Relationship between geochemical environments, nutritional resources, and faunal succession in whale-fall ecosystems

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ABSTRACT: Faunal succession in whale-fall communities is closely associated with the progress of decomposition of the whale carcass. The main nutritional resources supporting a whale-fall community change from whale matter to chemosynthetic products over time. To study the geochemical aspects of this nutritional succession, we sampled animals over time on and in sediments around carcasses of sperm whale *Physeter macrocephalus* in Sagami Bay (mobile scavenger to early sulfophilic stage) and off Cape Nomamisaki (sulfophilic stage), Japan (500 and 200–300 m water depths, respectively). In these 2 areas, stable carbon, nitrogen, and sulfur isotopes of the animal soft tissues were measured to precisely elucidate the nutritional resources for each animal. In Sagami Bay, mobile scavengers relied only on whale soft tissue. Infaunal animals at 2 wk after the deployment relied only on whale soft tissue, while infauna at 9 mo after the deployment relied on chemosynthetic products. Such changes in nutritional resources were consistent with the transition of the geochemical environment in the sediments. Off Cape Nomamisaki, vigorous microbial sulfate reduction and thioautotrophic primary production nourished the fauna around the carcasses. The fauna in this area consisted of chemosymbiotic bivalves and necrophagous animals with sulfide-tolerant metabolism. We conclude that the changes in microbial processes, biomass, and compositions in sediments influence faunal succession in whale-fall ecosystems via change in the available nutrition for the fauna.

KEY WORDS: Whale-fall ecosystem · Nutritional resource · Stable isotopes · Chemosynthetic product

1. INTRODUCTION

When a whale carcass sinks to the seafloor, a unique and dense animal community, called a whale-fall community, forms around it (e.g. Smith et al. 1989, Smith & Baco 2003). Faunal composition of whale-fall communities changes as carcass decomposition proceeds (Allison et al. 1991, Smith 1992, Deming et al. 1997, Smith et al. 2002, 2015, Smith & Baco 2003, Fujiwara et al. 2007, Glover et al. 2010,

Lundsten et al. 2010), with 4 stages commonly identified: a mobile scavenger stage, an enrichment opportunist stage, a sulfophilic stage, and a reef stage.

Faunal succession ultimately is driven by abiotic (geochemical; Treude et al. 2009, Onishi et al. 2018b) and biotic (e.g. facilitation processes; Smith & Baco 2003) factors, which may be caused by a change in the available nutritional resources for the animals. Bennett et al. (1994) hypothesized that the nutritional resources used by the animals in a whale-fall community are divided into 5 types: (1) whale organic material (e.g. soft tissues and lipids in the bones), (2) free-living heterotrophic and chemoautotrophic bacteria, (3) invertebrate-endosymbiotic sulfur-oxidizing chemoautotrophic bacteria, (4) tissue of primary consumers, and (5) detrital particles suspended in currents or deposited in sediments around the bones. Animals appearing in the mobile scavenger and enrichment opportunist stages rely primarily on whale organic material for their nutrition (Smith & Baco 2003), while animals appearing in the sulfophilic stage rely mainly on chemosynthetic organic matter (Smith & Baco 2003, Fujiwara et al. 2009, 2010, Alfaro-Lucas et al. 2018). Animals appearing in the reef stage primarily rely on phytoplanktonic organic matter via filter-feeding of sinking particles (Smith & Baco 2003). Thus, the main nutritional resources supporting a whale-fall community are likely to change according to the successional stage, which may be one of the key factors driving faunal succession.

Many studies have proposed that whale falls can be evolutionary and dispersal stepping stones for some fauna inhabiting deep-sea hydrothermal vents, methane seeps, and other organic falls because a whale-fall community may sustain chemosynthesis-based assemblages of animals during the sulfophilic stage (Smith et al. 1989, 2015, 2017, Distel et al. 2000, Jones et al. 2006, Fujiwara et al. 2010, Miyazaki et al. 2010, Lorion et al. 2013, Thubaut et al. 2013, Kiel 2017). Thus, biological and geochemical studies of the whale-fall community are important to understand the formation of chemosynthesis-based animal communities around whale falls and their relationships with hydrothermal vents and methane seeps. Smith & Baco (2003) and Alfaro-Lucas et al. (2018) discussed how the different nutritional resources varied with carcass size or during the different successional stages. However, the relationship between the available nutritional resource and faunal succession has not been fully investigated yet.

