Introduction

The food web structures in deep-sea chemosynthetic-based communities including hydrothermal vents, methane seeps and whale-fall communities differ significantly from those in deep-sea photosynthetic-based communities (Tunnicliffe 1991, Van Dover 2000) because the primary producers in food webs in chemosynthetic-based communities are chemoautotrophic bacteria. Such biological communities have large populations of invertebrate animals, vesicomyid clams, provannid gastropods and siboglinid polychaetes that harbor symbiotic bacteria in their bodies (e.g., Fisher 1990, Maruyama et al. 2008). The food webs in these communities have been analyzed based on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ stable isotope evidence (e.g., Fisher et al. 1994, Van Dover 2002, Mizota & Yamanaka 2003, Yamanaka et al. 2003), and three trophic categories, the primary producers, primary consumers and omnivores, have been roughly distinguished in vent communities (Van Dover 2000). There are, however, limited analytical data on the food webs in chemosynthetic-based communities around Japan.

A chemosynthesis-based community associated with methane seepage is distributed at depths of 800 to 1,300 m at the Off Hatsushima Island site in Sagami Bay (Okutani & Egawa 1985, Hashimoto et al. 1989, Fujikura et al. 2002). The community is composed of approximately 30 benthic species (Fujikura et al. 2002, 2008). To estimate energy flow for invertebrates harboring symbionts, the $\delta^{13}C$ value similar to that of the mytilid mussel Bathymodiolus platifrons Hashimoto & Okutani, no symbiotic bacteria, and very small radula (probably useless). In bait trap experiments, the whelk swarmed toward crushed B. platifrons. These results strongly suggest that P. buccinoides feeds on B. platifrons. The ecological niche of the whelk is that of a carnivore or scavenger. B. platifrons was distributed on other outcrops at this seep site, but P. buccinoides did not occur elsewhere. Thus, the distribution pattern of this whelk is not determined solely by its dietary habits.

Key words: Bathymodiolus platifrons, chemosynthesis-based community, dietary habit, Phymorhynchus buccinoides, stable isotope

Abstract: The Turrid whelk Phymorhynchus buccinoides aggregates Okutani, Fujikura & Sasaki on only four outcrops in a grey and black sediment area (25 m$^2$) at a depth of 1180 m at the Off Hatsushima Island seep site, Sagami Bay, Japan. To determine the food web structure of the deep-sea chemosynthesis-based community, we conducted the dietary food habits of the whelk by a combination of in situ behavioral observations, stable isotope compositions, symbiotic bacteria in their soft part body parts, in situ bait trap experiments, and anatomic examinations. The whelks had a $\delta^{13}C$ value similar to that of the mytilid mussel Bathymodiolus platifrons Hashimoto & Okutani, no symbiotic bacteria, and very small radula (probably useless). In bait trap experiments, the whelk swarmed toward crushed B. platifrons. These results strongly suggest that P. buccinoides feeds on B. platifrons. The ecological niche of the whelk is that of a carnivore or scavenger. B. platifrons was distributed on other outcrops at this seep site, but P. buccinoides did not occur elsewhere. Thus, the distribution pattern of this whelk is not determined solely by its dietary habits.

Key words: Bathymodiolus platifrons, chemosynthesis-based community, dietary habit, Phymorhynchus buccinoides, stable isotope

Turrids whelk, Phymorhynchus buccinoides feeds on Bathymodiolus mussels at a seep site in Sagami Bay, Japan

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only in food webs but also in determining the distribution patterns of organisms. To find out predator-prey interactions among P. buccinoides and other benthic organisms in the seep community, we therefore conducted five examinations including in situ observations, stable isotope analysis, the presence of symbiotic bacteria, reaction to bait and anatomy of P. buccinoides radula. In the present study, we discuss the possibility that dietary habit is one of the limiting factors on the occurrence of P. buccinoides on only four outcrops. In addition, the ecological niche of P. buccinoides is also discussed.

