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Relationship Between the Lifestyle of a Siboglinid (Pogonophoran) Polychaete, *Oligobrachia mashikoi,* and the Total Sulfide and Nitrogen Levels in its Habitat

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A gutless polychaete of the family Siboglinidae, *Oligobrachia mashikoi*, known in the past as a beard worm of the group Pogonophora, inhabits Tsukumo Bay of the Noto Peninsula in the Sea of Japan. Photographs were taken of this polychaete projecting about one third of the length of its tentacles outside of its tube. The tube protruded several mm from the sea bottom. These are the first field photographs of beard worms. The trophosome of this beard worm harbors sulfur-oxidizing bacteria. In fact, the muddy sediment where this worm inhabits smells slightly of hydrogen sulfide. Total sulfide levels, which can be an indicator of the generation of hydrogen sulfide gas, were measured at 10 locations in the bay. Furthermore, at the location which this species inhabits, the total sulfide levels in the vertical direction were determined. In addition, the total nitrogen levels, which can indicate the quantity of organic substances, were measured. The sediment inhabited by this worm was determined to have total sulfide levels of 0.24-0.39 mg/g dry mud, measured in the form of acid-volatile sulfide-sulfur. The total nitrogen levels were 1.0-1.5 μg/mg dry mud. These values suggest that the bottom of Tsukumo Bay has not been deteriorated by eutrophication. The levels were, however, highest in the surface layer of the sediment. These results suggest that hydrogen sulfide is generated in the surface of the sediment by sulfate-reducing bacteria, and that *O. mashikoi* appears to able to live in an environment that contains a slight amount of sulfide.

Key words: beard worm, tentacles, sulfide, nitrogen, levels in sediment, lifestyle

INTRODUCTION

Caullery (1914) gave the name *Siboglinum weberi* to a peculiar worm that lacks a mouth and digestive tract after the Dutch ship “Siboga”, which had been used to collect the specimens in 1899-1900 in Indonesian waters. Although he placed the species in the family Siboglinidae, he did not specify the phylum to which this family belongs. Uschakov (1933) classified a worm, *Lamellisabella zacchsi,* collected from the Sea of Okhotsk as a polychaetous annelid. After that, although the phylogenetic positions of Caullery’s and Uschakov’s worms changed, Beklemishev (1944) noted their tentacles, which appeared to grow as a beard from the ventral side of the cephalic lobe. As a result, in his book, he created a new Phylum, Pogonophora. In these animals, however, the opisthosoma of the end of the body is composed of a metameric structure with chitinous setae, as in the polychaetous Annelida. Therefore, there was some doubt as to whether the Pogonophora is close to Annelida (Liwanow and Porfirjewa, 1967).

Recently, Rouse and Fauchald (1997) conducted a cladistic analysis using 10 additional characters among six animal phyla, including Pogonophora, Echiura, and Annelida (Polychaeta). They found that that Pogonophora was positioned in one family among Polycheata. Therefore, they argued that this animal group should be systematically moved to the original position described in 1914, *i.e*., the family Siboglinidae and not the phylum Pogonophora (Rouse, 2001). At present, annelid taxonomists place Siboglinidae in Sabeliida (Plychaeta: Palpata: Canalipalpata) (Bartolomaeus *et al*., 2005). Recent biochemical and molecular data support this placement (Kojima *et al*., 1993; Black *et al*., 1997; McHugh, 1997; Halanych *et al*., 1998). Halanych (2005) reviewed the data and reported that the animal group known as Pogonophora is classified into three groups: Frenulate (beard worms), Monifera (Sclerolinum),

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and Vestimentifera (tube worms).

Instead of lacking a mouth and a digestive tract, adults of these groups harbor chemosynthetic bacteria in the posterior part of the body, called the trophosome. They live on carbohydrates produced by the bacteria. Recently, we showed that hemoglobin produced by *Oligobrachia mashikoi* has binding sites for hydrogen sulfide (Numoto *et al*., 2005). Therefore, the bacteria in this species must be sulfur-oxidizing bacteria. On the other hand, in *Siboglinum poseidoni*, the symbiotic bacteria were determined to be methane-oxidizing by an intake experiment of radioactive carbon and an ultrastructural study of the bacteria (Schmaljohann and Flügel, 1987). These studies strongly suggested that, in siboglinid polychaetes, oxidizing substrates such as hydrogen sulfide or methane are taken into the body from the environment and transported to the bacteria by blood stream.