Stable isotope compositions of biogenic elements (such as carbon, nitrogen, and sulfur) in soft tissue have been widely used for understanding nutritional resources for animals in chemosynthesis-based ecosystems, including whale falls (e.g. Smith & Baco 2003, Yamanaka et al. 2015, Alfaro-Lucas et al. 2018, Onishi et al. 2018a). Carbon and sulfur isotopic composition ($\delta^{13}C$ and $\delta^{34}S$) reflect diet sources due to small isotopic fractionation during assimilation, while nitrogen isotopic composition ($\delta^{15}N$) is systematically enriched in $\delta^{15}N$ (about +3‰) relative to that of the diet with increasing trophic level and thus is useful for determining the trophic position of taxa and the trophic structure of communities (DeNiro & Epstein 1981, Minagawa & Wada 1984). The 5 resources hypothesized by Bennett et al. (1994) could potentially be isotopically distinguished. Whale organic material should have similar $\delta^{13}C$ and $\delta^{34}S$ values to marine primary producers, i.e. phytoplankton (about −20‰ and +20‰, respectively; Rau et al. 1982, Fry & Sherr 1984, Gearing et al. 1984), and relatively higher $\delta^{15}N$ values than phytoplankton (about +5‰; Altabet & Francois 1994, Voss et al. 2001, Smith & Baco 2003, Alfaro-Lucas et al. 2018, Onishi et al. 2018b). On the other hand, the isotopic compositions of chemosynthetic organic matter by thioautotrophic bacteria are clearly different from those of phytoplankton due to different elemental sources and isotope fractionations (Mizota & Yamanaka 2003, Yamanaka et al. 2015). The $\delta^{13}C$ and $\delta^{15}N$ values of chemosynthetic organic matter have been reported to be about −35 and 0‰, respectively (Ruby et al. 1987, Nelson & Fisher 1995, Yamanaka et al. 2015). The $\delta^{34}S$ of chemosynthetic organic matter is identical to that of hydrogen sulfide utilized (Fry et al. 1983), and the $\delta^{34}S$ of hydrogen sulfide generated by microbes is often negative (Kaplan & Rittenberg 1964, Sim et al. 2011). Therefore, the $\delta^{13}C$, $\delta^{15}N$, and ^{34}S values in animal soft tissues in whale-fall communities can be used to indicate the available nutritional resources. Especially, the sulfur isotope is more powerful than carbon to identify sources of chemosynthetic origin.

To investigate how nutritional resources used by species in whale-fall communities change over time, we sampled the animals around whale carcasses implanted on the seafloor in 2 Japanese waters: Sagami Bay at a depth of ~500 m, on the Pacific Ocean side of the central part of Honshu Island; and off Cape Nomamisaki at a depth of ~200–300 m, on the East China Sea side of southeastern Kyushu Island. Detailed biological and geochemical studies around these whale carcasses were previously reported (Fujiwara et al. 2007, Aguzzi et al. 2018, Onishi et al. 2018b). We sampled the epifaunal and

infaunal animals around the carcasses and analyzed stable carbon, nitrogen, and sulfur isotopic compositions in the soft tissues of the animals near the whale carcasses to assess the nutritional resources for each animal. The purpose of this study is to evaluate the nutritional resources for the animals around the whale carcasses, and reveal the factors causing faunal succession.

2. MATERIALS AND METHODS

2.1. Study areas

One stranded sperm whale carcass *Physeter macrocephalus* was deployed on the shelf slope at a depth of 489 m in Sagami Bay (35° 5.58′ N, 139° 10. 27′ E) in June 2012 (our Fig. 1; Aguzzi et al. 2018). Observations and sampling using the remotely operated vehicle (ROV) 'Hyper-Dolphin' and R/V 'Natsushima' belonging to the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) were done in June 2012 (at 2 wk after deployment), August

2012 (at 2 mo), and March 2013 (at 9 mo). Many scavengers, such as snub-nosed eel *Simenchelys parasitica* and giant isopods *Bathynomus doederleinii*, were visible after 2 wk (Aguzzi et al. 2018, Onishi et al. 2018b). Between 2 wk and 2 mo, siboglinid worms *Osedax* spp. appeared on the whale bone surface exposed to ambient seawater, whereas at 9 mo, the animal community consisted of dense populations of the scavengers and *Osedax* worms. Sulfide concentrations in the sediment beneath the whale carcass were low at 2 wk and 2 mo (<0.5 mmol S kg^{-1} dry sediment), while a large amount of sulfide (up to 1 mmol S kg^{-1} dry sediment) was detected at 9 mo (Onishi et al. 2018b).

