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# A Compilation of the Stable Isotopic Compositions of Carbon, Nitrogen, and Sulfur in Soft Body Parts of Animals Collected from Deep-Sea Hydrothermal Vent and Methane Seep Fields: Variations in Energy Source and Importance of Subsurface Microbial Processes in the Sediment-Hosted Systems

# 10

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## Abstract

The stable isotopic signatures of biophilic elements, such as carbon, nitrogen, and sulfur, exhibited in animal soft body parts are excellent indicators for evaluating the pathways of energy and food sources. Thioautotrophic and methanotrophic nutrition prevailed in deep-sea hydrothermal vent and methane seep areas results in sulfide-sulfur and methane-carbon isotopic ratios. In this study, we reevaluated the carbon, nitrogen, and sulfur isotope compositions of animals taken from deep-sea hydrothermal vents and methane seep areas in order to understand the detailed pathways of energy and food sources for the habitants. The results showed that most animals collected from sediment-starved hydrothermal areas rely on thioautotrophic nutrition, using hydrogen sulfide dissolved in venting fluids as the sole primary energy source. On the other hand, animals from sediment-covered hydrothermal vent and cold seep fields show some variations in energy sources, of both hydrothermal and microbial origins. Sediment-covered areas tend to be enriched in biomass and diversity relative to sediment-starved areas. The results suggest that fluid discharged through sediments to the seafloor are strongly affected by subsurface microbial processes and result in increased biomass and diversity of the seafloor animal community.

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**Keywords**

Chemosynthesis-based animals • Food ecology • Hydrothermal vent community • Methane seep community • Stable isotopes

**10.1 Introduction**

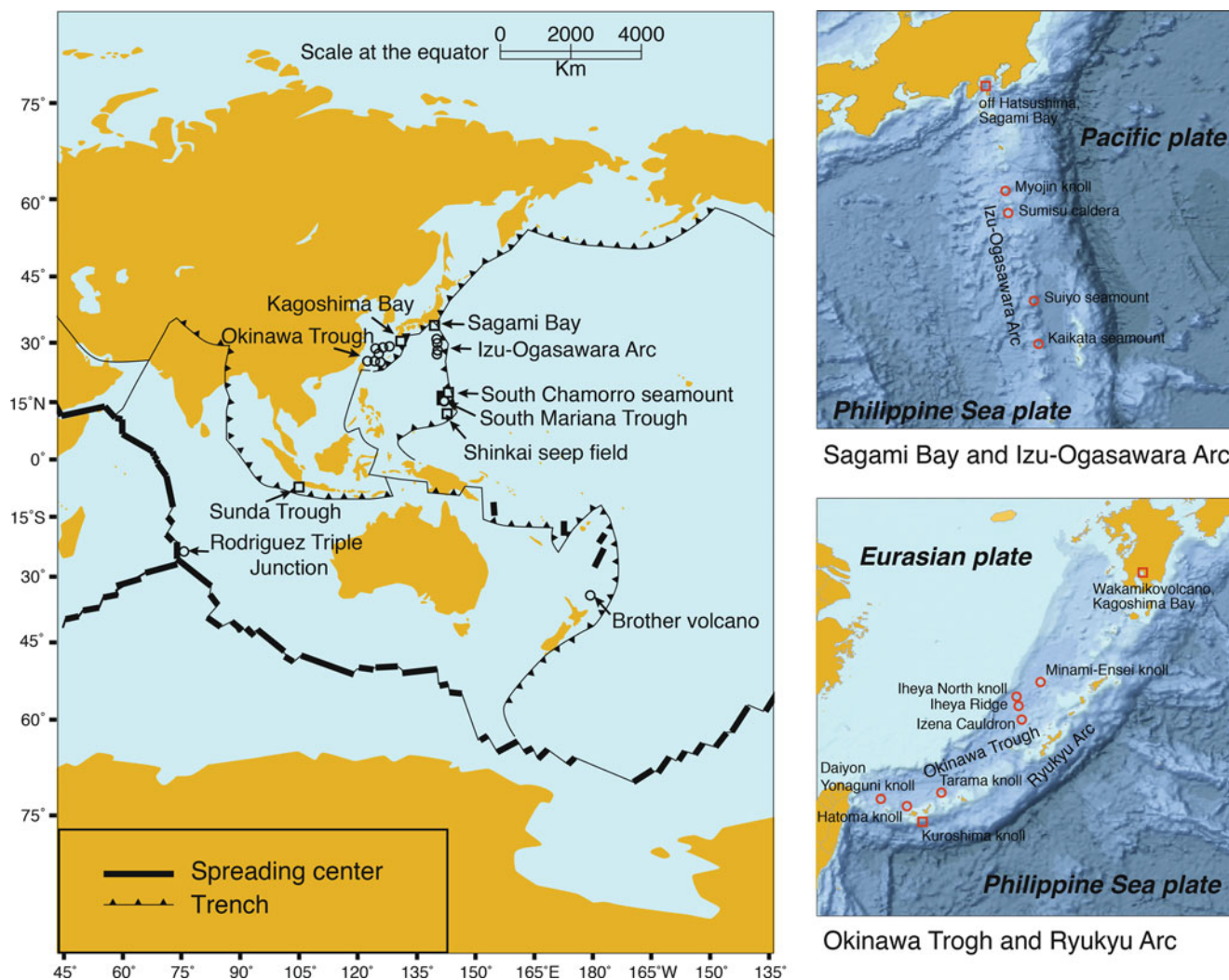
Since the first discovery of hydrothermal vent communities in 1977, the stable isotopic signatures of chemosynthesis-based animal species have been used to evaluate the isolation of vent communities from the usual marine food web systems that are supported by photoautotrophic primary production (e.g., Nelson and Fisher 1995; Mizota and Yamanaka 2003). Average carbon isotopic composition of marine photoautotrophic products produced by phytoplankton have been documented at  $\delta^{13}\text{C} = \sim -22\text{‰}$ , although they show a wide range ( $\delta^{13}\text{C} = -16$  to  $-28\text{‰}$ ), reflecting a variety of carbon fixation pathways together with physico-chemical conditions (e.g., Rees et al. 1978). The sulfur isotopic compositions of common marine animals, supported by the same photoautotrophic production, reflect the signature of sulfates dissolved in seawater, which is uniform throughout the oceans ( $\delta^{34}\text{S} = +21\text{‰}$ ) (Rees et al. 1978) and the sole nutrient source of sulfur for primary producers. On the other hand, it is reported that the carbon isotopic ratios of thioautotrophic microbes that use the Calvin cycle involving RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) for carbon fixation have a relatively narrow range of  $\delta^{13}\text{C}$  values, namely  $-35 \pm 5\text{‰}$ , and it is known that other types of thioautotrophic microbes have significantly higher  $\delta^{13}\text{C}$  values ( $\geq -20\text{‰}$ ) (e.g., Nelson and Fisher 1995; Markert et al. 2007). Furthermore, sulfur isotopic ratios of thioautotrophic microbes reflect sulfide nutrition with a limited kinetic isotope effect ( $\sim -5\text{‰}$ ) through the cell membrane (Fry et al. 1983). In natural environments,  $\delta^{34}\text{S}$  values of sulfides, which are mainly derived from volcanism and bacterial sulfate reduction, are clearly lower than those of sulfate-sulfur dissolved in seawater (e.g., Thode 1988; Canfield 2001). In the case of methanotrophic microbes, which are another important primary producer in the seep food web, carbon isotopic ratios reflect methane nutrition, while sulfur isotopic ratios reflect seawater sulfate-sulfur, similar to photoautotrophs. Some of the methane derived from pyrolysis of organic matter have carbon isotopic ratios similar to those of photoautotrophic products. Nevertheless, microbial methane, which prevails in anoxic sediments, has significantly lower  $\delta^{13}\text{C}$  values ( $\leq -45\text{‰}$ ), whereas abiotic methane has distinguishably high  $\delta^{13}\text{C}$  values ( $> -20\text{‰}$ ) (e.g., Schoell 1988).

However, the nitrogen sources for chemosynthesis-based animals are not well understood (Kennicutt et al. 1992; Fisher et al. 1994). Previously reported  $\delta^{15}\text{N}$  values for soft tissues from thiotrophic and methanotrophic animals range

from  $-20$  to  $+7\text{‰}$  (e.g., Nelson and Fisher 1995; Mizota and Yamanaka 2003). Such negative  $\delta^{15}\text{N}$  values of marine animal soft tissues have been reported only in communities consisting of chemosynthesis-based animals (e.g., Saino and Ohta 1989; Fiala-Médioni et al. 1993; Mizota and Yamanaka 2003) and cyanobacteria, which have the ability to fix dinitrogen ( $-3$  to  $+0\text{‰}$ ) (Minagawa and Wada 1984; Carpenter et al. 1997). The nitrogen nutrition of symbiotic bacteria is not well understood.

Stable isotopic signatures are quite useful indicators for distinguishing chemosynthesis-based animals from the phototrophic food web. Their isotopic signatures can possibly be used to identify the energy source for chemosynthesis. Mizota and Yamanaka (2003) reviewed the carbon, nitrogen, and sulfur isotopic compositions of chemosynthesis-based animals and the associated methane and sulfide data published prior to 2003, and discussed the flow of chemical energy from emitting fluids to the animal community. In the review, the importance of environmental isotopic data sets of sulfide-, methane-, and nitrogen-issuing species, was emphasized. Nevertheless, environmental isotopic data sets have not been fully integrated. Some animal clusters have been found far from vents where significant concentrations of sulfide and methane have been detected. Furthermore,  $\delta^{15}\text{N}$  values of nitrate, nitrite, and ammonium from the environment have not been reported. Most reported geochemical data from hydrothermal and seep fields are derived from venting fluids and visible seepages. This implies that the reported values are almost comparable to the end-member (i.e., deep-seated source) values. Therefore, it is difficult to directly compare the isotopic data to the soft body parts of animals, especially in sediment-hosted systems (i.e., methane seeps and sediment-covered hydrothermal fields), where emitting fluids penetrate through thick clastic sediments and are subsequently subjected to subsurface microbial transformation. An obvious example is methane seep communities dominated by thiotrophic animals, which use microbial hydrogen sulfide derived from sulfate reduction with methane as an electron donor (e.g., Mizota and Yamanaka 2003).

In addition, reduced chemical species, such as hydrogen sulfide and methane discharged from the seafloor, are incorporated by chemosynthetic and methanotrophic microbes, and the resulting microbial products have been considered to support not only vent- and seep-endemic animal communities but also common benthic and epibenthic animals. In the case of hydrothermal systems, discharge of these chemicals mainly originates from venting chimneys. In fact, at hydrothermal fields lacking sediment cover,



**Fig. 10.1** Maps showing the sample locations of this study. *Open circles* indicate hydrothermal fields. *Open squares* indicate methane seep fields. The Wakamiko site in Kagoshima Bay and the Shinkai seep field of Southern Mariana are categorized as methane seeps

hydrothermal discharge is mostly confined to venting chimneys and underlying hydrothermal mounds. Hydrothermal fields covered by thick clastic sediments have a few additional pathways for hydrothermal fluid discharge, e.g., diffusion into aquifers in overlying sediments. The fluids diffused within the sediments provide reduced chemical species and other nutrients to subsurface microbes. Some of the microbes may be grazed upon, thereby supporting benthic animals, and the others may generate reduced chemical species once more. Such secondary chemicals are also thought to support chemosynthesis-based animals that inhabit hydrothermal field and methane seep communities mentioned above.

Since 2003, reconnaissance surveying and sampling of chemosynthesis-based animals using submersibles from Japan and other countries is ongoing, and the following isotopic analyses have been made. In this Chapter, we have compiled the latest isotopic data from soft tissues of diverse

animals and environments located near hydrothermal vents and methane seeps of the extensive areas indicated in Fig. 10.1. We discuss the variations in energy and food sources of the environments from the view point of “TAIGA” (sub-seafloor fluid flow system, Urabe, Chap. 1), together with the importance of sub-seafloor microbial processes.