The plumes in tube worms have been reported so far to function as gills and to be the site of gas exchange, including hydrogen sulfide (Childress *et al*., 1984; Felbeck and Turner, 1995; Goffredi *et al*., 1997a, b and 1999; Andersen *et al*., 2002). On the other hand, the functions of the tentacles (beard) in beard worms have not been examined, although the plume in tube worms and the tentacles in beard worms are embryologically homologous (Southward, 1988). In beard worms, an ultrastructural study of the tentacles has been conducted (Gupta and Little, 1969). In addition, there are two reports in which the fine structure of the tentacles in beard worms was compared to that of tentacles of a brachiopod and a polycheate (Reed and Cloney, 1977; Gardiner, 1978). These are, however, general descriptions and do not specify the function of the tentacles of beard worms. Only Ivanov (1963) surmised that the tentacles of beard worms must function as gills because of the pinnules on the tentacles. Rouse (2001) conducted a cladistic analysis by regarding the tentacles of Annelida, Pogonophora, and Vestimentifera as the palpus. Southward *et al.* (2005) doubted, however, whether the similarities are really homologous, since the fine structure of the tentacles differs between polychaetes and beard worms, and the tentacles of beard worms do not function to collect food as they do in polychaetes. Therefore, there is no consensus on the function of the tentacles in beard worms.

Schulze and Halanych (2003) compared the ecology of beard worms with that of tube worms and noted their different lifestyles. Worms in the former group prefer soft anoxic sediment and bury the bulk of their bodies with their soft tubes in the mud. The ends of the tubes are open, and the opisthosoma is used as a tool for digging into the mud. They are not adapted for an environment with a high concentration of hydrogen sulfide. On the other hand, the latter group prefers hard sediment and exist on it with a relatively hard tube. The end of the tube is closed, and the opisthosoma is used as an anchor to fix the body. They have adapted to an environment with high levels of hydrogen sulfide. Therefore, it may be premature to state that the function of the tentacles is the same in beard worms and tube worms.

In the present study, in Tsukumo Bay of the Noto Peninsula, which juts into the Sea of Japan, we photographed by scuba diving a beard worm, *O. mashikoi*, which was projecting its tentacles out of its tube buried in the sea bottom. As noted above, this species harbors sulfur-oxidizing bacteria. In fact, the sediment smells slightly of hydrogen sulfide. Therefore, total sulfide levels in the sediments were measured at 10 locations in Tsukumo Bay as an indicator of the generation of hydrogen sulfide gas. In addition, in the sediment inhabited by this beard worm, the total sulfide levels were also determined in the vertical direction. Furthermore, the total nitrogen levels, which indicate the quantity of organic substances, were measured. These analyses are the first conducted in the habitat of a beard worm. We will discuss the relationship between the lifestyle of this beard worm and the data obtained.

MATERIALS AND METHODS

Tsukumo Bay is 250 m wide, 1,300 m long, and 25 m deep in the central region (Fig. 1). In summer 2004, we dived several times in the habitat of the beard worm and took pictures *in situ*. In June 2005 and 2006, we collected sediment from the surface of the sea bottom from 10 locations in the bay using a grab sampler (Poner type: DIK-190A-A1; Kaiki Rika Kogyo Co., Ltd., Saitama, Japan) (Fig. 1; Table 1). This sampler can take a 2-liter volume of mud. Of the 10 locations, the habitat of the beard worm inhabits location 7 (137°14'29"E 37°18'29"N).

The total sulfide levels of the mud at each location were determined with gas detector tubes (201H type and 201L type) using a kit (Gastec Corp, Kanagawa, Japan). The analysis was repeated three times using the same mud sample. The average value of total sulfide was expressed as acid-volatile sulfide-sulfur (AVS-S) (mg/g dry mud).