Twelve carcasses of stranded sperm whales *P. macrocephalus* were towed and sunk in waters off Cape Nomamisaki (31° 18.52′ N to 31° 23.87′ N, 129° 58. 77′ E to 129° 59.52′ E) in February 2002 (our Fig. 1; Fujiwara et al. 2007). The whale carcasses settled on the seafloor at depths between 200 and 300 m on the shelf slope 9 d after stranding. Observation and sampling using the ROV 'Hyper-Dolphin' were done around 5 of these whales (whales numbered 2, 6, 7,
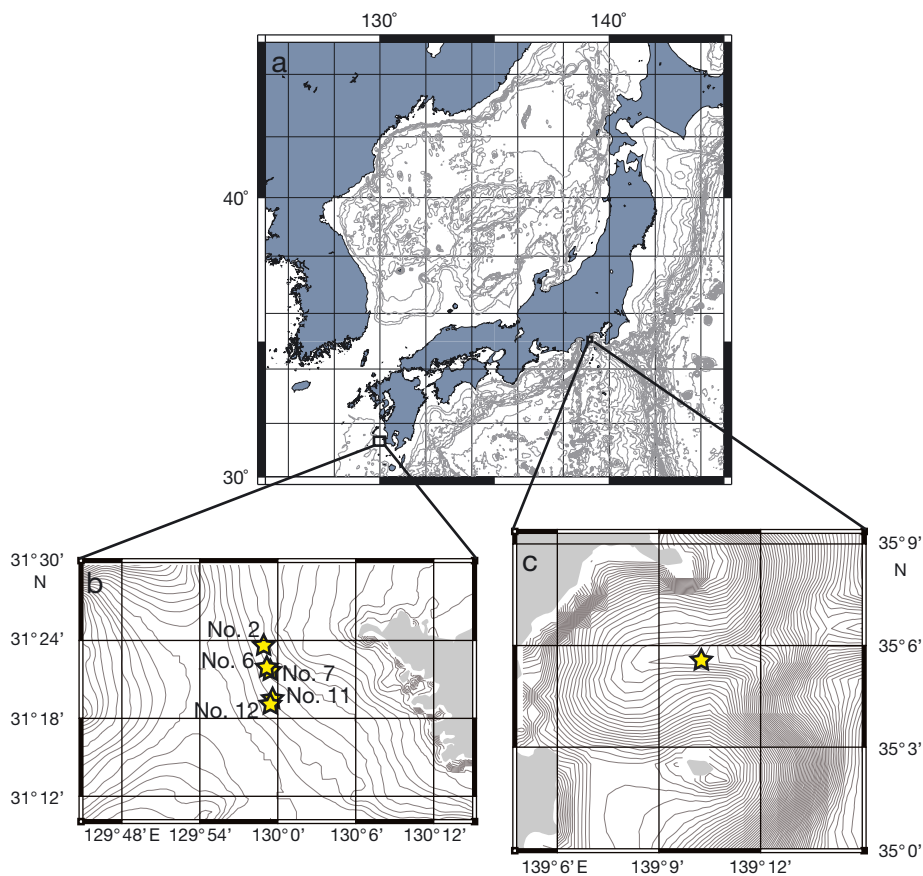

Fig. 1. (a) Whale-fall study sites, Japan. Stars: locations of whale carcasses (*Physeter macrocephalus*) deployed (b) off Cape Nomamisaki and (c) in Sagami Bay (modified from Onishi et al. 2018b)

11, and 12) in July 2003 (at 17 mo after deployment), July 2004 (at 29 mo), July–August 2005 (at 41 mo), June 2007 (at 64 mo), and August 2008 (at 78 mo). Mytilid mussels *Adipicola pacifica* were common during the dive surveys for 5 yr (Okutani et al. 2003, Fujiwara et al. 2007). The mytilid mussel *Adipicola crypta* had colonized the bones buried in sediments and the solemyid clam *Solemya pervernicosa* was in sediments beneath the bones (Fujiwara et al. 2007). Sulfide concentrations were high for at least 3 yr in the sediments beneath the whale carcasses (up to 40 mmol S kg^{-1} dry sediment; Onishi et al. 2018b).