## 10.2 Materials and Methods

### 10.2.1 Geological Background of the Sample Materials

#### 10.2.1.1 Okinawa Trough

The Okinawa Trough (Fig. 10.1) is a typical back-arc rifting basin between the east margin of the East China Sea and the Ryukyu Islands (e.g., Letouzey and Kimura 1986). The trough is filled with thick clastic sediments derived from surrounding

continents and islands, together with volcanoclastic sediments associated with arc and back-arc volcanism in this area (Tsugaru et al. 1991; Ishibashi et al. Chap. 29). Vigorous hydrothermal activities associated with volcanism have been observed in the trough, and the chemistry of emitting fluids has been characterized by high volatiles (i.e., CO<sub>2</sub> and methane) and ammonium concentrations, suggesting the significant interaction of magma with sedimentary organic matter (e.g., Ishibashi and Urabe 1995; Kawagucci, Chap. 35). Therefore, many of the hydrothermal activities in the Okinawa Trough are interpreted as a typical sediment-hosted system. Chemosynthesis-based animal communities have been found around the hydrothermal venting area, and they have been characterized by the presence of dense *Bathymodiolus* mussels, vestimentiferan tubeworms, and galatheid crab communities (e.g., Fujikura et al. 2012; Watanabe and Kojima, Chap. 40).

Animal samples were obtained from the following hydrothermal fields: Minami-Ensei knoll, Izena Hole, Iheya Ridge, Iheya North knoll, Tarama knoll, Daiyon-Yonaguni knoll, and Hatoma knoll (Fig. 10.1).

#### 10.2.1.2 Izu-Ogasawara Arc

The Izu-Ogasawara Islands (Fig. 10.1) are a volcanic arc associated with subduction of the Pacific Plate beneath the Philippine Plate. Deep-sea hydrothermal activities have been identified in the calderas and craters of the submerged volcanoes (e.g., Glasby et al. 2000). The hydrothermal areas lack clastic sediments except for a small amount of volcanoclastic sediment that fills in the craters and calderas (e.g., Iizasa 1993; Takano et al. 2004). Relative to the fluids emitting from the Okinawa Trough, the resulting chemistry of the hydrothermal fluids is characterized by significantly lower methane and ammonium concentrations, i.e., a sediment-starved system (e.g., Ishibashi and Toki 2012). Chemosynthesis-based animal communities have been found around the fluid venting areas and have been characterized by occurrence of dense *Bathymodiolus* mussels (e.g., Fujikura et al. 2012).

Animal samples were collected from the following hydrothermal fields: Myojin Knoll, Sumisu caldera, Suiyo seamount, and Kaikata seamount (Fig. 10.1).

#### 10.2.1.3 Additional Hydrothermal Fields

Animal samples were also collected from the following hydrothermal fields and used for isotopic analyses: three sites at the southern most portion of the Mariana back-arc spreading center in the South Mariana Trough (the Snail, Archaean, and Yamanaka sites), Brothers Volcano in the Kermadec Arc (New Zealand), and Hakuho Knoll at the Rodriguez Triple Junction (Central Indian Ridge) (Fig. 10.1). The lack of clastic sediments in the hydrothermal fields reflects ammonium concentrations < 20 μM/kg (Kato

et al. 2009; de Ronde et al. 2011; Gamo et al. 2001), due to minimal contribution of sedimentary organic matter to the hydrothermal fluids. Bio-diversity of the animal communities observed at the Snail and Yamanaka sites in the South Mariana Trough was very low (Kojima and Watanabe, Chap. 26). Dense *Alviniconcha* snails (Wheat et al. 2003), crabs (*Gandalfus* sp.) and sea anemones (*Marianactis* sp.) have been identified. High bio-diversity, including dense long-necked barnacles (*Volcanolepas osheai*), shrimp (*Alvinocaris* sp.), and vestimentiferan tubeworms (*Lamellibrachia* sp.), has been observed in the animal community at the Brother Volcano site (Kermadec Arc), while many types of animals, such as *Rimicaris* shrimp, *Bathymodiolus* mussels, *Alviniconcha* snails (de Ronde et al. 2011), and Brachyuran crabs, have been observed at the Kairei hydrothermal field on Hakuho Knoll (Central Indian Ridge) (Hashimoto et al. 2001; Watanabe and Beedesse, Chap. 16).

#### 10.2.1.4 Sagami and Kagoshima Bays and Kuroshima Knoll

Sagami and Kagoshima Bays and Kuroshima Knoll (Fig. 10.1) are areas with significantly high methane flux from the seafloor (Masuzawa et al. 1992; Yamanaka et al. 2013). Sagami Bay and Kuroshima Knoll are located on the fore-arc regions, which are related to subduction of the Philippine Plate, where compression stress associated with subduction is a possible driving force for methane seepage. In Kagoshima Bay, methane rich gas is emitted from fumaroles, together with volcanic gas associated with the volcanic activity of the Wakamiko volcano. Sedimentary organic matter has been considered a primary source for methane as a result of microbial metabolism or pyrolysis. Chemosynthesis-based animal communities have been found around the seepages and have been characterized by the occurrence of dense *Bathymodiolus* mussel, *Calyptogena* clam, and vestimentiferan tubeworm communities (e.g., Fujikura et al. 2012).

#### 10.2.1.5 Additional Methane Seep Fields

Methane seepages from the seafloor have been reported from South Chamorro Seamount (Northern Mariana Fore-arc) (Yamanaka et al. 2003b), Shinkai Seep Field (Southern Mariana Fore-arc) (Ohara et al. 2012), and the western edge of Sunda Trough (off southwest Java) (Okutani and Soh 2005) (Fig. 10.1), and characteristic animal communities have also been reported around the seepages. *Calyptogena* clams have been observed at all of these sites, while *Bathymodiolus* mussels are primarily observed on the South Chamorro Seamount. The South Chamorro Seamount is a large serpentine mud volcano, while the Shinkai Seep Field is an outcrop of serpentinized peridotite (Ohara et al. 2012). This suggests that methane emitting from these sites



may be an abiotic product of the reaction between carbon dioxide and dihydrogen, which are expected to occur during serpentinization (Berndt et al. 1996). Serpentinization of the seafloor may be the cause of hydrothermal activity at sites such as the Rainbow and Lost City hydrothermal fields (Kelley et al. 2005). A detailed geochemical study has recently been conducted on the Shinkai Seep Field (Ohara et al. 2012). Therefore, in this study we have categorized this system as a methane seep.

Methane seepage at the Sunda Trough is located in a fore-arc basin covered with thick clastic sediments, suggesting that the tectonic background is comparable with the cold seepages of Sagami Bay. This means that the primary source of methane is expected to be organic matter of sedimentary origin.

### 10.2.2 Animal, Sediment, and Fluid Sampling Procedures

The animal samples used in this study are listed in Table 10.1. In addition to common marine benthic animals, such as sponges, sea cucumbers, starfishes, and fishes, well known chemosynthesis-based animals, including three bivalves (*Calyptogena*, *Bathymodiolus*, *Acharax*), two shrimps (*Alvinocaris*, *Limicaris*), two tubeworms (*Lamellibrachia*, *Alaysia*), one gastropoda (*Alviniconcha*), one galatheid crab (*Shinkaia*), among others, were analyzed for the carbon, nitrogen, and sulfur isotopic ratios in their soft tissues.

The sampling areas of the animals used in this study are also indicated in Table 10.1. The deep-sea hydrothermal fields of Okinawa Trough, Izu-Ogasawara Arc, Mariana Trough in the North Pacific Ocean, Kermadec Arc in the South Pacific Ocean, and Rodriguez Triple Junction in the Indian Ocean, and the methane seeps (cold seeps) of Kagoshima and Sagami Bays, Ryukyu and Mariana fore-arcs in the Pacific Ocean, and Sunda Trough in the Indian Ocean, were subjected to sample collection using submersibles (ROVs/HyperDolphin and Kaiko, DSV/Shinkai 6500 belonging to JAMSTEC and RV/Ropos belonging to Canadian Scientific Submersible Facility).

During the dive studies, animal samples were collected with a suction sampler or manipulator equipped with the submersible. Fluid and sediments used for geochemical characterization were collected using all-titanium ALVIN-type fluid samplers (Von Damm et al. 1985), gas-tight WHATS fluid samplers (Tsunogai et al. 2003), multi-cylinder type ROCS fluid samplers (Yamanaka et al. 2013), and NISKIN bottles equipped with the submersibles. The fluid and sediment samples were collected as close as possible to the animal habitat in order to evaluate the relationship between the animal soft bodies and the energy sources for the chemosynthesis-based animal communities, using isotopic signatures.

### 10.2.3 Analytical Procedures

Soft tissues of bivalve and gastropoda samples were dissected into gill, foot, mantle, adductor and viscera sections, and subjected to isotopic analyses. For vestimentiferan tubeworm samples, the soft body parts were dissected into gill, vestimentifera, and trophosome sections, and the trophosome sections were used for the analyses. Muscle samples were separated from the crustacean species. Other animals, such as limpets, sponges, polychaetes, etc., were used as whole-body samples due to small size or complex anatomy. The tissues dissected for analyses were treated with 1 M hydrochloric acid to remove carbonates, then freeze-dried prior to grinding. Preparation for sulfur isotopic measurements in the soft tissues was described previously (Mizota et al. 1999; Yamanaka et al. 2000a, b). Briefly, to remove excess seawater sulfate, the dissected soft tissues were dialyzed repeatedly in cellulose bags against 1 M lithium chloride solution at 5 °C. Dialyzed samples were then freeze-dried and pulverized. Samples of large size ( $\geq 1$  g dry weight) were placed in a Parr bomb 1108 Oxygen Combustion Vessel (a stainless steel vessel filled with oxygen gas under high-pressure (30 kg/cm<sup>2</sup>) and a few milliliters of distilled water). After combustion, the dried samples were completely converted into gas, and all sulfur compounds were trapped as sulfates in the distilled water within the vessel. The resulting sulfates were precipitated as BaSO<sub>4</sub>. The dried soft tissue samples were measured for carbon, nitrogen, and sulfur isotopic ratios using an EA/irMS (IsoPrime coupled with Euro Vector EA3000, GV Instruments, UK, and Delta Plus coupled with CE Instrument NA2500, Thermo Quest, USA). Small-sized samples for sulfur isotope analysis were wrapped within tin capsules and directly subjected to EA/irMS.

Sulfide-sulfur were recovered from the substrate sediments by treatment with warm ( $\leq 80$  °C) 30 % hydrogen peroxide solution to generate sulfates, which were finally converted into BaSO<sub>4</sub>. Dissolved sulfide in the hydrothermal fluids was fixed on board in order to precipitate zinc sulfide, and then it was converted to sulfate for recovery as BaSO<sub>4</sub> precipitate. The BaSO<sub>4</sub> precipitate was converted into SO<sub>2</sub> gas, as described by Yanagisawa and Sakai (Yanagisawa and Sakai 1983).

The concentration and carbon isotope composition of the dissolved methane in the water samples were determined following the methods of Tsunogai et al. (2000), using an isotope-ratio-monitoring-GC/MS (MAT 252, Thermo Quest, USA). The methane content in the samples was calculated by comparing the <sup>44</sup>CO<sub>2</sub> output with that of a working standard gas containing c. 500 ppm methane in nitrogen.

All of the isotopic values were expressed using  $\delta$  notation as a per mill deviation (‰) from international reference materials (VPDB for  $\delta^{13}\text{C}$ , CDT for  $\delta^{34}\text{S}$  and atmospheric N<sub>2</sub> for  $\delta^{15}\text{N}$ , respectively). Analytical errors associated with the overall process of these determinations were 0.2, 0.3, and 0.3‰, respectively.