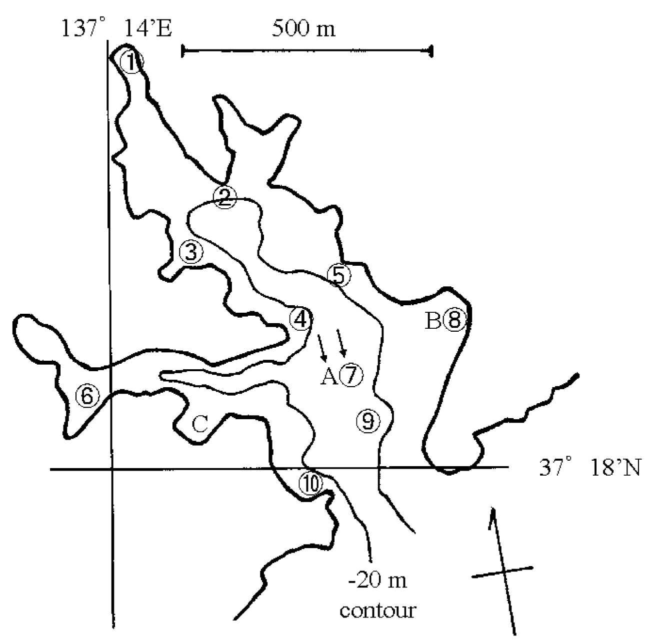

Fig. 1. Map of Tsukumo Bay, showing sampling locations 1 to 10, in which surface mud of the sea bottom was collected, and A to C, in which mud samples in the vertical direction were also collected. Arrows indicate the locations inhabited by the beard worm *Oligobrachia mashikoi*.

Table 1. The depth (m) of water at sampling locations 1–10.

Locations	1	2	3	4	5	6	7	8	9	10
Depth	8	21	18	18	15	12	25	5	25	12

Furthermore, in November 2005 and March and June 2006 at location A (=location 7) and newly established locations B and C, mud samples were collected using a core sampler (Phleger corer, Rigo Co., Ltd., Saitama, Japan), to determine the total sulfide levels in the vertical direction. At location A, the mud of each 5-cm core from the surface to a 35-cm depth was taken and analyzed at least three times, usually six to nine times. *Oligobrachia mashikoi* did not inhabit locations B and C. Sediment B is composed of mud, sand, and small stones. Sediment C is similar to that at location A, although the water current is stronger at the latter location. At locations B and C, the 10-cm core from the surface to a 35-cm depth was determined three times.

At locations A, B, and C, the total nitrogen levels of the surface and in the vertical direction were measured by a modified Kjeldahl method at 220 nm using a UV-visible recording spectrophotometer (Simazu Corp., Tokyo). The 5-cm mud core from the surface to a 40 cm depth was analyzed twice. The average value was expressed as µg/mg dry mud.

RESULTS

Scuba diving

When divers reached the sea bottom, a large quantity of detritus was suspended. After the visibility cleared, the divers moved slowly against the water current. The beard worms were observed projecting their tentacles from their tubes (Fig. 2a, b). Some worms reacted to the light by quickly withdrawing their tentacles, while others did not. Each individual, however, withdrew its tentacles when the divers approached within 10 cm. When the photographs were observed in detail, they showed that the worms were projecting 1/3 of the length of the tentacles from their tubes, which protruded several mm from the sediment. Although detritus was attached to each tube, the reddish body of the worm was observed through the tube (Fig. 2a, b). The color of the tentacles varied from red to reddish purple. The mud that the worms inhabited was so soft that the divers could thrust their arms to shoulder depth into the sediment. In the most densely populated areas, the tubes stood at a slant in the mud and sometimes trailed along each other. In sparsely populated areas, the tubes stood vertically. The tube length and diameter were 39.2±1.82 cm (av.±SE) and 0.60±0.018 mm, respectively (30 individuals, dried specimens).

Total sulfide

The numerical data obtained from June 2005 and 2006 were almost the same. In the surface layer, the AVS-S concentration at location 1, which is at the head of the bay, was 1.05-1.10 mg/g, the highest among the sampling locations. At location 10, the entrance of the bay, this value was 0.02-0.01 mg/g, the lowest among the locations. The value of AVS-S at location 7 inhabited by the beard worm was 0.39-0.24 mg/g. Fig. 3 shows the results obtained in November 2005.