2.2. Sample collection

Animal sampling was conducted using a suction sampler and a scoop operated by the manipulators of the ROV 'Hyper-Dolphin'. Each organism was identified on board based on morphology before being frozen and preserved at −80°C in a deep-freezer for on-shore analyses. The on-shore isotope analyses were done within several months from each sampling.

We collected some of the dominant animals in and on the sediments around the whale carcasses in Sagami Bay, including mobile scavengers, such as snub-nosed eels *Simenchelys parasitica*, giant isopods *B. doederleini*, and echinoids (Cidaridae gen. sp.). In addition, unidentified ophiuroids (2 wk), sipunculids (2 wk), gammarids (9 mo), and cumaceans (9 mo) were collected from the sediment beneath the carcass at 9 mo after deployment (see Table 1).

Around the whale carcasses off Cape Nomamisaki, chemosymbiotic mytilid mussels *Adipicola pacifica* and *Adipicola crypta* and solemyid clam *Solemya pervernicosa* were collected from the surface of the whale bones and in the sediment beneath the bones during the 5 yr of investigation. *Osedax japonicus* and *Protodrilus puniceus* were collected from the bones whereas the lancelet *Asymmetron inferum* was collected from the sediments beneath the bones 29, 41, or 78 mo after deployment (see Table 1).

2.3. Analytical methods

Large animal samples were dissected on board or in the on-shore laboratory for isotope analyses, while small animal samples were combined and then used for analyses. For the carbon and nitrogen isotope measurements, the soft or bulk tissues of animal samples were treated with 1 N HCl to remove trace car-

bonates, and then treated with chloroform and methanol mixed solvent (3:1 v/v) to remove lipids. The treated samples were lyophilized and pulverized. For sulfur isotope measurement, soft or bulk tissues were dialyzed repeatedly using 1 M LiCl solution to remove excess seawater sulfate. All organic sulfur species in the dialyzed samples were converted to sulfate using a Parr Bomb 1108 apparatus and then recovered as $BaSO_4$ precipitate by addition of 0.5 M $BaCl_2$ (e.g. Mizota et al. 1999, Yamanaka & Mizota 2001).

All carbon, nitrogen, and sulfur isotopic ratios were measured using an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS; IsoPrime EA; GV Instruments). All isotopic values are expressed using δ notation in per mille deviation (‰) from international reference materials: Vienna Peedee belemnite (VPDB) for $\delta^{13}C$, atmospheric N_2 for $\delta^{15}N$, and Cañon Diablo troilite (CDT) for $\delta^{34}S$. Analytical errors associated with the overall process of these determinations were ±0.2, ±0.3, and ±0.3‰ for C, N, and S isotopic ratios, respectively. Isotopic values were examined using mean comparison *t*-tests or Kruskal-Wallis test to evaluate the statistical significance (p < 0.05) of the differences observed.

2.4. Chemosynthetic organic matter in nutritional resources

The relative contributions of chemosynthetically derived carbon (CDC) and sulfide-derived sulfur (SDS) in the nutritional resources for the animals can be evaluated by carbon and sulfur isotopes assuming 2-endmember isotope mass balance models (Alfaro-Lucas et al. 2018):

$$CDC \text{ or } SDS \, (\%) = (\delta X_{sample} - \delta X_{whale}) \times 100/(\delta X_{COM} - \delta X_{whale}) \quad (1)$$

where δX is respectively the $\delta^{13}C$ or $\delta^{34}S$ values of each animal ('sample'), whale organic material, and chemosynthetic organic matter (COM), as represented by the subscripts. Because this model may overestimate or underestimate the proportions in CDC (CDC_{max}) or SDS (SDS_{min}), respectively, the CDC_{min} and SDS_{max} were calculated by replacing δX_{whale} with δX_{SOM} (where SOM is sedimentary organic matter).