Table 10.1 Animal samples discussed in this paper

Type	Area	Field	Site (marks in figures)	Longitude	Latitude	Depth (m)	Category	Genus (Order or Family)	Species	Food ecology <sup>a</sup>	Submersible type	Dive #	Year/ Month	Note												
															Organism	Sampling Info										
Hydrothermal system	Okinawa Trough	Minami-Ensei knoll	(MEn)	127°-38.392'E	28°-23.476'N	701-709	Sea sponge	(Demospongiae)	?	Heterotroph (F)	HyperDolphin	1327, 1328	2011/9													
															Izema Hole	JADE (IzJ)	127°-04.884'E	27°-16.296'N	1,305	Crab	<i>Shinkaita</i>	<i>crossneri</i>	Heterotroph and ectosymbiosis	HyperDolphin	1184	2010/9
																	127°-04.500'E	27°-15.936'N	1,536	Crab	<i>Paralomis</i>	<i>verrilli</i>	Heterotroph (C)	HyperDolphin	1193	2010/9
																	127°-04.500'E	27°-15.936'N	1,536	Crab	<i>Paralomis</i>	?	Heterotroph (C)	HyperDolphin	1193	2010/9
																	127°-04.884'E	27°-16.296'N	1,305	Shrimp	<i>Alvinocaris</i>	<i>longirostris</i>	Heterotroph and ectosymbiosis	HyperDolphin	1184	2010/9
																	127°-04.877'E	27°-16.299'N	1,536	Shrimp	<i>Opaepele</i>	<i>lolii</i>	Heterotroph and ectosymbiosis	HyperDolphin	1192	2010/9
																	127°-04.494'E	27°-15.942'N	1,520	Bivalve	<i>Bathymodiolus</i>	<i>platifrons</i>	Endosymbiosis (M)	HyperDolphin	1188	2010/9
																	127°-04.877'E, 127°-04.500'E	27°-16.299'N, 27°-15.936'N	1,536	Gastropoda	<i>Provanna</i>	?	Heterotroph (G)	HyperDolphin	1192, 1193	2010/9
																	127°-04.884'E	27°-16.296'N	1,300-1,535	Limpet	<i>Lepetodrilus</i>	<i>nux</i>	Heterotroph (G)	HyperDolphin	1184, 1192, 1193	2010/9
																	127°-04.877'E, 127°-04.500'E	27°-16.299'N, 27°-15.936'N	1,536	Limpet	<i>Bathymacraea</i>	<i>secunda</i>	Heterotroph (G)	HyperDolphin	1192, 1193	2010/9
																	127°-04.878'E	27°-16.302'N	1,306	Fish	(Zoarcidae)	?	Heterotroph (C)	HyperDolphin	1192	2010/9
																	127°-04.500'E	27°-15.936'N	1,536	Starfish	(Goniasteridae)	?	Heterotroph (D)	HyperDolphin	1193	2010/9
																	127°-04.500'E	27°-15.936'N	1,536	Starfish	(Asteroidea)	?	Heterotroph (D)	HyperDolphin	1193	2010/9
																	127°-04.884'E	27°-16.296'N	1,305	Sea sponge	(Demospongiae)	?	Heterotroph (F)	HyperDolphin	1184	2010/9
																	127°-04.500'E	27°-15.936'N	1,536	Sea sponge	(Hexachitellida)	?	Heterotroph (F)	HyperDolphin	1193	2010/9
		127°-04.500'E	27°-15.936'N	1,536	Sea sponge	(Demospongiae)	?	Heterotroph (F)	HyperDolphin	1193	2010/9															
		127°-04.494'E	27°-15.942'N	1,520	Polychaeta	<i>Branchipolynoe</i>	<i>pettiboneae</i>	Heterotroph (P)	HyperDolphin	1188	2010/9	Parasite on <i>Bathymodiolus</i> mussels														
		127°-04.799'E	27°-16.226'N	1,335	Polychaeta	<i>Paradiatsyche</i>	?	Endosymbiosis (T)	HyperDolphin	1193	2010/9															
		Hakurei (IzH)	127°-04.134'E, 127°-04.141'E	27°-14.940'N, 27°-14.944'N	1,593	Crab	<i>Shinkaita</i>	<i>crossneri</i>	Heterotroph and ectosymbiosis	HyperDolphin	1191, 1311	2010/9, 2011/8														
		Ihaya Ridge (IhR)	127°-04.089'E	27°-14.815'N	1,617	Bivalve	<i>Bathymodiolus</i>	<i>japonicus</i>	Endosymbiosis (M)	HyperDolphin	1329	2011/10														
			126°-58.188'E	27°-33.018'N	1,399	Crab	<i>Shinkaita</i>	<i>crossneri</i>	Heterotroph and ectosymbiosis	HyperDolphin	1183	2010/9														
			126°-58.188'E	27°-33.018'N	1,399	Shrimp	<i>Alvinocaris</i>	<i>longirostris</i>	Heterotroph and ectosymbiosis	HyperDolphin	1183	2010/9														
			126°-58.188'E	27°-33.018'N	1,399	Bivalve	<i>Calyptogena</i>	<i>okutanii</i>	Endosymbiosis (T)	HyperDolphin	1183	2010/9														

	126°58.188'E	27°33.018'N	1,399	Bivalve	<i>Acharax</i>	?	Endosymbiosis (T)	HyperDolphin	1183	2010/9
	126°58.188'E	27°33.018'N	1,399	Tube worm	<i>Alaysia</i>	?	Endosymbiosis (T)	HyperDolphin	1183	2010/9
Iheya North Knoll	127°04.494'E	27°15.942'N	1,520	Crab	<i>Shinkaia</i>	<i>erosnieri</i>	Heterotroph and ectosymbiosis	HyperDolphin	1188	2010/9
	127°04.494'E	27°15.942'N	1,520	Bivalve	<i>Calyplogena</i>	<i>soyoae</i>	Endosymbiosis (T)	HyperDolphin	1188	2010/9
	127°04.494'E	27°15.942'N	1,520	Bivalve	<i>Bathymodiolus</i>	<i>platifrons</i>	Endosymbiosis (M)	HyperDolphin	1188	2010/9
	126°53.993'E	27°47.286'N	993	Tube worm	<i>Lamellibrachia</i>	?	Endosymbiosis (T)	HyperDolphin	222	2003/9
	126°53.993'E	27°47.286'N	993	Barnacle	<i>Ashinkailepas</i>	<i>sepiophilica</i>	heterotroph (F)	HyperDolphin	222	2003/9
Tarama knoll	124°32.165'E	25°05.469'N	1,732	Shrimp	<i>Alvinocaris?</i>	?	Heterotroph and ectosymbiosis	HyperDolphin	1322	2011/9
	124°32.349'E	25°05.576'N	1,556	Fish	<i>Liparis</i>	?	Heterotroph (C)	HyperDolphin	1034	2009/7
	124°32.165'E	25°05.469'N	1,732	Fish	<i>Liparis</i>	?	Heterotroph (C)	HyperDolphin	1322	2011/9
	124°32.310'E	25°05.554'N	1,588	Starfish	(Asteroidea)	?	Heterotroph (D)	HyperDolphin	1034	2009/7
	124°32.310'E	25°05.554'N	1,588	Sea sponge	(Hexachimellida)	?	Heterotroph (F)	HyperDolphin	1034	2009/7
	124°32.136'E	25°05.326'N	1,850	Soft coral	(Alcyonacea)	?	Heterotroph (F)	HyperDolphin	1108	2010/4
	124°32.124'E	25°05.303'N	1,862	Sea cucumber	<i>Eryniaster</i>	<i>eximia</i>	Heterotroph (D)	HyperDolphin	1108	2010/4
	124°32.364'E	25°05.631'N	1,500	Octopus	<i>Benitochtopus</i>	?	Heterotroph (C)	HyperDolphin	1033	2009/7
Daiyon-Yonaguni knoll	122°41.999'E	24°50.931'N	1,336	Crab	<i>Shinkaia</i>	<i>erosnieri</i>	Heterotroph and ectosymbiosis	Shinkai2000	1273	2001/5
	122°41.999'E	24°50.931'N	1,336	Bivalve	<i>Bathymodiolus</i>	<i>platifrons</i>	Endosymbiosis (M)	Shinkai2000	1273	2001/5
Hatoma knoll	123°50.369'E	24°51.451'N	1,523	Crab	<i>Shinkaia</i>	<i>erosnieri</i>	Heterotroph and ectosymbiosis	Shinkai2000	1352	2002/5
	123°50.369'E	24°51.451'N	1,523	Shrimp	<i>Alvinocaris</i>	<i>longirostris</i>	Heterotroph and ectosymbiosis	Shinkai2000	1352	2002/5
	123°50.369'E	24°51.451'N	1,523	Shrimp	<i>Lebbeus</i>	<i>washingtonianus</i>	Heterotroph (D)	Shinkai2000	1352	2001/5
	123°50.369'E	24°51.451'N	1,523	Crab	<i>Munidopsis</i>	<i>ryukyuensis</i>	Heterotroph (D)	Shinkai2000	1352	2002/5
	123°50.365'E	24°51.558'N	1,484	Crab	<i>Paralomis</i>	?	Heterotroph (C)	Shinkai2000	1361	2002/6
	123°50.369'E	24°51.451'N	1,523	bivalve	<i>Bathymodiolus</i>	<i>platifrons</i>	Endosymbiosis (M)	Shinkai2000	1270	2001/5
Izu-Ogasawara arc	139°52'E	32°06'N	1,274	Crab	<i>Gandalphas</i>	<i>yunohana</i>	Heterotroph (D)	HyperDolphin	185	2003/6
	139°52.081'E	32°06.278'N	1,303	Bivalve	<i>Bathymodiolus</i>	<i>septemdiem</i>	Endosymbiosis (T)	HyperDolphin	1284	2011/6

(continued)

Table 10.1 (continued)

Type	Area	Field	Site (marks in figures)	Organism				Sampling Info						
				Longitude	Latitude	Depth (m)	Category	Genus (Order or Family)	Species	Food ecology <sup>a</sup>	Submersible type	Dive #	Year/ Month	Note
		Sumisu caldera	(Sm)	140°04.258'E	31°28.179'N	686	Bivalve	<i>Bathymodiolus septemdieteri</i>	<i>septemdieteri</i>	Endosymbiosis (T)	HyperDolphin	84	2002/3	
				140°04.258'E	31°28.179'N	686	Sea sponge	<i>Characella</i>	?	Endosymbiosis (T)	HyperDolphin	84	2002/3	Provided from Dr. Lindsay
				140°04.258'E	31°28.179'N	686	Tube worm	<i>Lamellibrachia</i>	?	Endosymbiosis (T)	HyperDolphin	84	2002/3	
		Suiyo Seamount	(Sy)	140°38.668'E	28°34.268'N	1,381	Bivalve	<i>Bathymodiolus septemdieteri</i>	<i>septemdieteri</i>	Endosymbiosis (T)	HyperDolphin	1285	2011/6	
		Kaikata Seamount	(Ki)	141°04.3'E	26°42.5'N	508	Crab	<i>Ganadafus yunohana</i>	<i>yunohana</i>	Heterotroph (D)	HyperDolphin	186	2003/6	Provided from Dr. Tsuchida
				141°04.250'E	26°42.500'N	448	Crab	<i>Leptodius exaratus</i>	<i>exaratus</i>	Heterotroph (D)	Shinkai2000	1234	2000/11	Provided from Dr. Tsuchida
				141°04.250'E	26°42.500'N	448	Fish	<i>Symphurus orientalis</i>	<i>orientalis</i>	Heterotroph (C)	Shinkai2000	1234	2000/11	Provided from Dr. Tsuchida
	South Mariana Trough	Mariana backarc spreading center	Snail site (Sn)	143°37.195'E	12°57.167'N	2,860	Crab	<i>Ganadafus yunohana</i>	<i>yunohana</i>	Heterotroph (D)	Shinkai6500	793	2003/10	
				143°37.167'E	12°57.185'N	2,861	Gastropoda	<i>Alviniconca hesvri</i>	<i>hesvri</i>	Endosymbiosis (T)	ROPOS	776	2004/3	
			Archaean site (Ar)	143°37.901'E	12°56.370'E	2,990	Gastropoda	<i>Phymorhynchus</i>	?	Heterotroph (D)	ROPOS	781	2004/3	
			Yamanaka site (Ym)	143°56.789'E	12°56.660'N	2,827	Bermacle	<i>Neoverruca brachyleptadoformis</i>	<i>brachyleptadoformis</i>	Heterotroph (F)	ROPOS	779	2004/3	
				143°56.796'E	12°53.638'N	2,828	Sea anemone	<i>Marianactis</i>	?	Heterotroph (D)	<i>ditto</i>	774	2004/3	
	Kermadec Arc	Brothers Volcano	Lower Cone site (Br)	179°04.302'E	34°52.722'S	1,336	Tube worm	<i>Lamellibrachia</i>	sp.1	Endosymbiosis (T)	Shinkai6500	854	2004/11	
				179°04.302'E	34°52.722'S	1,336	Tube worm	<i>Lamellibrachia</i>	sp.2	Endosymbiosis (T)	<i>ditto</i>	854	2004/11	
				179°04.302'E	34°52.722'S	1,336	Bermacle	<i>Vulcanolepas oshaei</i>	<i>oshaei</i>	Heterotroph (F)	<i>ditto</i>	854	2004/11	
	Rodriguez Triple Junction, Central Indian Ridge	Hakuo Knoll	Kaiko field (RtK)	70°02.40'E	25°19.16'S	2,432, 2,442	Bivalve	<i>Bathymodiolus marisindicus</i>	<i>marisindicus</i>	Endosymbiosis (T)	Kaiko	168, 169	2000/8	
				124°11.547'E	24°07.798'N	638, 624	Bivalve	<i>Bathymodiolus hirtus</i>	<i>hirtus</i>	Endosymbiosis (M)	Shinkai2000	1355, 1364	2002/5, 6	
				124°11.535'E	24°07.805'N	638, 624	Bivalve	<i>Bathymodiolus securiformis</i>	<i>securiformis</i>	Endosymbiosis (M)	Shinkai2000	1355, 1364	2002/5, 6	
				124°11.547'E	24°07.798'N	638, 624	Bivalve	<i>Calypptogena kawamurai</i>	<i>kawamurai</i>	Endosymbiosis (T)	Shinkai2000	1355, 1364	2002/5, 6	
				124°11.535'E	24°07.805'N	638, 624	Bivalve	<i>Calypptogena satsuma</i>	<i>satsuma</i>	Endosymbiosis (T)	HyperDolphin	686	2007/6	
	Kyushu Island Trough	Kagoshima Bay	Wakamiko (KgW)	130°48.082'E	31°39.742'N	102	Tube worm	<i>Lamellibrachia</i>	<i>satsuma</i>	Endosymbiosis (T)	HyperDolphin	1293	2011/6	
				139°13.396'E	35°00.926'N	928	Shrimp	<i>Alvinicaris longirostris</i>	<i>longirostris</i>	Heterotroph and ectosymbiosis	HyperDolphin	1293	2011/6	
				139°13.380'E	35°00.940'N	910	Bivalve	<i>Bathymodiolus platifrons</i>	<i>platifrons</i>	Endosymbiosis (M)	HyperDolphin	1291	2011/6	