The AVS-S concentrations in the vertical direction at locations A-C were highest on the surface. This result corresponded among the data from November 2005 and March and June 2006. The value on the surface at location A was 0.46 mg/g in November 2005, 0.27 mg/g in March 2006 and 0.60 mg/g in June 2006. At 5-10 cm below the surface, the

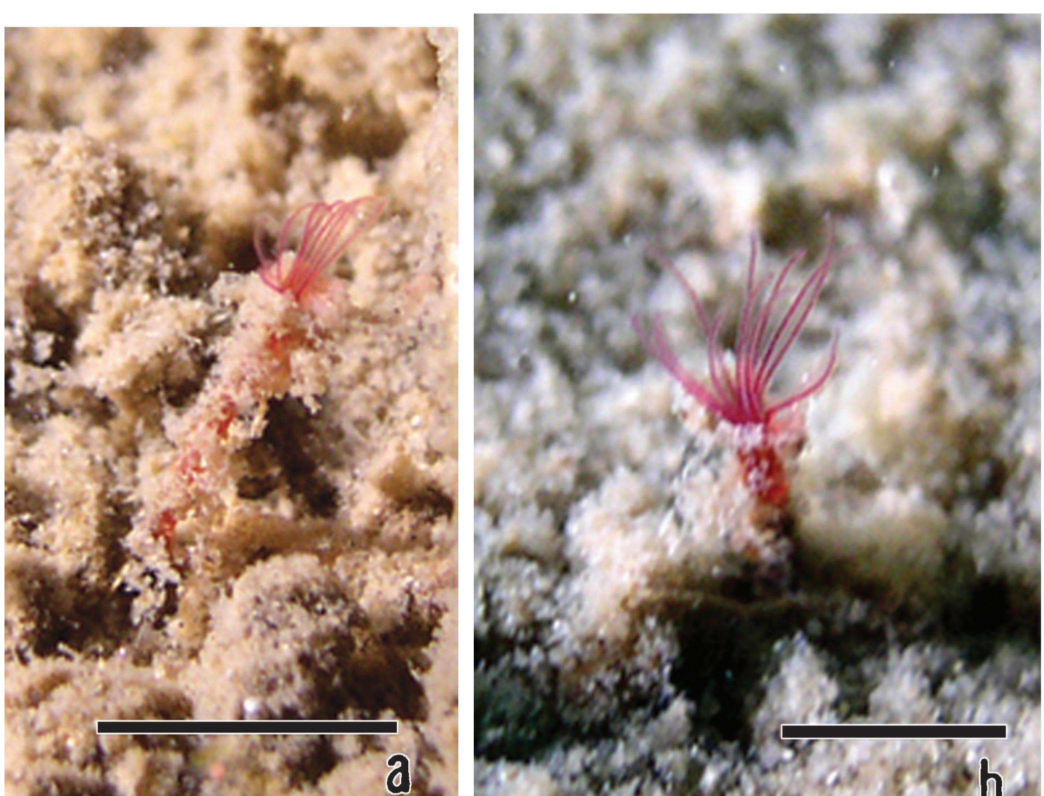

Fig. 2a, b. Photographs of the beard worm *O. mashikoi* taken on site. Each bar indicates 10 mm.

values decreased to one half or less the surface level, 0.15-0.19, 0.14-0.12, and 0.19-0.17 mg/g, respectively. In deeper samples, the values were lower by about 0.07, 0.01, and 0.10 mg/g, respectively. Fig.4 shows the data from November 2005.

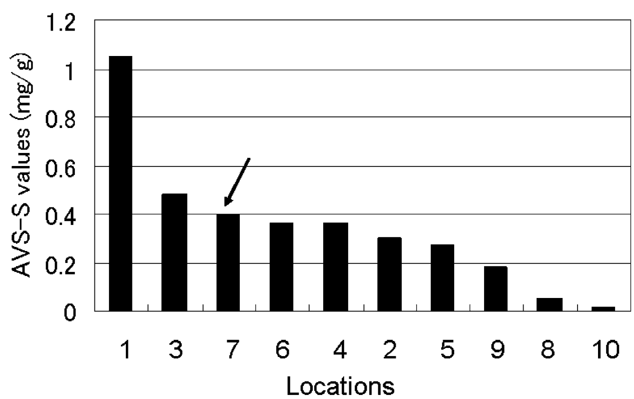

Fig. 3. Total sulfide levels in the surface layer of the sea bottom at sampling locations 1 to 10. Columns are arranged in order of the levels. Arrow shows the location that the beard worm inhabits.