**Materials & Methods**

**Sample collection and in situ observations**

Phymorhynchus buccinoides and Oenopota sagamiana Okutani & Fujikura of the Turridae family of Gastropoda, Calyptogena soyoae Okutani/C. okutani Kojima & Ohta of the Vesicomyidae family of Bivalvia and Bathymodiolus platifrons Hashimoto & Okutani of the Mytilidae family of Bivalvia, which are all endemic species of chemosynthesis-based communities, were collected using a suction sampler attached to the Remotely Operated Vehicle (ROV) Hyper-Dolphin (dives #524 and #525 on 12 and 13 March 2006 and dives #623 and #629 on 10 and 14 December 2006, respectively) at the Off Hatsushima Island seep site (35°00.2'N, 139°13.49'E, 1180 m deep), Sagami Bay, central Japan. Unidentified Anthozoa representing nonendemic species of chemosynthesis-based communities were also collected near the seep site. In situ observations were conducted using an ultrahigh-sensitivity HDTV camera (Hitachi Electronics Services Co., Ltd., Tokyo, Japan) and a digital still camera (DPC-7000, Deep Sea System International, Inc., Falmouth, MA, USA) on the ROV.

**Stable isotope analysis**

The δ13C, δ15N and δ34S stable isotope compositions of an unidentified Actinaria, B. platifrons, C. soyoae/C. okutani, O. sagamiana and P. buccinoides were analyzed (Table 1). Preparation for carbon, nitrogen and sulfur isotopic measurements followed the procedures described in previous studies (Mizota et al. 1999, Yamanaka et al. 2008). The dissected soft tissues of each specimen were centrifugally washed repeatedly with 0.1 M LiCl solution to eliminate seawater sulfates and then freeze-dried. Carbon and nitrogen isotope compositions were determined using an elemental NA2500 analyzer coupled with an isotope ratio mass spectrometer (Thermo Electron Corp., Waltham, MA, USA) at the Prefectural University of Kumamoto, Tsukide, Kumamoto, Japan. For sulfur analysis, a part of the freeze-dried samples was pulverized and then combusted in a Parr bomb #1108 stainless steel vessel filled with oxygen gas under high pressure (30 kg cm−2) and a few milliliters of distilled water. After combustion, the dried samples were completely converted to gas, and sulfur was trapped as sulfate ion in the distilled water in the vessel. The resulting sulfate was recovered as BaSO4 precipitate with the addition of 0.5 M BaCl2 solution. The sulfur isotope composition of the BaSO4 separate was determined using a continuous-flow isotope ratio mass spectrometer (IsoPrime EA, GV Instruments, Manchester, UK) installed at Okayama University, Tsushima-naka, Okayama, Japan.

All values are expressed as common δ13C, δ15N and δ34S.

**Table 1.** δ13C, δ15N and δ34S stable isotope compositions of benthic species in/around the Off Hatsushima seep site, Sagami Bay, Japan

| Taxon                | N Part analyzed | Habitat | δ13C (‰) | δ15N (‰) | δ34S (‰) | Symbiont                      |
|----------------------|-----------------|---------|----------|----------|----------|-------------------------------|
| Mollusca             |                 |         |          |          |          |                               |
| Gastropoda           |                 |         |          |          |          |                               |
| Turridae             |                 |         |          |          |          |                               |
| Phymorhynchus buccinoides | 6          | Foot    | −58.6±4.4 | −4.0±1.2 | +13.8±0.6 | No microbe in gills (Present study) |
| Oenopota sagamiana   | 6              | Foot    | −30.9±3.6 | +2.0±1.5 | +6.8±0.1 | No microbe in gills (Endow et al. 1992) |
| Bivalvia             |                 |         |          |          |          |                               |
| Vesicomyidae         |                 |         |          |          |          |                               |
| Calyptogena soyoae/C. okutani | 6 | Gill    | −36.6±0.9 | −6.6±2.2 | −27.5±4.9 | Thioautotroph (Kuwahara et al. 2007, Maruyama et al. 2008) |
| Mytilidae            |                 |         |          |          |          |                               |
| Bathymodiolus platifrons | 2          | Gill    | −69.2±1.4 | −11.2±1.1 | +20.4±0.6 | Methanotroph (Fujiwara et al. 2000) |
| Cnidaria             |                 |         |          |          |          |                               |
| Anthozoa             |                 |         |          |          |          |                               |
| Unidentified species | 2              | Whole body | −15.2±0.9 | +15.0±0.7 | +11.8±0.2 | Unknown                      |