139°13.494'E, 35°00.222'N, 1,180, 910	Bivalve	<i>Bathymodiolus japonicus</i>	Endosymbiosis (M)	HyperDolphin 524, 1291	2006/3, 2011/6
139°13.380'E 35°00.940'N					
139°13.322'E 35°00.955'N	Bivalve	<i>Calyptogena okutani</i>	Endosymbiosis (T)	HyperDolphin 1291	2011/6
139°13.322'E 35°00.955'N	Bivalve	<i>Calyptogena soyoae</i>	Endosymbiosis (T)	HyperDolphin 1291	2011/6
139°13.482'E 35°00.222'N	Bivalve	<i>Acharax johnsoni</i>	Endosymbiosis (T)	HyperDolphin 528	2006/3
139°13.482'E 35°00.222'N	Bivalve	(Thyasiridae)	?	HyperDolphin 528	2006/3
139°13.482'E 35°00.222'N	Bivalve	<i>Conchoecia bisecta</i>	Endosymbiosis (T)	HyperDolphin 528	2006/3
139°13.458'E 35°00.168'N	Gastropoda	<i>Provanna glabra</i>	Heterotroph (C)	HyperDolphin 525	2006/3
139°13.458'E, 35°00.168'N, 1,173, 1,172	Gastropoda	<i>Margarites shinkai</i>	Heterotroph (D)	HyperDolphin 525, 528	2006/3
139°13.482'E 35°00.222'N					
139°13.494'E 35°00.222'N	Gastropoda	<i>Phymorhynchus buccinoides</i>	Heterotroph (D)	HyperDolphin 524	2006/3
139°13.458'E 35°00.168'N	Gastropoda	<i>Bathymacaea nipponica</i>	Heterotroph (D)	HyperDolphin 525	2006/3
139°13.494'E 35°00.222'N	Gastropoda	<i>Oenopota sagamiata</i>	Heterotroph (D)	HyperDolphin 524	2006/3
139°13.458'E 35°00.168'N	Starfish	<i>Ophiuroidea</i>	?	HyperDolphin 525	2006/3
139°13.560'E 35°00.054'N	Sea anemone	<i>Actinaria</i>	?	HyperDolphin 524	2006/3
139°13.482'E 35°00.222'N	Tube worm	<i>Lamellibrachia</i>	?	HyperDolphin 528	2006/3
139°13.482'E 35°00.222'N	Tube worm	<i>Alaysia</i>	?	HyperDolphin 528	2006/3
139°13.458'E 35°00.168'N	Polychaeta	<i>Nicomache ohtai</i>	Heterotroph (D)	HyperDolphin 525	2006/3
146°00.210'E 13°46.999'N	Bivalve	<i>Bathymodiolus</i>	?	Kaiko 165	2000/6
143°02.94'E 11°39.09'N	Bivalve	<i>Calyptogena maritana</i>	Endosymbiosis (T)	Shimkai6500	1234
105°47.202'E, 7°24.600'S, 2,390, 2,100	Bivalve	<i>Calyptogena garuda</i>	Endosymbiosis (T)	Shimkai6500	716, 2002/10
105°46.998'E 7°24.600'S	Bivalve			727	Dr. Soh

\*Reported food ecology in previous literature: heterotroph (C): Carniver, Scavenger and/or Grazer, (D): Detritus and sediment feeder, (F): Filter feeder, (G): Grazer, (P) Parasite, ectosymbiosis (T) and endosymbiosis (T): harboring only thioautotrophic endosymbiont, endosymbiosis (M): harboring only methanotrophic endosymbiont, endosymbiosis (d): harboring both thiotrophic and methanotrophic endosymbionts

## 10.3 Analytical Results for Isotopic Composition

### 10.3.1 Isotopic Compositions of Animal Samples from Hydrothermal Fields

The analytical results for the isotopic compositions of animal soft body parts and issuing fluids are summarized in Tables 10.2 and 10.3. Diagrams of the relationships among nitrogen and sulfur isotopic ratios vs. carbon isotopic ratios are shown in Figs. 10.2 and 10.3. Abbreviations in the figures indicate the locations of the samples (red-colored abbreviations indicate sediment-starved hydrothermal fields, see Table 10.1), and the asterisks to the right of the abbreviations indicate the species that harbor methanotrophic endosymbionts. The diagrams also indicate the approximate ranges of common marine organisms that rely on phototrophic products (insert enclosed by green dotted box). The  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  plot in Fig. 10.2 shows that many animals, especially crustacean species, were among the range of common marine organisms, while the  $\delta^{13}\text{C}$  vs.  $\delta^{34}\text{S}$  plot (Fig. 10.3) indicates that most samples had lower  $\delta^{34}\text{S}$  values ( $< +15\text{‰}$ ), relative to the common marine organisms. *Calyptogena* clams, indicated by open circles, had a typical thioautotrophic range of  $\delta^{13}\text{C} = -35 \pm 5\text{‰}$  and  $\delta^{34}\text{S} \ll +15\text{‰}$ , while *Bathymodiolus* mussels, which harbor thioautotrophic and/or methanotrophic endosymbionts in their gill tissues, showed wider  $\delta^{13}\text{C}$  values ranging from  $-50$  to  $-25\text{‰}$  and significantly low  $\delta^{15}\text{N}$  values ( $< 0\text{‰}$ , Fig. 10.2). Nearly all methanotrophic mussels had high  $\delta^{34}\text{S}$  values, close to  $+15\text{‰}$ , while the thiotrophic mussels had significantly lower  $\delta^{34}\text{S}$  values, relative to the other mussels. Vestimentiferan tubeworms, indicated by open cross symbols, also had a wide range of  $\delta^{13}\text{C}$  values ( $-35$  to  $-12\text{‰}$ ), indicating that they harbor thioautotrophic endosymbionts with various types of carbon fixation pathways in addition to the Calvin cycle. Certain animal samples (*Alvinocaris* shrimp, *Alaysia* tubeworms at Iheya Ridge field, *Ashinkailepas* barnacles at Iheya North knoll, *Bathymodiolus* mussels at Sumisu caldera, and *Lamellibrachia* tubeworms at Brother seamount) had significantly lower  $\delta^{34}\text{S}$  values ( $< -10\text{‰}$ ), relative to the hydrogen sulfide issuing from the associated hydrothermal vents.

### 10.3.2 Isotopic Compositions of Animal Samples from Methane Seep Fields

Analytical results for animal soft tissues and issuing fluids are summarized in Table 10.3. Figures 10.4 and 10.5 show relationships comparable to those shown for the hydrothermal fields. Samples without abbreviations represent

collections off Hatsushima, in Sagami Bay. Nearly all data were beyond the range of common marine organisms. *Calyptogena* clams, as shown by open circles, were located in the typical thioautotrophic range, as were the specimens obtained from hydrothermal fields, with one exception: *Calyptogena soyoae* off Hatsushima ( $\delta^{34}\text{S} = +16.8\text{‰}$ ). Except for two groups of samples from Kuroshima knoll ( $\delta^{34}\text{S} = +10.6, +7.2$  for gill tissues), methanotrophic *Bathymodiolus* mussels had high  $\delta^{34}\text{S}$  values, which are comparable to those of common marine organisms. Some of the bivalves (*Bathymodiolus*, *Calyptogena*, and *Acharax*) showed significantly low  $\delta^{15}\text{N}$  values  $< -5\text{‰}$ . *Bathymodiolus* mussels also showed significantly low  $\delta^{15}\text{N}$  values as well as the lowest  $\delta^{13}\text{C}$  values. It appeared that the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of these samples are positively correlated ( $R^2 = 0.85$ ).

### 10.3.3 Stable Isotopic Composition of the Issuing Fluids Associated with Animal Communities

#### 10.3.3.1 Hydrogen Sulfide

The  $\delta^{34}\text{S}$  values of hydrogen sulfide dissolved in the hydrothermal fluids ranged from nearly 0 to  $+12\text{‰}$  (Table 10.2), whereas those from methane-rich seeps were less than  $-20\text{‰}$ , except for the fumarolic gas emitting from Wakamiko submarine volcano (Table 10.3). Thiotrophic animals, which are known to harbor only thioautotrophic endosymbionts or feed on thioautotrophic products, are expected to have  $\delta^{34}\text{S}$  values close to that of the associated hydrogen sulfide. Therefore, the apparent sulfur isotopic fractionation between the soft tissue of thiotrophic animals and the associated hydrogen sulfide collected from the hydrothermal systems should be zero or nearly zero. As shown in Fig. 10.6, most  $\delta^{34}\text{S}$  values of the animals were lower than those of the hydrogen sulfides, while some animals showed values slightly higher than those of the hydrogen sulfides. On the other hand, methanotrophic species, which are known to harbor methanotrophic endosymbionts, and heterotrophs relying on phototrophic products are expected to have high  $\delta^{34}\text{S}$  values, ranging from  $+15$  to  $+21\text{‰}$ , since they assimilate seawater sulfates with  $\delta^{34}\text{S}$  values of  $\sim +21\text{‰}$ . Figures 10.7 and 10.8 show the apparent differences between the  $\delta^{34}\text{S}$  values of animal soft tissues and seawater-sulfate for samples from hydrothermal systems and methane seeps, respectively. The diagrams show that, with some exceptions, especially bivalves inhabiting methane seeps (*Bathymodiolus japonicas*, *B. platifrons*, *B. hirtus*, and *B. securiformis* in Fig. 10.8),  $\delta^{34}\text{S}$  values for all animal tissues were significantly lower than that of seawater-sulfate.