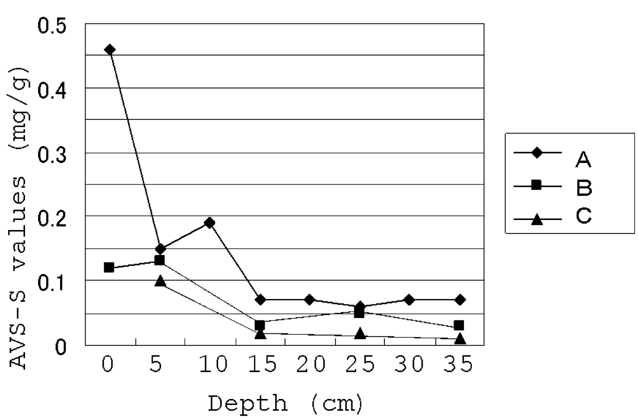

Fig. 4. Total sulfide levels in the mud in the vertical direction from 0 to 35 cm at sampling locations A, B, and C.

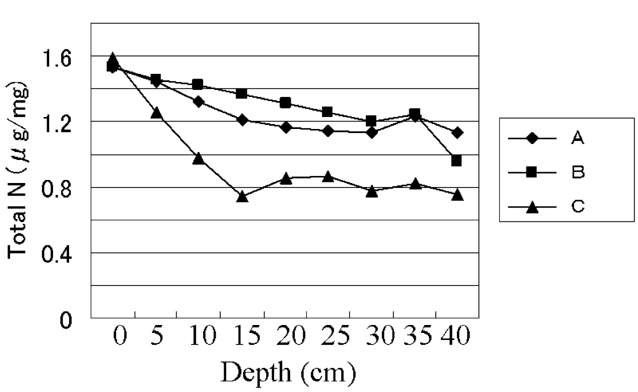

Fig. 5. Total nitrogen levels of the mud in the vertical direction from 5 to 35 cm at sampling locations A, B, and C.

Total nitrogen

At all three locations (A-C), the surface value tended to be the highest among the three times sampling periods, although the values fluctuated somewhat. The levels were about 1.00-1.50 μg/mg in November 2005, 0.23-0.37 μg/mg in March 2006, and 0.29-0.34 μg/mg in 2006. In deeper samples, at 40 cm, the values were somewhat lower, about 0.50-1.00, 0.19-0.31, and 0.22-0.35 μg/mg, respectively. The results from November 2005 are shown in Fig. 5.

DISCUSSION

One species (*Lamellibrachia* sp.) of tube worm that inhabits the hydrocarbon seeps is known to absorb hydrogen sulfide gas passing through the posterior part of the body, which is elongated into the sediment when hydrogen sulfide in the sea water is at too low a level for absorption from the plume, and when its concentration is sufficiently high in the sediment (Julian *et al*., 1999). In this tube worm, the permeability of hydrogen sulfide in the tube of the posterior part of the body is 2.5 times that of the tube in the trunk. The diameter of the tube is 1.4 mm, larger than that in *Oligobrachia mashikoi*. In addition, tubes are generally sturdier in tube worms than in beard worms. Thus, in *O. mashikoi*, which has a more delicate tube, it is possible that hydrogen sulfide is absorbed by passing through the tube into the body. At location 7, the hydrogen sulfide levels were the highest in the surface layer of the sediment. The worm projected 1/3 of the length of its tentacles from its tube, which protruded only several mm out of the sediment. Therefore, considering all of the findings, we believe that the life style of this beard worm reflects the distribution pattern of hydrogen sulfide in the sediment. The tubes in the surface layer of the sediment contain tentacles and cephalic lobe with a heart. In *O. mashikoi*, oxygen may be absorbed by the anterior part of the tentacles that project into oxygenated seawater, and hydrogen sulfide may be absorbed from the remaining part of the tentacles in addition to the anterior part of the body. Oxygen and hydrogen sulfide bound with hemoglobin must be transported by the blood stream produced by the heart to the body tissues and symbiotic bacteria, respectively. This idea does not conflict with existing reports on the functions of the plume in tube worms. On the other hand, Southward and Southward (1981) suggested the possibility that small beard worms, such as *O. mashikoi* can absorb organic matter dissolved in sea water into the body directly through the tube and integument. Therefore, even if the quantity of carbohydrates produced by the symbiotic bacteria is low, *O. mashikoi* may be able to absorb organic matter from the body elongated into the sediment. The lifestyle of the beard worm is illustrated in Fig. 6.

