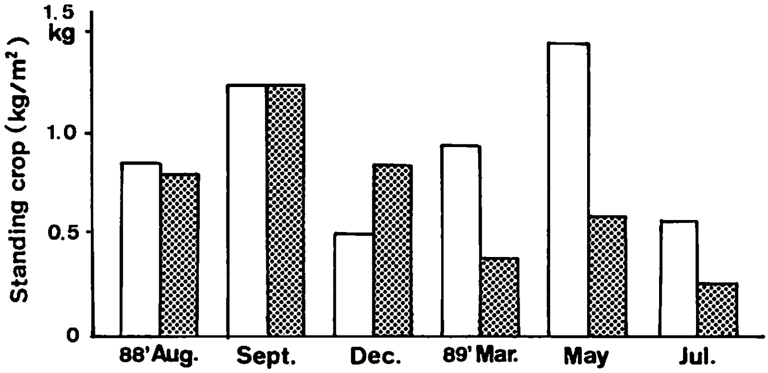

Fig.3 Seasonal changes of total algal standing crop on the inner vertical surface of Ozakai fishing-port waterbreak. White bar:0-1m; dotted bar:1-2m depth.

N=2) except for the samples of December.

The seasonal changes of each algal standing crop is shown in Figure 4. *G. amansii* distributed more abundantly in shallower areas all through the year (Fig.4A). *P. capillacea* also grew all year round (Fig.4B), but *H. charoides* only in summer (Fig.4C). *P. leptophyllum* flourished in autumn in deeper areas (Fig.4D). In addition, two species of small filamentous red algae were frequently observed: *Centroceras clavulatum* formed tufts near water level, and *Antithamnion japonicum* densely covered deteriorating algal uprights, especially of *Laurencia intermedia*. As well, *Peyssonellia* conchicola and some unidentified thin crustose coralline algae covered the vertical surface in places.

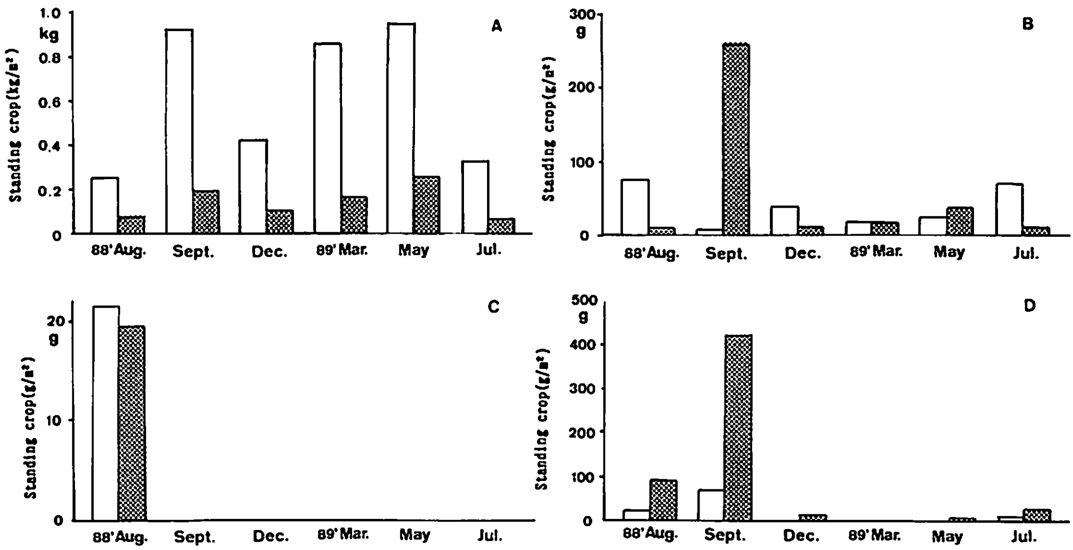

Fig.4 Seasonal changes of each dominant red algal standing crop on the inner vertical surface of Ozakai fishing-port waterbreak.
A:*Gelidium amansii*, B:*Pterocladia capillacea*, C:*Hypnea charoides*, D:*Plocamium leptopyllum*. White ber:0-1m; dotted bar:1-2m depth.

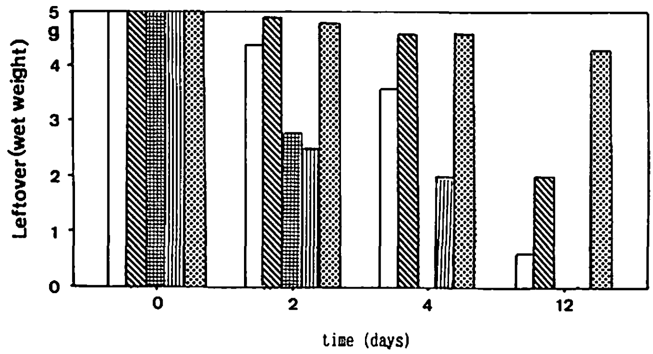

Fig.5 Changes of wet weight leftovers of five red algae fed to juvenile horned turban in the feeding experiment.
White:*Gelidium amansii*;Shaded:*Pterocladia capillacea*;Cross:*Hypnea charoides*;
Line:*Centroceras clavulatum*;Dotted:*Plocamium leptophyllum*.