Table 1. δ13C, δ15N and δ34S stable isotope compositions of benthic species in/around the Off Hatsushima seep site, Sagami Bay, Japan
notations, per mil variation (‰) relative to V-PDB, V-CDT and air dinitrogen, respectively. The analytical precision for δ^{13}C, δ^{15}N and δ^{34}S measurements was greater than ±0.2‰ during the present study.

**Symbiotic bacterial 16S rDNA sequencing**

DNA of the gill tissue from *P. buccinoides* was extracted and purified using the DNeasy tissue kit (QIAGEN, Hilden, Germany). The 1.4-kbp fragment of bacterial 16S rDNA was amplified in the PCR and sequenced. The oligonucleotide primers (Eubac27F and Euba1492R) of universal bacterial 16S rDNA were used (Delong 1992). PCR conditions were: 95 °C for 3 min; followed by 95 °C for 90 sec, 55°C for 90 sec and 72°C for 90 sec for 30 cycles; and 72°C for 10 min. The sequences of PCR-amplified fragments were confirmed by DNA sequencing.

**In situ bait traps experiments**

Some *B. platifrons* on a *P. buccinoides*-aggregated outcrop were crushed using the manipulators on the ROV (white arrow in Fig. 1A). A fresh *Scomber japonicus* Houttuyn was deployed as bait beside the *P. buccinoides*-aggregated outcrop (Fig. 1A). Four hours after crushing and deployment, we revisited the same site to observe whether the whelks reacted to the two types of bait.

**Anatomic examination**

Three *P. buccinoides* specimens were dissected to observe the radula. A ribbon of radular teeth was removed from the radular sac, cleaned in diluted commercial bleach, rinsed in water and ethanol, air-dried on scanning electron microscope (SEM) stabs with double-sided carbon tape and coated with platinum vanadium. Photographs of the radula were taken with an SEM (Hitachi S-2250N, Tokyo, Japan) at The University Museum, University of Tokyo (UMUT). The radula depicted was registered and deposited in the Department of Historical Geology and Paleontology, UMUT (voucher UMUT RM29688).

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**Fig. 1.** Outcrops at the Off Hatsushima seep site, Sagami Bay, Japan. A: Some *Bathymodiolus platifrons* specimens (white arrow) crushed with a manipulator. A fresh *Scomber japonicus* was deployed as bait beside the *Phymorhynchus buccinoides*-aggregated outcrop. B: Whole view of the *P. buccinoides*-aggregated outcrop. Several *P. buccinoides* concentrated near the basal outcrop. The surface of a white bacterial mat was covered with several mussels. C: No *P. buccinoides* occurred on other outcrops with *B. platifrons*. This outcrop was situated only 15 m from the *P. buccinoides*-aggregated outcrop. D: After four hours of manipulation, many *P. buccinoides* were attracted to the crushed *B. platifrons*. 
Results

In situ observations

Phymorhynchus buccinoides was distributed on four outcrops in a grey and black sediment area (25 m²) (Fig. 1B) 1.5 m in height and 1 m in diameter in whitish-grey and black sediments. The sediments indicated a reducing environment with a high sulfide concentration. Numerous Bathymodiolus platifrons and unidentified polychaete-like worms and zoarcid fishes also occurred on P. buccinoides-aggregated outcrops. Several mussels were covered with a white bacterial mat. White egg capsules of P. buccinoides of uniform size (11 mm in major axis, 5 mm in minor axis and 4 mm in height) were attached to the shell surfaces of mussels (Watanabe et al. in press). Approximately 100 P. buccinoides individuals were present on the basal P. buccinoides-aggregated outcrop (Fig. 1B). Some specimens were approaching mussels by extending a siphon and foot. Almost all of the other whelks were at rest in a “sit-and-wait” condition. Oenopota sagamiana were densely aggregated 10 m from the P. buccinoides-aggregated outcrops in whitish-grey and black sediments, and numerous Calyptogena soyoae/C. okutanii were buried in sediments, 20 m from P. buccinoides-aggregated outcrops. B. platifrons were also attached to other outcrops without P. buccinoides, only 15 m from the P. buccinoides-aggregated outcrops (Fig. 1C).