**Table 10.2** Carbon, nitrogen, and sulfur isotope compositions of benthic animal bodies sampled around hydrothermal fields and associated environmental geochemical data

Area	Field	Site	Genus (Order or Family)	Species	Soft body parts										References					
					Carbon					Nitrogen						Sulfur				
					Tissues	$\delta^{13}\text{C}_{\text{VPDB}}$ (%)	SD	$\delta^{15}\text{N}_{\text{Air}}$ (%)	SD	Number	Methane $\delta^{13}\text{C}_{\text{VPDB}}$ (%)	Ammonium $\delta^{15}\text{N}_{\text{Air}}$ (%)	Hydrogen sulfide $\delta^{34}\text{S}_{\text{CDT}}$ (%)							
Okinawa Trough	Mimami-Ensei knoll		(Demospongiae) ?	Bulk	-26.6		-0.6		+6.9	10.6	1	-26.0 to -24.7*	nr	*Kawagucci et al. (2013)						
			(Demospongiae) ?	Bulk	-29.7		-0.2		+11.9	1										
			(Demospongiae) ?	Bulk	-26.0		+2.7		+13.6	1										
Izena Hole	JADE		<i>Shinkaia crosnieri</i>	Muscle	-23.1	0.4	+5.2	0.3	-3.8	3		-41.0 to -36.0*	nr	*Ishibashi et al. (1995), **Kim et al. (1989), Sakai et al. (1990), Gamo et al. (2001)						
			<i>Paralomis verrilli</i>	Muscle	-24.2		+5.6		-5.4	1										
			?	Muscle	-24.8	1.1	+6.8	1.2	+7.5	2										
			<i>Alvinocaris longirostris</i>	Muscle	-21.0	1.6	+4.3	1.1	-0.7, +2.7	4										
				Egg	-19.7	3.5	+0.5	2.2	+1.1, +1.6	4										
			<i>Opaepele loihi</i>	Muscle	-17.6	0.7	-0.6	0.4	nd	3										
			<i>Bathymodiolus platifrons</i>	Foot	-30.4	0.9	-0.4	1.1	+7.5, +7.7	4										
				Gill	-30.1	0.9	0.0	0.8	+8.5, +9.1	3										
				Gill	-31.5	0.6	nd		+10.6	2										
				Mantle	-31.5	1.1	-0.2		+11.4	2										
			<i>Provanna ?</i>	Bulk	-25.3	0.9	+3.0	2.5	+5.7	4										
			<i>Lepetodrilus nux</i>	Bulk	-20.8	2.3	+2.3	0.4	+3.5	5										
			<i>Bathycyanea secunda</i>	Bulk	-26.6	1.3	+0.9	1.7	+8.6	4										
			(Zoarcidae) ?	Bulk	-25.7		+7.7		-4.1	1										
			(Goniasteridae) ?	Bulk	-42.3		+8.3		nd	1										
			(Asteroidea) ?	Bulk	-34.4		+8.3		+14.6	1										
			(Demospongiae) ?	Bulk	-21.2		+1.8		+5.6	1										
			(Hexactinellida) ?	Bulk	-29.6		+7.3		+10.9	7.1										
			(Hexactinellida) ?	Bulk	-30.6		+8.0		+6.9	1										
			(Demospongiae) ?	Bulk	-29.5		+7.9		nd	1										
			(Demospongiae) ?	Bulk	-38.5		-6.6		nd	1										
			<i>Branchipolynoe pettiboneae</i>	Bulk	-29.7	0.6	+6.4	0.5	+8.9	4										
			<i>Paradielychone ?</i>	Bulk	-33.4	1.1	+2.7	0.3	+3.6	3										
Hakurei			<i>Shinkaia crosnieri</i>	Muscle	-22.1	1.4	+4.7	1.3	+0.1	6		-32.0*	nr	*Kawagucci et al. (2010)						
			<i>Bathymodiolus japonicus</i>	Gill	-31.3	1.3	-3.3	0.1	-8.0	3										
Ihaya Ridge			<i>Shinkaia crosnieri</i>	Muscle	-23.7	0.7	+4.4	0.6	-1.1	3		-41.2*	nr	*Ishibashi et al. (1995), **Kim et al. (1990), Gamo et al. (1991)						
			<i>Alvinocaris longirostris</i>	Muscle	-26.1	2.0	+6.9	1.1	-21.0	3										
				Egg	-34.7	0.3	+3.3	0.8	-21.0	3										
			<i>Calyptogena okatani</i>	Gill	-35.2	0.2	+3.1	5.1	+7.7	2										
				Foot	-33.9	1.0	+4.0	2.9	+2.0	3										
			<i>Acharax ?</i>	Bulk	-31.7	0.5	+1.6	2.3	-6.5	3										

(continued)

Table 10.2 (continued)

Area	Field	Site	Genus (Order or Family)	Species	Soft body parts					Issuing fluids			References	
					Tissues	Carbon		Nitrogen		Sulfur	Methane	Ammonium		Hydrogen sulfide
						$\delta^{13}\text{C}_{\text{VPDB}}$ (%)	SD	$\delta^{15}\text{N}_{\text{Air}}$ (%)	SD					
Number	SD	Number	SD	Number	SD	Number	SD	Number	SD	Number	SD			
			<i>Alaysia</i> ?	Bulk	-17.5	0.5	+3.5	0.2	-22.0	3				
Iheya North Knoll			<i>Shinkaia crosnieri</i>	Muscle	-32.4		+4.1		+6.9	1	-54.0 to -52.8*	nr	+8.0 to +12.2**	*Kawagucci et al. (2011), **Yamanaka et al. (2000b)
			<i>Calypptogena soyocae</i>	Gill	-37.5*		+2.0		+1.8*	1				*Mae et al. (2007)
			<i>Bathymodiolus platifrons</i>	Mantle	-36.3*		+4.5		+0.4*	1				<i>ditto</i>
			<i>Bathymodiolus platifrons</i>	Gill	-45.2	0.9	-4.4	0.2	+13.3	3				
			<i>Bathymodiolus platifrons</i>	Gill	-49.2*	1.6*	-4.2*	0.3*	+13.4*	3				*Mae et al. (2007)
			<i>Bathymodiolus platifrons</i>	Mantle	-49.0*	2.9*	-3.7*	0.5*	+18.3*	3				<i>ditto</i>
			<i>Lamellibrachia</i> ?	Trophosome	-22.3	1.3	+2.9	0.9	nd	6				
			<i>Ashinkailepas sepiophilata</i>	Bulk	-22.8	0.4	+7.2	0.7	-11.0	5				
Taroma knoll			<i>Alvinocaris</i> ?	Muscle	-15.1		-0.6		+6.8	1	-38.4*	nr		*Inoue and Ueno, personal communication
			<i>Liparis</i> ?	Muscle	-22.3		+9.2		-7.3	1				
			<i>Liparis</i> ?	Muscle	-25.7		+13.6		+7.5	1				
			(Asteroidea) ?	Bulk	-32.4		+15.1		+8.7	1				
			(Hexactinellida) ?	Bulk	-34.3		+10.6		+5.0	1				
			<i>ditto</i>	Bulk	-35.1		+9.9		+4.8	1				
			<i>ditto</i>	Bulk	-32.1		+7.7		+2.5	1				
			(Aleyonacea) ?	Bulk	-26.3		+11.6		+13.8	1				
			<i>Eryniaster eximia</i>	Bulk	-20.7		+12.8		+14.6	1				
			<i>Benthocropus</i> ?	Muscle	-23.1		+14.0		+11.5	1				
Yonaguni knoll			<i>Shinkaia crosnieri</i>	Muscle	-22.9	0.0	+3.1	0.1	+5.4	3	-27.3 to -24.8*	nr	+13.0	*Konno et al. (2006)
			<i>Bathymodiolus platifrons</i>	Muscle	-17.3		+1.0		+4.6	1				
			<i>Bathymodiolus platifrons</i>	Gill	-25.1	0.1	-7.5	1.4	+13.5	2				
			<i>Bathymodiolus platifrons</i>	Mantle	-26.2	0.2	-5.9	1.0	+13.2	2				
			<i>Bathymodiolus platifrons</i>	Gill	-24.5*		-6.8*		+11.1*	1				*Naraoka et al. 2008
Hatoma knoll			<i>Shinkaia crosnieri</i>	Muscle	-24.6	1.6	+5.1	0.7	+8.3	6	-51.3 to -44.3*	nr	+8.0 to +12.0	*Naraoka et al. (2008)
			<i>Alvinocaris longirostris</i>	Muscle	-32.2	5.8	+5.2	1.2	+11.1	3.9				
			<i>Lebbeus washingtonianus</i>	Muscle	-30.8	0.1	+5.5	0.0	nd	2				
			<i>Manidopsis ryukyuensis</i>	Muscle	-28.3	0.8	+3.8	0.7	nd	4				
			<i>Paratomis</i> ?	Muscle	-26.7		+9.0		nd	1				
			<i>Bathymodiolus platifrons</i>	Gill	-44.8*		-4.4*		+13.9*	1				*Naraoka et al. (2008)
Izu-Ogasawara arc			<i>Gandalfus yunohana</i>	Muscle	-15.9	2.2	+8.1	0.4	nd	5	-16.3*	nr	+4.9 to +5.6**	*Tsunogai et al. (2000), **Yamanaka et al. (2000a, 2000b)
			<i>Bathymodiolus septemderim</i>	Gill	-34.4	0.7	+0.8	0.8	+2.9, +3.1	4				
Sumisu caldera			<i>Bathymodiolus septemderim</i>	Gill	-34.5		-4.2		-24.4	1	nr	nr	nr	

	<i>Characella</i>	?	Bulk	-34.8	+11.1	-5.9	1		Harboring thioautotrophic endosymbiont (Nishijima et al. (2010))
	<i>Lamellibrachia</i>	?	Trophosome	-12.9	+6.1	-18.5	1		
Suiyo Seamount	<i>Bathymodiolus septemdieterm</i>		Gill	-35.3	-2.4	-0.3	1	-7.4 to -5.3*	+3.1** **Yamanaka et al. (2000a, 2000b)
			mantle	-32.8	-1.9	+2.1	1		
			Gill	-36.0*	-5.1*	+4.3*	1		*Naraoka et al. (2008)
			Gill	-34.1	1.1	-1.9	1.7	-0.1	
Kaikata Seamount	<i>Gandalfus yunohana</i>		Muscle	-20.2	2.3	+9.9	0.7	nd	nr
	<i>Leptodius exaratus</i>		Muscle	-21.9	0.4	+8.4	0.6	nd	
	<i>Symphurus orientalis</i>		Muscle	-17.4	+10.1	nd	1		
South Mariana backarc Trough spreading center	<i>Gandalfus yunohana</i>		Muscle	-21.2	1.8	+9.7	1.2	+7.5	nr
	<i>Alviniconca hessri</i>		Muscle	-31.3	0.4	+3.6	0.8	+9.8	+7.0 - +8.7* (Sulfide minerals)
	<i>Phymorhynchus</i>	?	foot	-30.8	+4.8	+4.4	1	nr	-1.8 to +1.7* *Kakegawa et al. (2008) (Sulfide minerals)
	<i>Neoverruca brachylepadoformis</i>		Bulk	-21.8	0.7	+10.4	0.8	nd	+5.1 to +6.3* *Kakegawa et al. (2008) (Sulfide minerals)
	<i>Marianactis</i>	?	Bulk	-17.7	+9.7	+4.6	1		
Kermadec Arc	<i>Lamellibrachia</i>	?	Trophosome	-7.5	1	+1.9	0.4	nd	-8.0 to -4.8*
			Trophosome	-7.5	1	+1.9	0.4	nd	-37.0 to -33.9*
	<i>Lamellibrachia</i>	?	Trophosome	-12.9	0.3	+3.7	0.0	nd	
	<i>Vulcanolepas osheai</i>		Bulk	-13.0	1.7	+7.8	1.1	nd	
Central Indian Ridge	<i>Bathymodiolus marisindicus</i>		Gill	-31.3*	0.7*	-8.1*	1.5*	+5.3**	+6.8 to +7.0** *Van Dover et al. (2001), **Yamanaka et al. (2003a), ***Gamo et al. (2001)

Asterisks on the right shoulder of isotope values indicate cited data appeared in Reference column at the same line.  
nr not reported, nd not determined