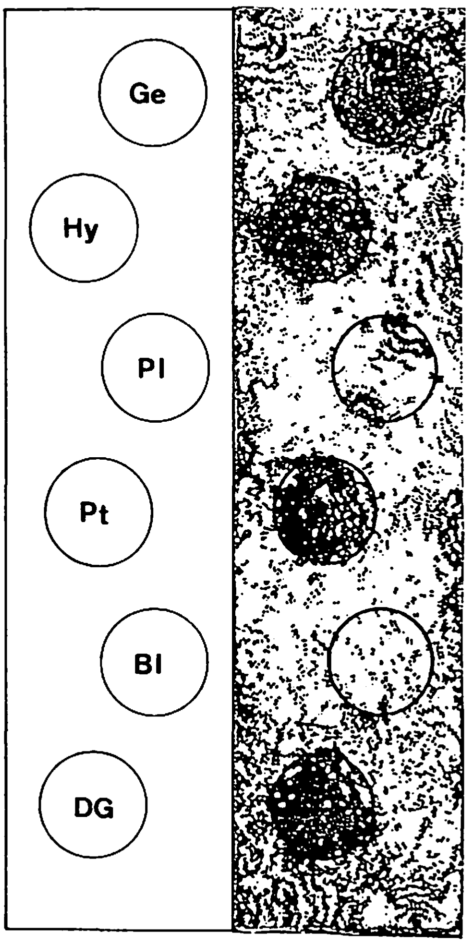

Fig.6 Typical assay result for juvenile horned turban feeding activity using Avicel plate method. Bl:Blank; DG:DGDG; Ge:*Gelidium amansii*;
Hy:Hypnea charoides; Pl:*Plocamium leptophyllum*; Pt:*Pterocladia capillacea*.

Table 2. Assay results of algal MeOH extracts for juvenile horned turban.

Species	Feeding Activity 10㎜(SH)				30−40㎜(SH)			
	−	±	+	++	−	±	+	++
Gelidium amansii	1	0	2	3	0	0	1	4
Pterocladia capillacea	4	0	2	0	1	0	0	4
Hypnea charoides	1	0	3	2	0	0	1	4
Plocamium leptophyllum	−	−	−	−	1	1	0	0

−: No or nearly the same number of traces are left inside the sample zone as those outside.
±: A few more traces are observed inside the sample zone than outside.
+: Clear difference of traces are observed between inside the sample zone and outside.
++:Almost all the Avicel in the sample zone has been grazed off.

Feeding Experiment As shown in Figure 5, juvenile horned turbans foraged *H. charoides* most rapidly, and *C. clavulatum*, *G. amansii*, *P. capillacea* in turn. *P. leptophyllum*, however, were hardly foraged over the 12-day experimental period.

The shells grazed slender branches of these algae, as if eating noodles, grasping the branches with their feet. In the case of *G. amansii*, the shells cut 1.2㎜-long branches (mean value, N=5) with their radulla and jaw plates. Bioassay and TLC of the algal MeOH extracts Typical assay plate is shown in Figure 6, and results were summarized in Table 2. Juvenile horned turbans of both size (10 and 30-40㎜ SH) grazed off within each sample zone of *G. amansii*, *H. charoides* and *P. capillacea* MeOH extracts on the Avicel plates. It seemed that palatability of larger shells (30-40㎜ SH) to these algae were clearer than that of smaller shells (10㎜ SH) . The larger shells, however, rarely grazed or even came close to the sample zone of *P. leptophyllum*.

The results of TLC analysis of the extracts showed that one of the most active fractions of each algal species (e.g., Fr.E in *H. charoides* extract, Fig.7) contains at least two potent feeding-stimulant glycerolipids, digalactosyldiacylglycerol (DGDG) and 6-sulfoquinovosyldiacylglycerol (SQDG) .

Discussion

Fishing−port waterbreaks have great potential as nursery grounds in the course of horned turban ranching. Marine algae grew abundantly on the inner

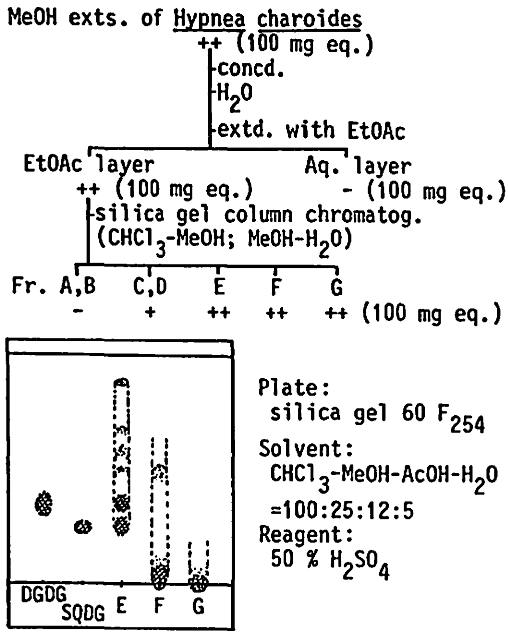

Fig.7 Procedure of TLC analysis and the typical analysis result.