Tsukumo Bay is calm all year around. The coast has a variety of trees, and many branches hang over the sea. Therefore, a large quantity of leaves falls into the sea in autumn. The sea bottom that this worm inhabits is slightly concave (Fig. 7), and sea water flows counter-clockwise. Consequently, organic matter such as decayed leaves accumulates heavily. Leaves contain amino acid residues, such as methionine and cysteine containing sulfur and supply sulfate (SO_4^{-2} ions) to the sediment. On the other hand, the existence of sulfate-reducing bacteria is universal on sea bottoms. Bacteria reduce the sulfate and generate hydrogen sulfide.

At location 1, the total sulfide concentration was extremely high compared to that at other locations. The area surrounding location 1 includes many houses and is a sightseeing area. In addition, a small river flows into the site. Therefore, we suspect that organic matter deposited by humans is present at location 1.

In Japan, appropriate levels of sulfide contained in the sea bottom of fish nurseries are regulated by law to ensure sustainable aquaculture production by the Japan Fisheries Cooperatives (Yokoyama, 2003). According to the law, the total sulfide levels in the bottom sediment measured as AVS-S concentration should be kept under 2.5 mg/g dry mud. For example, in two sea bream fisheries in the Seto Inland Sea, the total sulfide levels were 0.9±0.5 mg/g and 1.2±0.6 mg/g: the control values in two other areas without fisheries were 0.2±0.1 mg/g and 0.3±0.2 mg/g (Pawar *et al*., 2001). The total sulfide levels in Tsukumo Bay are thus similar to the control levels at appropriate fish farms. Yokota (2003) showed that, in these environments, the biomass of the macrobenthos attains maximum levels when the total nitrogen level is 1.2 μg/g dry sediment. This nitrogen level is similar to that we measured in November 2005. These facts demonstrate that Tsukumo Bay is not a reductive environment deteriorated by eutrophication, but rather an oxygenated and productive one inhabited by various organisms. *Oligobrachia mashikoi* appears to be able to live in environments, such as soft and muddy sediment that contain slight amounts of sulfide.

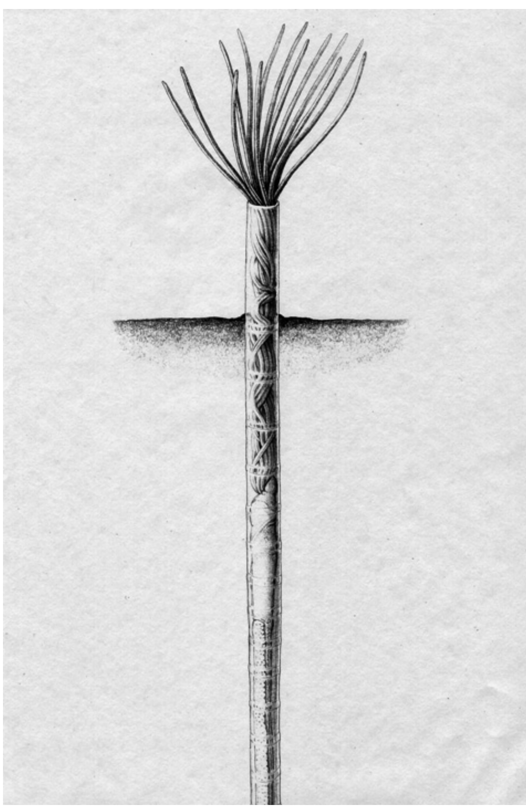

Fig. 6. Schematic illustration of the beard worm *O. mashikoi* inhabiting the surface of the sea bottom.

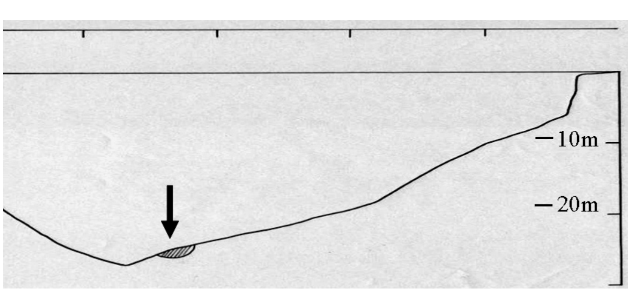

Fig. 7. Longitudinal section of Tsukumo bay (1300 m long). Arrow indicates the sea bottom inhabited by the beard worm *O. mashikoi*.

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