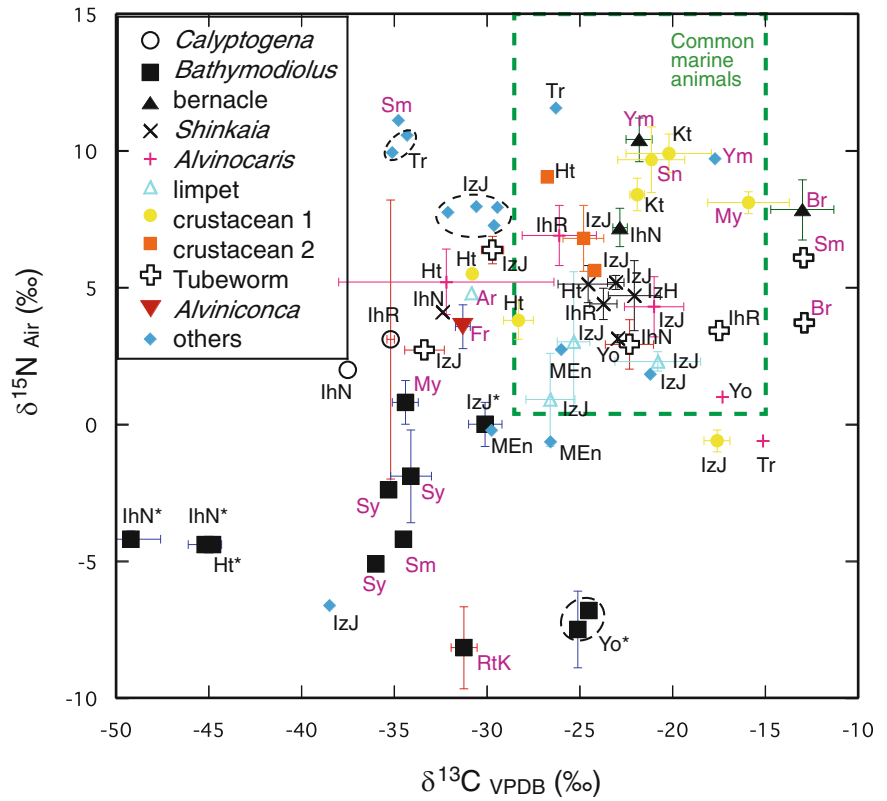




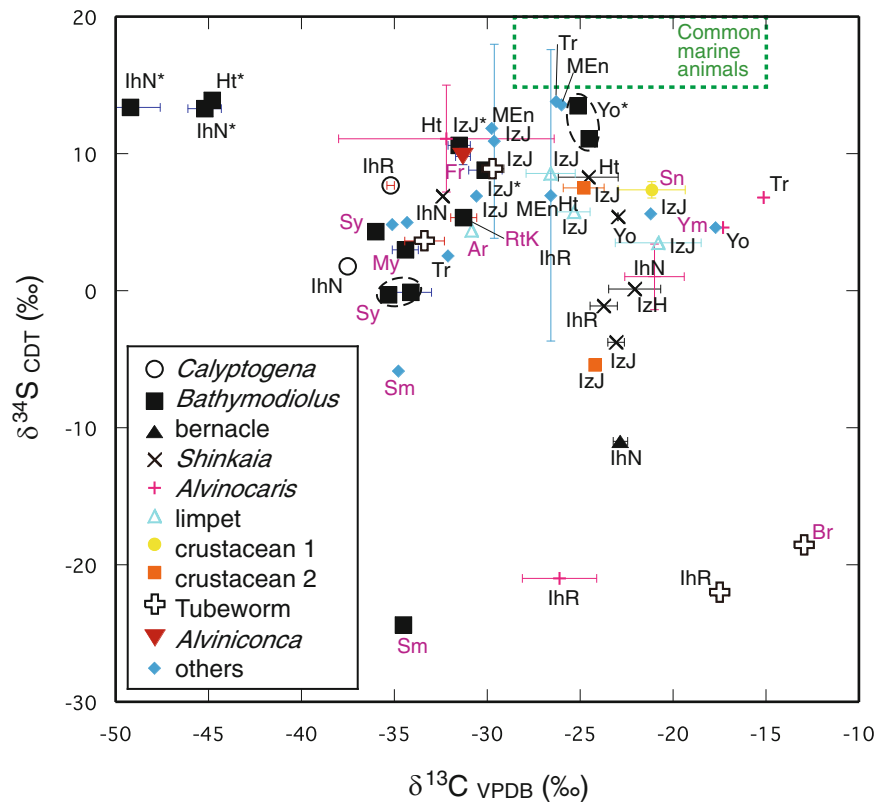
	(Thyasiridae) ?	Gill	-37.6	-7.0	nd	1		
	<i>Conchocelea bisecta</i>	Gill	-36.0	0.1	-8.5	0.1	nd	2
	<i>Provanna glabra</i>	Bulk	-30.7	0.6	-2.3	0.6	nd	2
	<i>Margarites shinkai</i>	Bulk	-32.9	0.1	-1.7	0.1	nd	2
	<i>Phymorhynchus buccinoides</i>	Bulk	-58.6	4.4	-4.0	1.2	+13.8	6
	<i>Bathymacrea nipponica</i>	Bulk	-25.7		-3.7		nd	1
	<i>Oenopota sagamitana</i>	Bulk	-30.9	3.6	+2.0	1.5	nd	6
	(Ophiuroidea) ?	Bulk	-32.8	1.3	-2.6	1.1	nd	4
	(Actinaria) ?	Bulk	-15.2	0.7	+15.0	0.7	nd	2
	<i>Lamelliobranchia</i> ?	Trophosome	-25.4	0.9	+4.1	0.9	+7.8	3
	<i>Lamelliobranchia</i> ?	Trophosome	-20.2	1.5	-0.1	1.8	-27.9, -17.9	
	<i>Alaysia</i> ?	Trophosome	-19.5	1.1	+4.2	1.1	nd	2
	<i>Nicomache ohtai</i>	Bulk	-33.3		+1.1		nd	1
Northern Mariana arc	<i>Bathymodiolus</i> ?	Gill	-18.9*	+1.8*	+10.6*		-14.6*	nr
		Foot	nr	nr	+10.4*			1
		Mantle	-21.4*	+3.1*	+10.2*			1
		Adductor	-19.1*	+2.7*				1
		Viscera	-20.2*	+2.9*				1
Southern Mariana arc	<i>Calypptogena mariana</i>	Gill	-34.6*	0.5*	+4.2*	1.4*	-9.0	nr
								1.0
								3
								nr
								nr
								nr
Sunda Trough	<i>Calypptogena garuda</i>	Gill	-35.5	0.3	-1.5	4.2	-12.3	4.2
		Foot	-34.4	1.2	+2.2	4.4	nd	2
								2
								2
								2
								2
								2
								2

Asterisks on the right shoulder of isotope values indicate cited data appeared in Reference column at the same line.  
nr not reported, nd not determined

**Fig. 10.2** Plot of carbon vs. nitrogen isotopic ratios for animal soft bodies from hydrothermal fields. Numerical data are shown in Table 10.2. Barnacles include *Ashinkailepas* and *Neoverruca*. Crustacean 1 includes two shrimps, *Lebbeus* and *Opaepele*, and three crabs, *Munidopsis*, *Gandalfus*, and *Leptodius*. Crustacean 2 includes the crab, *Paralomis*. Others include sea anemones, soft corals, and sea sponges. Abbreviations in the figures indicate the locations of the samples (red-colored abbreviations indicate sediment-starved hydrothermal fields, see Table 10.1)

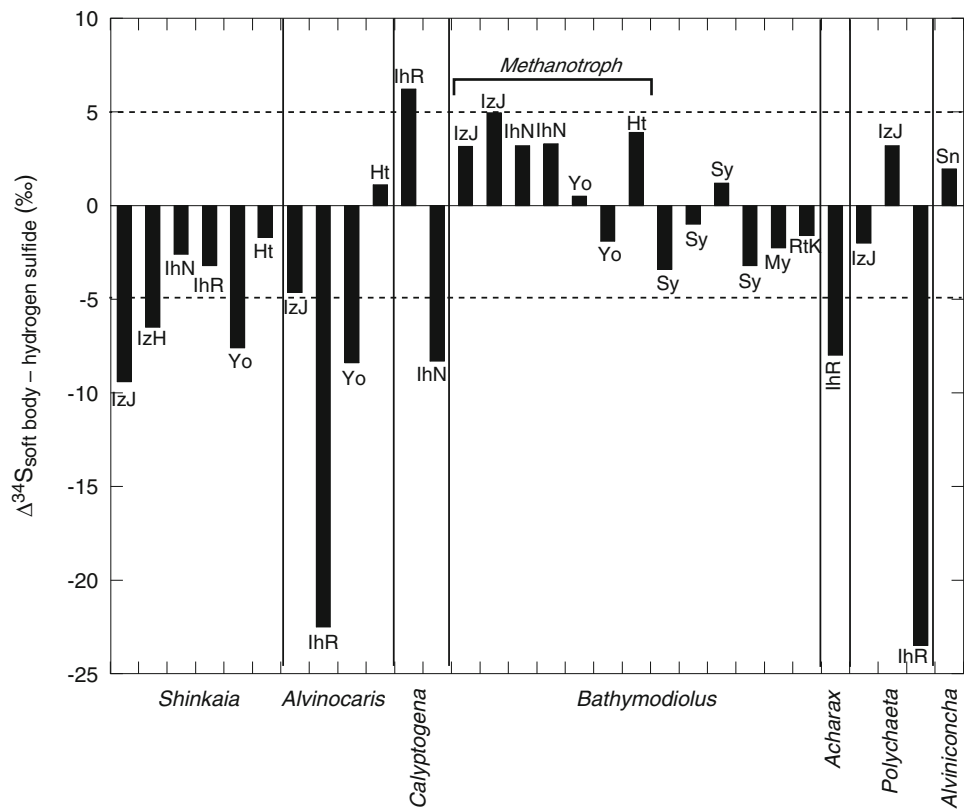


**Fig. 10.3** Plot of carbon vs. sulfur isotopic ratios for animal soft bodies from hydrothermal fields. Numerical data are shown in Table 10.2. Barnacles include *Ashinkailepas* and *Neoverruca*. Crustacean 1 includes two shrimps, *Lebbeus* and *Opaepele*, and three crabs, *Munidopsis*, *Gandalfus*, and *Leptodius*. Crustacean 2 includes the crab, *Paralomis*. Others include sea anemones, soft corals, and sea sponges. Abbreviations in the figures indicate the locations of the samples (red-colored abbreviations indicate sediment-starved hydrothermal fields, see Table 10.1)

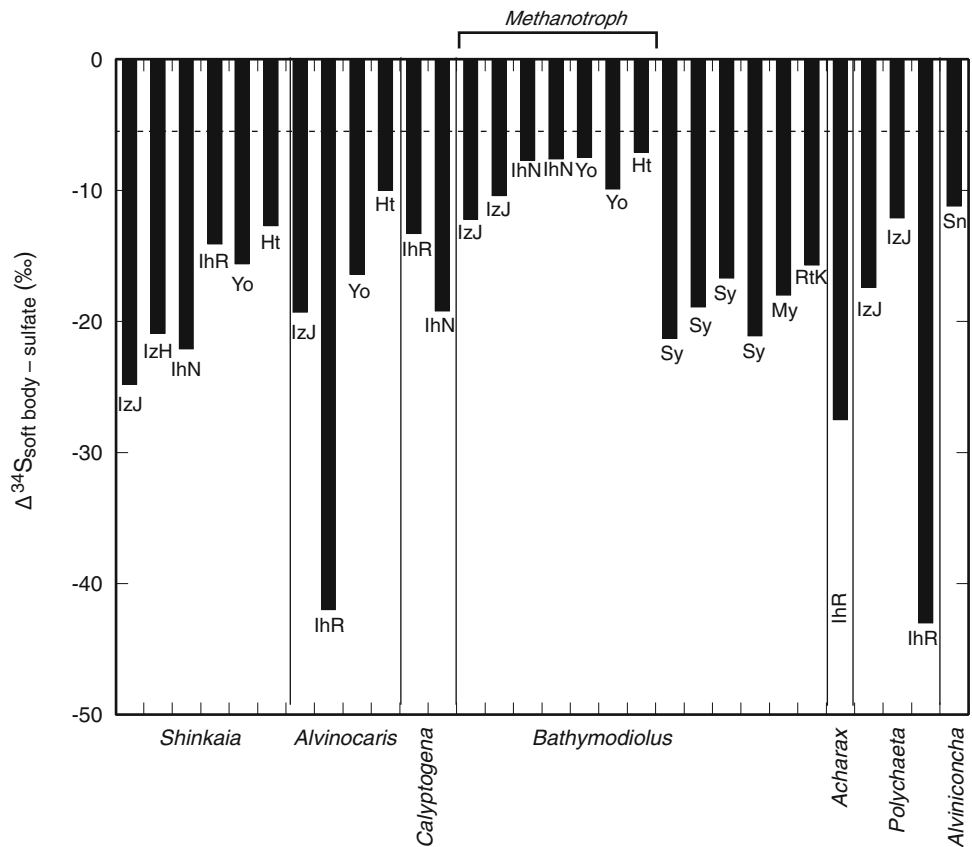




**Fig. 10.6** Apparent differences between the  $\delta^{34}\text{S}$  values of animal soft bodies and issuing hydrogen sulfide from hydrothermal fields. The two dotted lines indicate the possible range of variation in  $\delta^{34}\text{S}$  values of hydrogen sulfide and kinetic isotope fractionation involved with its assimilation. Abbreviations indicate the sample locations shown in Table 10.1