vertical surface. Among them, some red algal species, *C. clavulatum*, *G. amansii*, *P. capillacea* and *H. charoides* are palatable to horned turban as their natural food. Juvenile horned turbans can eat these algae easily, because the thalli of these algae are composed of slender branches. Especially the alga *H. charoides*, fragile enough to be torn or grazed easily, is palatable. The palatability of these red algae were confirmed by bioassay. MeOH extracts from *G. amansii*, *P. capillacea* and *H. charoides* gave the feeding stimulation to the shells. In the extracts, we found the preservation of at least two kinds of phagostimulant glycerolipids, DGDG and SQDG in *G. amansii* and *H. charoides*.

DGDG has been already isolated from other groups of algae, such as green *Ulva pertusa* (Sakata et al. 1988) and brown *Undaria pinnatifida* (Sakata and Ina 1985), while SQDG has been isolated from *U. pertusa* (Sakata et al., 1988). The isolation of these glycerolipids from red algal group (Rhodophyta) were the first time up to date.

H. charoides was the most palatable because of easiness for grazing (i.e., slenderness and fragileness of branches) and containing phagostimulants (and no feeding inhibitors), but this species appears only in summer. Thus, among these palatable algae, *G. amansii* is thought to be the most reliable algal food for released juvenile horned turban. This agar species is not only palatable, but grows abundantly nearly all the year through. Recently, agar beds in the protected area, mostly composed of *G. amansii*, have been noted as one of the best nursery grounds for horned turban ranching (Okabe et al., 1989). Our experimental results support these findings.

On the contrary, *P. leptophyllum* was hardly foraged, in spite of its slender structure. Avicel zones of MeOH extracts from this algae were rarely grazed. The absense of well-grazed zones of *P. leptophyllum* suggests the existence of feeding inhibitors for chemical defense. Further studies revealed that *P. leptophyllum* contains halogenated monoterpen, a deterrent of appetite for juvenile horned turbans and some other algiverous animals (Sakata, Iwase, Ina and Fujita, submitted).

If large number of juvenile horned turbans were released in a closed fishing-port waterbreak area, such an unpalatable alga might become prominent as this alga would proliferate after palatable algae were grazed. As well, if unpalatable algae were to form dense tufts, they could protect palatable algal juveniles from being grazed. Long-term and regular observation of algal flora on the inner vertical surface of fishing-port waterbreaks will be necessary to utilize these waterbreaks effectively or repeatedly as horned turban nursery grounds.

The total standing crop of minute (e.g., *A. japonicum*) and crustose red algae (e.g., *P. conchicola*) were small. In this study, however, minute species *C. clavulatum* was grazed well. We should also clarify the role or food value of these minute or crustose red algae for ranching juvenile horned turbans, as well as green and brown algae.

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サザエ稚貝の天然餌料としての漁港防波堤側面に生育する紅藻数種の重要性

藤田大介・岡田英男・坂田完三

サザエは富山県で最も重要な浅海性巻貝で，主産地の氷見では最近漁獲量及び能登地方からの移入量が急増しており，種苗生産並びに放流試験が試みられている。氷見市大境の漁港防波堤におけるサザエ稚貝の中間育成試験は極めて良い成長結果が得られているので，1988年の8月から1989年の7月にかけてこの防波堤内側面の海藻相を調査した結果，52種の海藻が同定された。紅藻が特に多く，中でも最も現存量が大きかったのはマクサで，オバクサともにほぼ1年中見られ，イバラノリは夏季に，ホソユカリは秋季に限って出現した。殻高15㎜のサザエ稚貝にイバラノリ，トゲイギス，マクサ，オバクサ及びホソユカリの5種の投与実験を行ったところ，この順によく摂餌されたが，ホソユカリは殆ど摂餌されなかった。また，この5種のうちトゲイギスを除く4種の海藻のメタノール抽出物をアビセル板上の試験円に塗布して稚貝（殻高10，30-40㎜）に対する摂餌活性評価を行ったところ，イバラノリ，マクサ及びオバクサの各抽出物を塗布した試験円では多数の歯跡が残されたのに対し，ホソユカリ抽出物の試験円では歯跡は希であった。オバクサを除く3種の抽出物を薄層クロマトグラフィーで調べたところ，いずれにも摂餌刺激性複合脂質ジガラクトシルジアシルグリセロールが含まれていた。この複合脂質はこれまで緑藻及び褐藻から見つかっていたが，紅藻では今回初めて存在が確認された。ホソユカリはこのような摂餌刺激物質を含有しながらも摂餌活性が低いため，摂餌忌避物質の存在が示唆された。一方，マクサはイバラノリほど摂餌されないが，忌避物質も含まず，周年，豊富に生育していることから，漁港防波堤におけるサザエの天然餌料として重要と考えられた。