In this model, we used the $\delta^{13}C$ and $\delta^{34}S$ of whale soft tissues as $\delta^{13}C_{whale}$ and $\delta^{34}S_{whale}$ in Sagami Bay and off Cape Nomamisaki reported by Onishi et al. (2018b). Also, $\delta^{13}C_{SOM}$ values in each area are reported in Onishi et al. (2018b), and $\delta^{34}S_{SOM}$ are

Table 1. Analytical results of stable carbon, nitrogen, and sulfur isotope ratios in the animals obtained from whale carcasses (*Physeter macrocephalus*). δ13C, δ15N, and δ34S represent mean values for all individuals combined. CDC: chemosynthetically derived carbon; n: number of individuals analyzed; nd: no data; (–) not applicable; SD: standard deviation; SDS: sulfide-derived sulfur; SOM/COM: sedimentary/chemosynthetic organic matter

Area	Fauna	Sampling time (after deployment)	n	Stable isotope composition (‰) δ13C	SD	δ15N	SD	δ34S	SD	Reliance on chemosynthetic production (%) CDCmin	SD	CDCmax	SD	SDSmin	SD	SDSmax	SD
Sagami Bay	*Simenchelys parasitica*	2 wk	3	−20.1	0.8	+15.8	0.8	+16.0	1.0	0	–	11.8	4.7	0	–	16.7	4.2
	Bathynomus doederleinii	2 mo	1	−18.5	–	+17.1	–	+16.9	–	0	–	2.4	–	0	–	12.9	–
		9 mo	1	−18.4	–	+16.0	–	+17.1	–	0	–	1.8	–	0	–	12.1	–
	Cidaridae sp.	9 mo	1	−19.4	–	+17.5	–	+18.2	–	0	–	7.7	–	0	–	7.5	–
	Ophiuroids	2 wk	2	−18.2	0.4	+11.8	0.6	nd	–	0	–	0.6	2.4	–	–	–	–
	Sipunculids	2 wk	1	−18.0	–	+14.3	–	+16.4	–	0	–	0	–	0	–	15.0	–
	Gammarids	9 mo	60 (combined)[a]	−23.7	2.1	+9.6	4.2	+11.8	0.9	20.4	14.8	33.1	12.4	16.0	4.8	34.2	3.8
	Cumaceans	9 mo	30 (combined)	−35.3	–	−2.1	–	+7.0	–	100	–	100	–	41.5	–	54.2	–
	Whale soft tissue		–	−18.1[b]	–	+13.4[b]	–	+14.8[b]	–								
	SOM		–	−20.8[b]	0.0	+6.8[b]	1.0	+21[c]	–								
	COM		–	−35[d]	5.0	0[d]	5.0	−4[b]	–								
Off Cape Nomamisaki	*Adipicola crypta*	17 mo	20 (combined)[a]	−36.0	1.1	+6.5	0.0	−1.6	0.4	100	–	100	–	100	–	100	–
		29 mo	5	−35.9	1.0	+7.5	1.0	+0.5	0.2	100	–	100	–	100	–	100	–
		41 mo	5	−37.3	1.4	+9.1	1.5	nd	–	100	–	100	–	100	–	–	–
		64 mo	2	−35.5	1.8	+6.2	0.6	−2.0	1.4	100	–	100	–	100	–	100	–
	Adipicola pacifica	17 mo	16 (combined)	−24.8	–	+5.7	–	+8.8	–	33.3	–	53.2	–	54.1	–	62.2	–
		29 mo	1	−26.2	–	+8.3	–	nd	–	42.5	–	59.6	–	–	–	–	–
		64 mo	4	−24.0	0.4	+4.5	1.3	+10.6	0.6	28.1	2.6	49.5	1.8	41.9	4.1	52.2	3.3
	Solemya pervernicosa	17 mo	7	−31.4	0.8	+3.3	1.4	+3.1	0.8	76.5	5.2	83.5	3.7	92.6	5.4	93.9	4.4
		29 mo	3	−31.5	1.0	+1.7	3.7	nd	–	77.1	6.5	83.9	4.6	–	–	–	–
		41 mo	1	−31.9	–	+2.7	–	nd	–	79.7	–	85.8	–	–	–	–	–
		78 mo	4	−33.2	0.9	+2.9	1.2	+2.9	4.1	88.2	5.9	91.7	4.1	93.9	27.7	95.0	22.8
	Osedax japonicus	29 mo	2	−14.6	0.3	+17.0	–	−7.6	–	0	–	6.0	1.4	100	–	100	–
	Asymmetron inferum	29 mo	2	−19.2	1.2	+5.1	3.1	+4.7	6.1	0	–	27.5	5.5	81.8	41.2	85.0	33.9
		41 mo	1	−20.0	–	+9.4	–	+3.5	–	2.0	–	31.2	–	89.9	–	91.7	–
		78 mo	4	−22.1	0.8	+7.9	0.9	+1.6	2.6	15.7	5.2	40.8	3.7	100	–	100	–
	Protodrilus puniceus	78 mo	3	−21.8	0.8	+12.6	0.1	+12.4	0.3	13.7	5.2	39.4	3.7	29.7	2.0	42.2	1.7
	Whale soft tissue		–	−13.2[b]	–	+16.4[b]	–	+16.8[b]	–								
	Whale bone		–	−18.4[b]	3.1	+15.3[b]	–	+16.9[b]	1.8								
	Bone lipid			−21.5[b]	2.3	+12.2[b]	3.2	–	–								
	SOM		–	−19.7[d]	–	nd	–	+21[c]	–								
	COM		–	−35[d]	5.0	0[d]	5.0	+2[b]	–								