Stable isotope analysis

The δ¹³C and δ³⁴S values for the soft tissues of P. buccinoides were −58.6‰ and +13.8‰, respectively, which were both similar to the values (−69.2‰ in δ¹³C and +20.4‰ in δ³⁴S) for B. platifrons relative to other animals (Table 1, Fig. 2B). The δ¹⁵N values of P. buccinoides were −4.0‰, which were approximately 7‰ enriched in δ¹⁵N relative to B. platifrons (−11.2‰) and were comparable to those for C. soyoae/C. okutanii (−6.6‰). On the other hand, the δ¹³C values in the soft tissues of O. sagamiana (−30.9‰) were close to those of C. soyoae/C. okutanii (−36.6‰), while the δ³⁴S value of O. sagamiana (+6.8‰) was clearly distinguished from that of C. soyoae/C. okutanii (−27.5‰) (Table 1, Fig. 2). The δ¹⁵N values of O. sagamiana were +2.0‰, which were approximately 9‰ enriched in δ¹⁵N relative to C. soyoae/C. okutanii. The isotopic signature of the unidentified Anthozoa was characterized by high δ¹³C (−15.2‰) and δ¹⁵N (+15.0‰) values relative to the other animals examined in this study, while the δ³⁴S value (+11.8‰) was similar to that of the other animals with the exception of C. soyoae/okutanii, which had a significantly lower δ³⁴S value (Table 1, Fig. 2).

Symbiotic bacterial 16S rDNA sequencing

In the bacterial 16S rDNA in the gill tissue of P. buccinoides, the band intensity of the PCR fragment of 16S rDNA was weak, and no typical chemosynthetic bacteria were detected. It is therefore concluded that the gill tissue of the whelk contained no chemosynthetic bacterial symbionts.

In situ bait trap experiments

As soon as B. platifrons were crushed by a manipulator, P. buccinoides crept toward the crushed mussels. But no individual reacted to the fresh Scomber japonicus bait within 20 min. After four hours of manipulation, P. buccinoides were attracted to swarm around the crushed B. platifrons (Fig. 1D). But we could not find the fresh S. japonicus bait. Animals such as fishes and crabs probably took it away.

Anatomic examination

The radula is toxoglossate and consists of a pair of sharp
teeth in each row (Fig. 3A). An individual tooth was approximately 110 μm in length for a specimen with a shell height of 48 mm (Fig. 3B–D), circular in cross section and rolled into a hollow harpoon-like tooth with a clear suture (s: Fig. 3B, D). The tip is sharply pointed and lacks denticles, and there are wide openings near the tip and base (ob, ot: Fig. 3D). The base is extended, with a width of 25 μm (Fig. 3C, D). The teeth are attached to a thin radular membrane (rm: Fig. 3A) only at their bases.