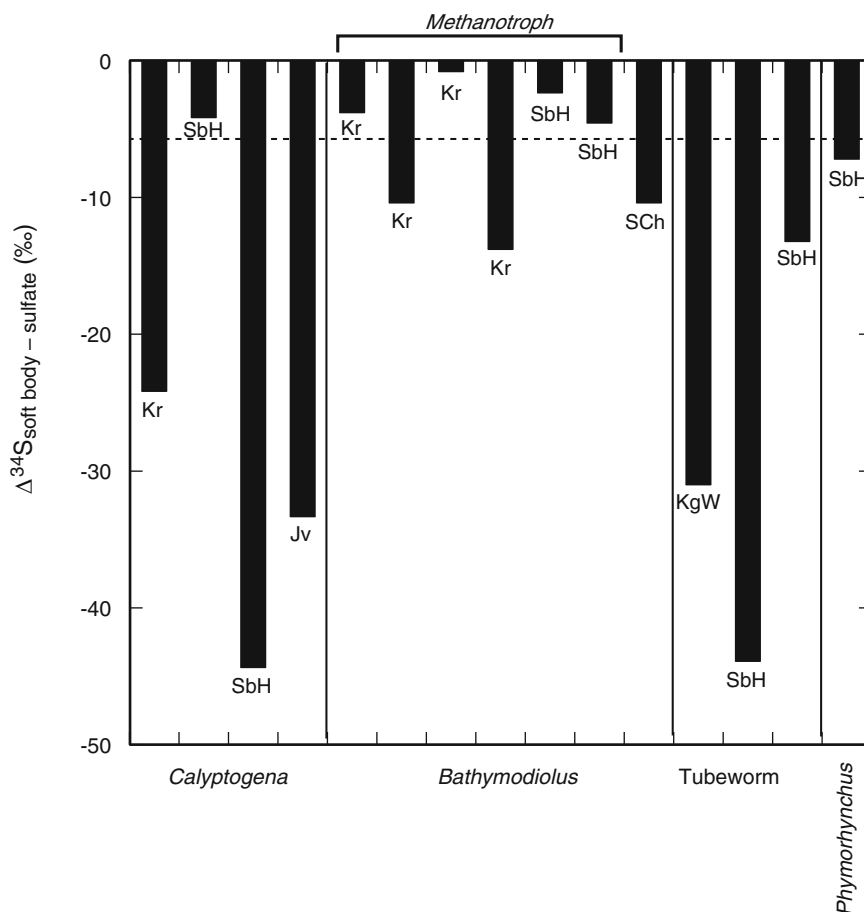


**Fig. 10.7** Apparent differences between the  $\delta^{34}\text{S}$  values of animal soft bodies from hydrothermal fields and seawater sulfate-sulfur ( $\delta^{34}\text{S} = +21 \text{‰}$ ). The dotted line indicates the possible range of kinetic isotope fractionation involved in the incorporation and subsequent assimilation of sulfates. Abbreviations indicate the sample locations shown in Table 10.1





**Fig. 10.8** Apparent differences between the  $\delta^{34}\text{S}$  values of animal soft body parts obtained from methane seep areas and seawater sulfate-sulfur ( $\delta^{34}\text{S} = +21\text{‰}$ ). The dotted line indicates the possible range of kinetic isotope fractionation involved with assimilation of sulfate. Abbreviations indicate the sample locations shown in Table 10.1



### 10.3.3.2 Methane

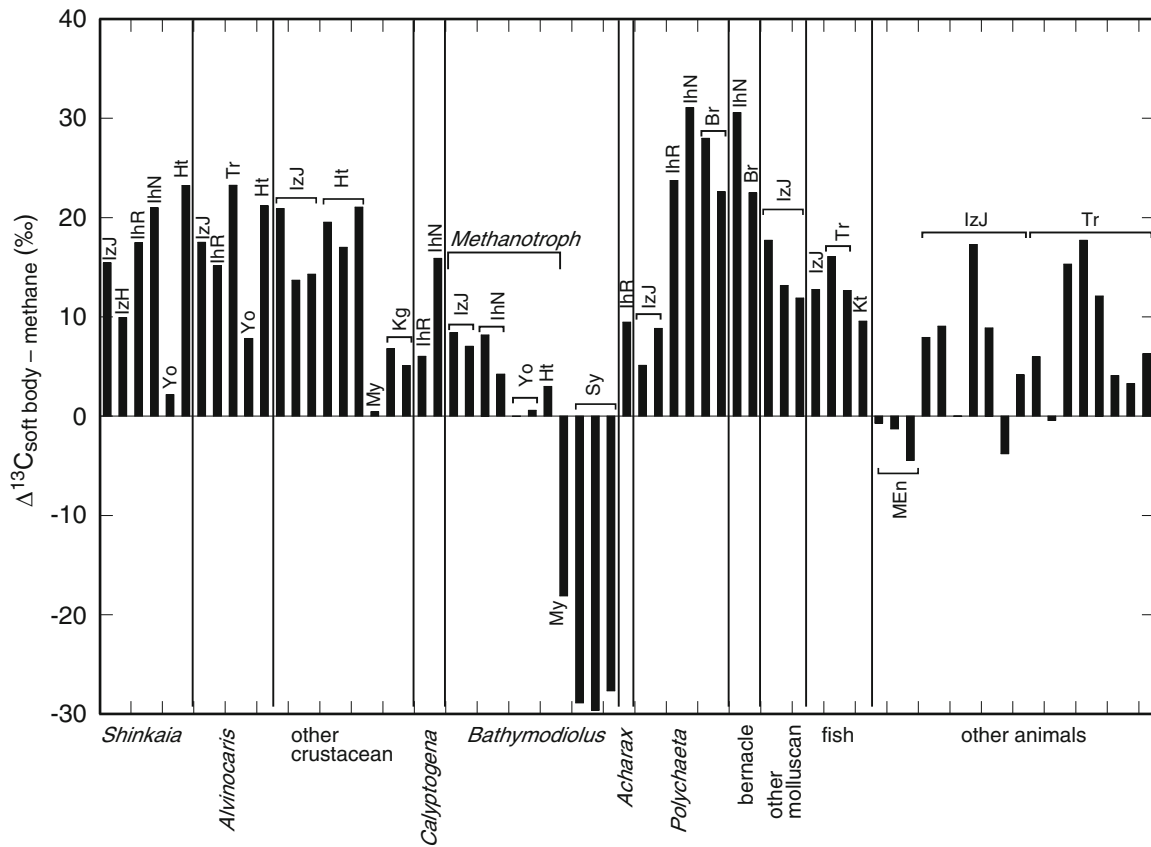
The  $\delta^{13}\text{C}$  values of dissolved methane in the venting fluids ranged from  $-54$  to  $-4\text{‰}$  (Tables 10.2 and 10.3). Methanotrophic species, which are known to harbor methanotrophic endosymbionts, are expected to show  $\delta^{13}\text{C}$  values close to that of the associated methane. Apparent differences between  $\delta^{13}\text{C}$  values of animal soft tissues and the associated methane are shown in Figs. 10.9 and 10.10. Most animal samples showed higher  $\delta^{13}\text{C}$  values relative to those of the associated methane.

## 10.4 Discussion

### 10.4.1 The Contribution of Thioautotrophic Nutrition to the Benthic Animal Community

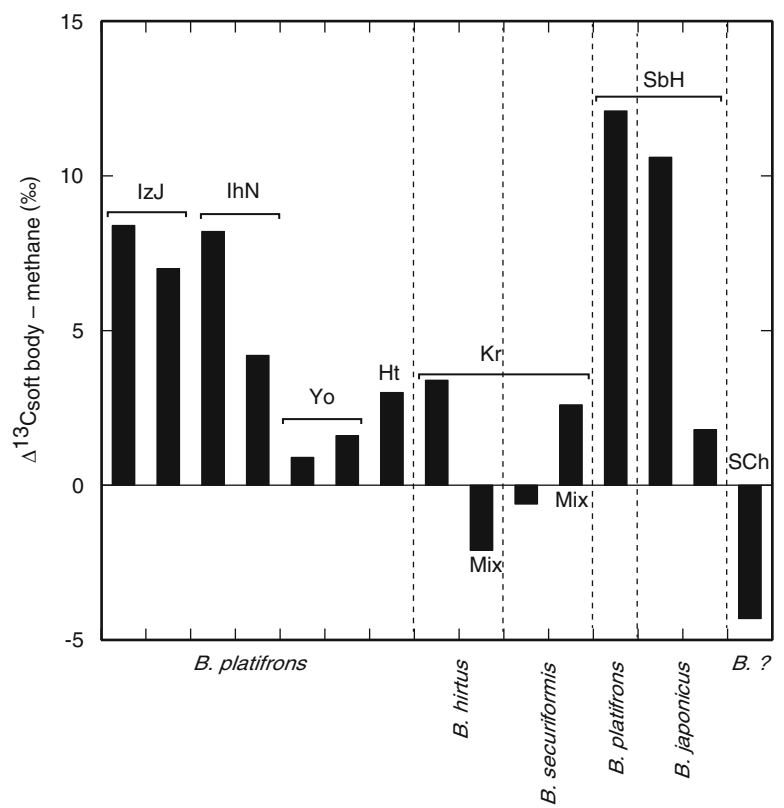
For benthic communities inhabiting the areas around hydrothermal vents and methane seeps, i.e., the discharge zone of TAIGA, major mechanisms that exploit their limited sulfur isotopic fractionation are considered to be a positive assimilation of sulfide-sulfur, since  $\delta^{34}\text{S}$  values of sulfide are usually lower than  $+12\text{‰}$ . As shown in

Figs. 10.3 and 10.5, many animal tissue samples had  $\delta^{34}\text{S}$  values lower than  $+15\text{‰}$ , suggesting that the animals rely nearly all or in part on thioautotrophic nutrition. Some animals (mostly mussels, such as *Bathymodiolus japonicas*, *B. platifrons*, *B. hirtus*, and *B. securiformis*), which have higher  $\delta^{34}\text{S}$  values ( $\geq +15\text{‰}$ ), are known to harbor methanotrophic endosymbionts in their gill tissue (Fujiwara et al. 2000; Fujikura et al. 2003). These results are quite reasonable, since some of the methanotrophic mussels inhabiting the Okinawa Trough have  $\delta^{34}\text{S}$  values of slightly less than  $+14\text{‰}$ . *Bathymodiolus* mussels are expected to have preserved their ability to feed via filtration (Page et al. 1990, 1991). Therefore, these results may suggest positive assimilation of thioautotrophic nutrition via filter feeding. Mussels from South Chamorro serpentine seamount also had low  $\delta^{34}\text{S}$  values ( $\sim +10\text{‰}$ ). The mussels are considered to harbor both thioautotrophic and methanotrophic endosymbionts (i.e., dual symbiosis) (Yamanaka et al. 2003b). The other heterotrophs, except for vent and seep endemic species, also have  $\delta^{34}\text{S}$  values of slightly  $<+15\text{‰}$  (Figs. 10.3 and 10.5), indicating that thioautotrophic nutrition at the vent and seep fields prevails not only with the endemic animals, but also with diverse animals inhabiting areas around the fields.