[a]Divided the population into half, and then composited them. Each composited sample was measured, and averaged; [b]Reported by Onishi et al. (2018b); [c]Assumed from seawater sulfate value (+21‰; Rees et al. 1978); [d]Assumed from Yamanaka et al. (2015)

assumed to be +21‰ because the $\delta^{34}S$ values of photosynthetic products typically have ca. +21‰ (cf. Yamanaka et al. 2015) as well as seawater sulfate (+21‰; Rees et al. 1978). Although the $\delta^{13}C_{COM}$ values were not analyzed, the $\delta^{13}C$ value of the chemoautotrophs are known to typically range between −40 and −30‰ (Ruby et al. 1987, Nelson & Fisher 1995). The $\delta^{13}C_{COM}$ values are assumed to be −35‰ in both study areas. Furthermore, the $\delta^{34}S_{COM}$ values are assumed to be identical to that of the sulfide utilized because of the small isotope effect during assimilation (Fry et al. 1983), and the $\delta^{34}S$ of sulfide in Sagami Bay and off Cape Nomamisaki are reported to be approximately −4 and +2‰ (Onishi et al. 2018b), respectively.

3. RESULTS

Mobile scavengers (*Simenchelys parasitica*, *Bathynomus doederleinii*, and Cidaridae sp.) in Sagami Bay (Table 1, Fig. 2a,b) had similar isotopic compositions,

although their sampling times were different. The $\delta^{13}C$ and $\delta^{34}S$ values of these taxa (−20.1 ± 0.8 to −18.4‰ and +16.0 ± 1.0 to +18.2‰, mean ± SD, respectively) were in between the whale soft tissue (−18.1 and +14.8‰, respectively) and sedimentary organic matter (−20.8 and +21‰, respectively), while their $\delta^{15}N$ values (+15.8 ± 0.8 to +17.5‰) were a few per mille higher than that of the whale soft tissue (+13.4‰). The $\delta^{13}C$ and $\delta^{34}S$ values led to the low CDC (up to 11.8 ± 4.7%) and SDS (up to 16.7 ± 4.2%) calculated. On the other hand, infaunal species in Sagami Bay had different isotopic compositions at different sampling times (*t*-test, p < 0.05 for both $\delta^{13}C$ and $\delta^{15}N$ of the infauna at 2 wk and 9 mo). The isotopic compositions in ophiuroids and sipunculids at 2 wk after deployment were similar to those of the mobile scavengers. The CDC (up to 0.6 ± 2.4%) and SDS (up to 15.0%) values of the infauna and epifauna at 2 wk were also low. On the other hand, gammarids and cumaceans at 9 mo were characterized by lower $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values than those of whale soft tissue. The $\delta^{13}C$ and $\delta^{15}N$ values of the cumaceans
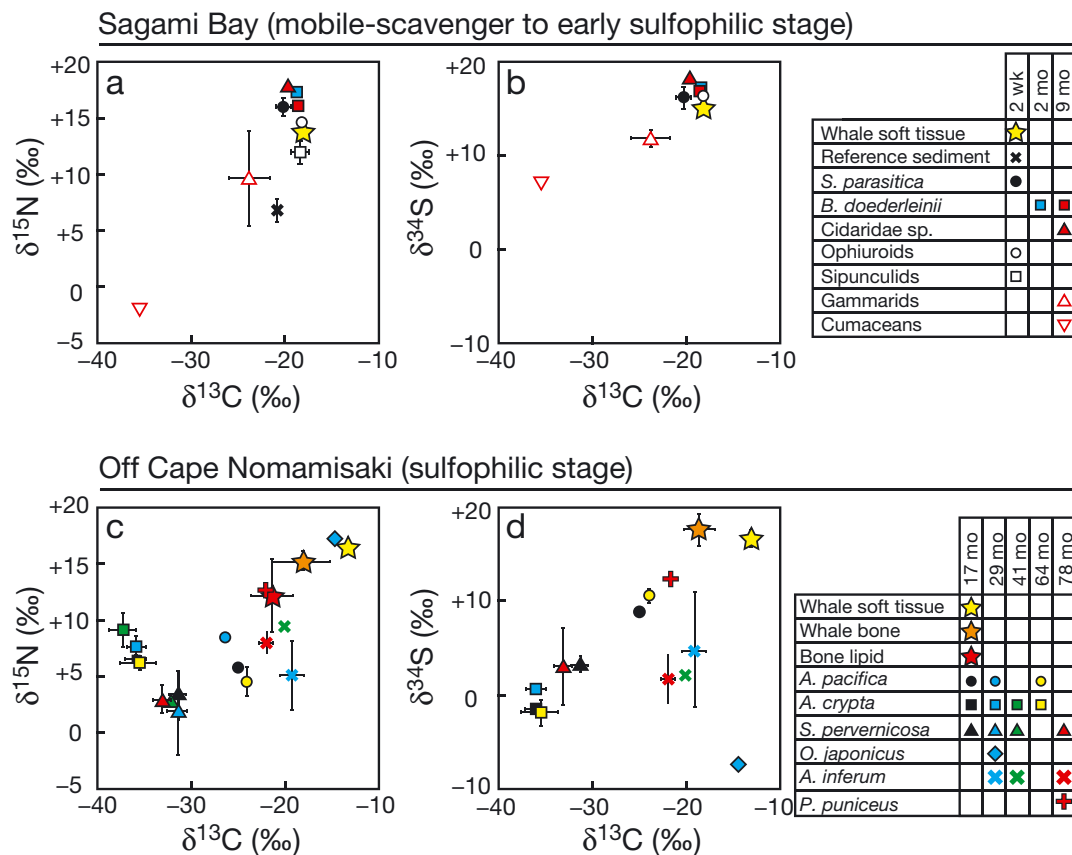