**Discussion**

Because the δ¹³C and δ¹⁵N values of common marine primary producers range from −31 to −10% (−22 to −17% for most) (Sackett et al. 1965, Degens et al. 1968) and >−3‰ (usually positive values) (Minagawa & Wada 1984, Carpenter et al. 1997), respectively, the low δ¹³C and δ¹⁵N values in the present study except for the unidentified Anthozoa specimen indicate that seep-endemic animals can be distinguished from marine photosynthesis-based ecosystem-endemic ones. The lower values for seep-endemic species are often observed (Mizota & Yamanaka 2003), and it is believed that the primary producers in such environments, i.e., chemoautotrophic and methanotrophic bacteria, have unique isotope compositions associated with their metabolic pathways and/or carbon sources (Nelson & Fisher 1995). In particular, methanotrophs in seep environments where bacterial methane emissions are prevalent have significantly low δ¹³C values of less than −40‰ because the δ¹³C values of bacterial methane are generally lower than −45‰ (Schoell 1988). Samples of *Bathymodiolus platifrons*, which harbor only methanotrophic endosymbionts (Fujiwara et al. 2000), have very low δ¹³C values of −69.2‰. *Phymorhynchus buccinoides* also has low δ¹³C values of −58.6‰ which are similar to those of *B. platifrons*. These results therefore indicate that: 1) *P. buccinoides* mainly feeds on *B. platifrons*; 2) *P. buccinoides* mainly feeds on organisms relying on methane bacteria like *B. platifrons*; 3) *P. buccinoides* mainly feeds on methane bacteria; and/or 4) *P. buccinoides* relies on symbionts (methane bacteria) in its body for energy acquisition.

The degree of δ¹⁵N enrichment during a single feeding process at the trophic level ranged from +1.3 to +5.3‰ (Minagawa & Wada 1984). The somewhat 7‰ higher δ¹⁵N enrichment values of *P. buccinoides* compared with *B. platifrons* indicate that this whelk is at a higher trophic level than *B. platifrons*, and the negative δ¹⁵N values (−4‰) suggest that *P. buccinoides* is nearly independent from the phototrophic food web, as mentioned above. Symbiont-harboring bivalves including *B. platifrons* (−11.2‰) and Calyptogena soyoae/C. okutanii (−6.6‰) had lower δ¹⁵N values. Similar lower values are observed for solenomyid bivalves, which rely on symbiotic bacteria (Yamanaka et al. 2008). Yamanaka et al. (2008) suggest lower δ¹⁵N values attribute isotopic fractionation of assimilation process from ammonia using glutamine synthetase (Yoneyama et al. 1993) or glutamine dehydrogenase (Lee & Childress 1994).

The sulfur isotope composition of common marine animals is similar to that of seawater sulfate-sulfur (δ³⁴S=+21‰; Rees et al. 1978), because their primary source of sulfur as an inorganic nutrient is seawater sulfate ion and little isotopic fractionation occurs during stimulatory sulfate reduction and assimilation through the food web (Fry 1983, 1988, Peterson & Howarth 1987). Marine animals with low δ³⁴S values of less than +15‰ are suggested to depend on minor but not negligible contributions of magmatic and/or bacterial sulfide (Yamanaka et al. 2003), which show low δ³⁴S values of less than +10‰ (usually lower than 0‰), as a nutritional source (Thode 1991). *P. buccinoides* has slightly lower δ³⁴S values (+13.8‰), suggesting a minor contribution from magmatic and/or bacterial sulfide. However, the δ³⁴S values of the whelk were still close to those of *B. platifrons* (+20.4‰) and photosynthesis-based marine animals. The δ³⁴S values of the unidentified Anthozoa (+11.8‰) were also lower than those of the photosynthesis-based marine animals, suggesting that thioautotrophic production relying on sulfide in a seep environment is an important food source in addition to materials from the euphotic zone.

The large biomass of benthic animals in deep-sea chemosynthesis-based communities including provannid gastropods, vesicomyid bivalves, *Bathymodiolus* mussels and siboglinid polychaetes, relies on symbiotic bacteria in their bodies for nutrition (Fisher 1990, Maruyama et al. 2008). However, no such bacteria were found in *P. buccinoides* gills. Based on the results of *in situ* bait trap experiments, this whelk swarmed toward crushed *B. platifrons* but no benthic species was attracted to a fresh fish (Fig. 1). The present results thus strongly suggest that *P. buccinoides* feeds on *B. platifrons*.
Most members of the conoidean gastropods (Turridae, Conidae and Terebridae) have a toxoglossate radula, and the dietary habits of these families are aggressively carnivorous or predatory (see Kohn 1998 for a review). A typical predatory conid snail, Conus tulipa (Linnaeus), has relatively large radular teeth of approximately 4–5 mm in length, which is 6.6% of its 60.6–71.0 mm shell length (Yoshiba 2002). In P. buccinoides, the radula is very small at a length of 0.11 mm, which is 0.23% of its shell length. Therefore, it is assumed that this is difficult for this whelk to prey on other mega-benthic animals because its radular teeth are too small. We did not observe P. buccinoides preying on living B. platifrons during in situ observations. In a preliminary investigation, no material was found in the stomach of this whelk (Sasaki et al. 2007). P. buccinoides does not actively creep over the substrate but takes a “sit-and-wait” position. This whelk may thus be a scavenger that feeds on only Bathymodiolus mussel remains.