**Fig. 10.9** Apparent differences between the  $\delta^{13}\text{C}$  values of animal soft body parts and associated methane obtained from hydrothermal areas. Abbreviations indicate the sample locations shown in Table 10.1

**Fig. 10.10** Apparent isotopic fractionation of  $\delta^{13}\text{C}$  values of animal soft bodies and issuing methane from methane seep fields. The mussels harbor methanotrophic symbionts in their gill tissues. Abbreviations indicate the sample locations shown in Table 10.1. "Mix" indicates that the sample specimens form a mixed colony of *B. hirtus* and *B. securiformis*



One group of *Calyptogena soyoae* from the off Hatsushima site showed quite high  $\delta^{34}\text{S}$  values ( $\sim +16.5 \pm 6.5$  ‰). Masuzawa (1996) reported similar high  $\delta^{34}\text{S}$  values ( $\sim +14$  ‰) from other *Calyptogena soyoae* in the area, although the  $\delta^{34}\text{S}$  value of hydrogen sulfide from that habitat has not been documented. The spatial distribution of  $\delta^{34}\text{S}$  values for sedimentary sulfide is often heterogeneous within the anoxic environment (e.g., Yamanaka et al. 1999). Paired analysis of sulfur in clams and substrate sediments is required for further study.

The above results also suggest that the sediment-hosted hydrothermal vent and methane-rich seep fields provide thioautotrophic nutrition (i.e., sulfur-oxidizing bacteria and heterotrophic organisms that rely on them) derived from the suspended particles and detritus in the surface sediments. In fact, significant amounts of sulfur-oxidizing microbe cells have been identified in the bottom water around hydrothermal fields in the Okinawa Trough (Yanagawa et al. Chap. 6). Such high biomass flux of the sulfur-oxidizing microbes is supported by microbial sulfide production, suggesting that sub-seafloor bacterial sulfate reduction using methane or sedimentary organic matter as electron donors (Masuzawa et al. 1992) is quite active at the discharge zone of TAIGA.

From the above-mentioned stable isotopic data, it is possible to estimate the contribution of thioautotrophic nutrition to benthic animal communities. Nevertheless, the primary source of hydrogen sulfide is not only hydrothermal but also bacterial. The  $\delta^{34}\text{S}$  values of bacterial hydrogen sulfide vary widely (e.g., Thode 1988). Therefore, it is difficult to accurately estimate the contribution of thioautotrophic nutrition. The  $\delta^{34}\text{S}$  values for sulfur of animal soft tissue are less than +10 ‰, indicative of a significant contribution from thioautotrophic nutrition.

#### 10.4.2 Variations in the Carbon Isotopic Ratios of the Benthic Animal Community

The carbon isotopic ratios for the animal soft tissues varied widely (Figs. 10.2, 10.3, and 10.4, and Tables 10.2 and 10.3). This may reflect the variation in  $\delta^{13}\text{C}$  values of the methane ( $-71$  to  $-6$  ‰) issuing from hydrothermal vents and cold seeps. In particular,  $\delta^{13}\text{C}$  values of methanotrophic *Bathymodiolus* mussels varied widely, ranging from  $-70$  to  $-20$  ‰. The  $\delta^{13}\text{C}$  values for most animal soft tissue samples were higher than those of the associated methane (Figs. 10.9 and 10.10). This can be explained by the following two scenarios: (1) the contribution of methane as a carbon source for chemosynthesis-based animals, except for methanotrophic species, is insignificant at many discharge zones or (2) the  $\delta^{13}\text{C}$  values of methane are increasing due to the selective incorporation of isotopically light

methane during biological oxidation (e.g., Silverman and Oyama 1968) before delivery to methanotrophs.

In the former case (1), chemoautotrophs fix  $\text{CO}_2$  and therefore do not directly assimilate  $^{13}\text{C}$ -depleted methane. Dissolved inorganic carbon (mainly bicarbonate) is usually abundant in the bottom seawater. This means that the signature of  $^{13}\text{C}$ -depleted  $\text{CO}_2$  originating from  $^{13}\text{C}$ -depleted methane oxidation is difficult to detect. In the latter case (2), methane emitted at the sediment-hosted systems undergoes biological oxidation and incorporation during penetration of the sediments, resulting in methane that is enriched in  $^{13}\text{C}$  relative to the primary source to be delivered to methanotrophic animals (mainly *Bathymodiolus* mussels).

Another plausible explanation for the methanotrophic species that are enriched in  $^{13}\text{C}$ , relative to the associated methane, is the heterotrophic assimilation of organic matter via filter feeding, as demonstrated by Page et al. (1990). The  $\delta^{13}\text{C}$  values of methane originating from pyrolysis of organic matter and microbial methanogenesis, relative to that of common organic matter, are usually depleted in  $^{13}\text{C}$ . Heterotrophic and thiotrophic animals are enriched in  $^{13}\text{C}$  compared to methane from these sources. In these cases, the soft tissue is probably depleted in  $^{34}\text{S}$  due to the dependence on thioautotrophic nutrition. In fact, the mussels inhabiting the Okinawa Trough were slightly depleted in  $^{34}\text{S}$ , suggesting a positive incorporation into thioautotrophic nutrition, as mentioned above.

On the other hand, some animal soft tissues depleted in  $^{13}\text{C}$ , relative to the associated methane (Figs. 10.9 and 10.10), were found at the field where emitted methane showed quite high  $\delta^{13}\text{C}$  values ( $\geq -10$  ‰) in an abiotic source (the sediment-starved Myojin and Suiyo seamounts and serpentine-hosted South Chamorro seamount). Furthermore, the mussels of South Chamorro harbor thioautotrophic endosymbionts in addition to methanotrophs, which assimilate abiotic methane enriched in  $^{13}\text{C}$ . Therefore, resulting  $\delta^{13}\text{C}$  values of the mussels decreased relative to those of the associated methane. Its dual symbiosis has also been supported by their low  $\delta^{34}\text{S}$  values (+10.6 ‰ for gill tissues), relative to seawater-sulfate (Table 10.3).

Some thiotrophic vestimentiferan tubeworms have slightly higher  $\delta^{13}\text{C}$  values ( $\geq -20$  ‰). It is known that this animal has another carbon fixation pathway instead of the Calvin cycle (Nelson and Fisher 1995). For example, the rTCA cycle is a process that accompanies the insignificant isotopic fractionation during carbon fixation (Markert et al. 2007). Some heterotrophic animals that have high  $\delta^{13}\text{C}$  values may feed via mechanisms similar to the tubeworms.

One species of limpet, *Phymorhynchus buccinoides*, obtained from off Hatsushima, has been reported to feed predominantly on the dead bodies of *Bathymodiolus* mussels (Fujikura et al. 2009). Their  $\delta^{13}\text{C}$  values are significantly lower ( $\sim -60$  ‰, Table 10.3) than those of the other

heterotrophs. Although such specific heterotrophs relying on a single vent or seep endemic species have rarely been reported until recently (Van Dover 2000), carnivores, scavengers, and parasites relying on a single vent or seep animals are likely found in the vent and seep communities. In fact, parasitical polychaeta, *Branchiopolynoe pettiboneae*, collected from inside the shell of *Bathymodiolus* mussels obtained from JADE site, Izena Hole have similar  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values to the associate mussels with  $\sim 6\text{‰}$  enriched in  $^{15}\text{N}$  (Table 10.2). With further study, these animals will continue to be identified.

#### 10.4.3 Nitrogen Isotopic Ratios of Symbiotic Bivalves

Nitrogen isotopic ratios of animals have been used for the estimation of trophic levels in studies of food web structures (Minagawa and Wada 1984). Usually, higher trophic levels are believed to have elevated  $\delta^{15}\text{N}$  values. Such enrichment in  $^{15}\text{N}$  with increasing trophic level is also common in vent and seep communities, while symbiotic mussels, such as *Calypptogena* clams and *Bathymodiolus* mussels, still have significantly low  $\delta^{15}\text{N}$  values (Figs. 10.2 and 10.4). Such trends were recognized in early studies of the chemosynthesis-based animal community, and the reasons for such low values have been debated (e.g., Van Dover 2000), although the detailed mechanism is not well understood. Some plausible explanations have been proposed, e.g., assimilation of ammonium, which prevails in anoxic sediments, may be an important process. During the assimilation of ammonium nutrition, isotopic fractionation may occur (e.g., Hübner 1986; Yoneyama et al. 1993; Lee and Childress 1994). Isotopic fractionation during the assimilation of ammonium depends on its concentration and the types of enzymes used in ammonium assimilation. The reported values range from  $-20$  to  $+4\text{‰}$  ( $\delta^{15}\text{N}_{\text{ammonium}} - \delta^{15}\text{N}_{\text{organism}}$ ) (Hübner 1986; Yoneyama et al. 1993). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of most animal tissues, especially the *Bathymodiolus* mussels inhabiting the methane seeps, are highly correlated ( $R^2 = 0.85$ ) (Fig. 10.4). The increasing  $\delta^{13}\text{C}$  values may be due to isotopic fractionation during microbial oxidation and/or assimilation of methane nutrition. The  $\delta^{15}\text{N}$  values of source ammonium are also plausibly increased by microbial consumption, because isotopically light ammonium is preferentially incorporated. Therefore, this implies that  $\delta^{15}\text{N}$  values of ammonium increase together with  $\delta^{13}\text{C}$  values of methane due to bacterial isotopic discrimination until the ammonium in source fluids reaches the mussel habitat.

Among the seep fields, the carbon isotopic ratios of methane have reflected the methane origin. However, in our limited data set, *Bathymodiolus* mussels obtained from

methane seeps off Hatsushima showed a positive correlation between carbon and nitrogen isotopic ratios. This implies that the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the mussels reflect subsurface microbial processes, which preferentially incorporate isotopically light methane together with ammonium. Ammonium is a plausible source of nitrogen nutrition for methanotrophic communities. Due to a lack of isotopic data for ammonium in this habitat, further studies are needed.

#### 10.4.4 Competition for Energy Sources and the Role of Filter Feeding by *Bathymodiolus* Mussels

Two *Bathymodiolus* species (*B. hirtus* and *B. securiformis*) have been observed at the Kuroshima knoll seep site, where they usually form small colonies composed of one species (separate-type). Nevertheless, some colonies form a mixture of both species (mixed-type). Both species are believed to harbor methanotrophic bacteria as their sole endosymbionts (Fujikura et al. 2003). In fact, sulfur isotopic compositions for specimens from separate-type colonies show a range typical of methanotrophic animals ( $\delta^{34}\text{S} \geq +15\text{‰}$ ). The  $\delta^{34}\text{S}$  values of specimens from the mixed-type colonies are about  $10\text{‰}$  lower than those from the separate-type colonies. Both carbon and nitrogen isotopic ratios for two types of samples are relatively similar. Such low  $\delta^{34}\text{S}$  values may be due to the assimilation of thioautotrophic nutrition via filter feeding. Mussels of the mixed-type colonies may compete with each other for methane nutrition as an energy source. They may compensate for the shortage of methanotrophic nutrition derived from the endosymbionts by filter feeding.

### 10.5 Summary

Benthic animal communities supported by thioautotrophic and methanotrophic nourishment are widely distributed over the discharge area of TAIGA, and they have high biodiversity including common marine benthos. As we have summarized the nutritional sources of the benthic animals discussed in this study in the Supplementary document (Suppl. 10.1), microbial methane and hydrogen sulfide generated within the sedimentary layer are important energy sources for microbes, in addition to abiogenic methane and hydrogen sulfide dissolved in the issuing fluids. Although some hydrothermal vent and methane seep endemic species harbor endosymbiotic bacteria and lack digestive organs, *Bathymodiolus* mussels still have ability to feed in the Okinawa Trough and some other methane seep fields. The data suggest that the sediment covered discharge area of TAIGA is also abundant in suspended and/or free-living

microbes, namely thioautotrophs, which support huge biomass production and biodiversity on the deep seafloor.

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