Bathymodiolus platifrons is distributed on other outcrops at the Off Hatsushima seep site. Although individuals are present on a couple of outcrops only 15 m from the P. buccinoides-aggregated outcrops, no P. buccinoides were found there (Fig. 1C). This indicates that the distribution pattern of P. buccinoides is not determined solely by its dietary habits. The whitish-grey and black sediments of the P. buccinoides-aggregated outcrops suggest a strongly reducing environment. Calyptogena soyae cannot live in these sediments due to the high concentration of hydrogen sulfide in pore water (Hashimoto et al. 1995). A thick filamentous bacterial mat had mussels on its surface (Fig. 1B), indicating that seep chemical flux and concentrations around the P. buccinoides-aggregated outcrops differ from the conditions at other seeps at the Off Hatsushima site. It will be necessary to investigate chemical environments just above the P. buccinoides-aggregated outcrops in the future to determine the precise factors governing the distribution of P. buccinoides.

Ten species of Phymorhynchus, comprising Phymorhynchus aff. alberti, P. buccinoides, Phymorhynchus carinatus, Phymorhynchus major, Phymorhynchus mokalevi, Phymorhynchus ovatus, Phymorhynchus starmeri, Phymorhynchus warreni, Phymorhynchus yamamii, and Phymorhynchus yamamii, have been found in deep-sea hydrothermal vents and seeps (Okutani et al. 1993, Okutani & Ohta 1993, Fisher et al. 1994, Sysoev & Kantor 1995, Beck 1996, Warén & Bouchet 2001). We know that, Phymorhynchus sp. from the East Pacific Rise vent sites feeds on the gastropoda Neomphalus fretterae McLean because its radula were found in the whelk stomach (Warén & Bouchet 1989). Warén & Bouchet (2001) analyzed the esophageal contents and reported that P. ovatus from the Mid-Atlantic Ridge vent sites feeds on Bathymodiolus mussels and worm-shaped animals. Detailed anatomic observations of P. hyfifluxi showed that it is not piscivorous or ver-nivorous but rather molluscivorous (Beck 1996). P. starmeri from the North Fiji Basin vent sites is probably not an endemic member of deep-sea chemosynthesis-based communities based on its concurrence with nonchemosynthesis-based communities in the Izu-Ogasawara (Bonin) Island area (Okutani et al. 1993, Hasegawa et al. 2000). However, P. buccinoides is apparently an endemic member of deep-sea chemosynthesis-based communities because the distribution of this species is restricted to a seep site and it feeds on Bathymodiolus mussels.

In the hydrothermal vent community at the Galapagos Rift in the eastern Pacific, there are three trophic levels: 1) chemoautotrophic microorganisms as the primary producers; 2) symbiont-containing invertebrates consisting of mussels, clams and tubeworms as the primary consumers; and 3) other mega-benthic animals including a turrid whelk as omnivores (Van Dover 2000). This turrid whelk is probably Phymorhynchus sp. as reported by Fisher et al. (1994) and its dietary habits were estimated based on the stable isotope composition alone (Fisher et al. 1994). In the present study, the dietary habits of P. buccinoides were determined based not only on isotopic evidence but also on the results of in situ bait trap experiments and anatomic examination. Applying Van Dover’s categories to the seep community at the Off Hatsushima Island site, C. soyae/C. okutanii and B. platifrons are the primary consumers. However, P. buccinoides is not an omnivore but a carnivore or scavenger.

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