Fig. 2. Carbon vs. nitrogen and carbon vs. sulfur isotopic compositions of animal samples obtained from sediments around whale carcasses (*Physeter macrocephalus*) implanted (a,b) in Sagami Bay and (c,d) off Cape Nomamisaki. See Table 1 for genus names

(−35.3 and −2.1‰, respectively) were within the range of the chemosynthetic organic matter (−35 ± 5‰ and 0 ± 5‰, respectively; Ruby et al. 1987, Nelson & Fisher 1995, Yamanaka et al. 2015), and their ^{34}S value (+7.0‰) was lower than that of the whale. These isotopic compositions yielded high CDC (100%) and SDS (41.5 to 54.2%) in this taxon. The δ^{13}C, δ^{15}N, and δ^{34}S values of the gammarids (−23.7 ± 2.1‰, +9.6 ± 4.2‰, and +11.8 ± 0.9‰, respectively) were intermediate between those of the whale soft tissue and the cumaceans. The CDC (20.4 ± 14.8 to 33.1 ± 12.4%) and SDS (16.0 ± 4.8 to 34.2 ± 3.8%) were also intermediate between the epifaunal species and the cumaceans.

The δ^{13}C, δ^{15}N, and δ^{34}S values of each animal species collected from off Cape Nomamisaki varied little with sampling time (Kruskal-Wallis test, $p > 0.05$ for each animal), except for δ^{13}C of *Solemya pervernicosa* and δ^{15}N of *Adipicola crypta* ($p < 0.05$; Table 1, Fig. 2c,d). The chemosymbiotic bivalves (*Adipicola pacifica*, *Adipicola crypta*, and *S. pervernicosa*; Fujiwara et al. 2009, 2010) had lower δ^{13}C, δ^{15}N, and δ^{34}S values than those of the whale tissues and sedimentary organic matter (Onishi et al. 2018b). δ^{13}C values of *Adipicola crypta* (−37.3 ± 1.4 to −35.5 ± 1.8‰) and *S. pervernicosa* (−33.2 ± 0.9 to −31.4 ± 0.8‰) were within the range of those of chemosynthetic organic matter (−35 ± 5‰; Ruby et al. 1987, Nelson & Fisher 1995). Their CDC (≥76.5 ± 5.2%) and SDS (≥92.6 ± 5.4%) were very high. The δ^{13}C, δ^{15}N, and δ^{34}S values of *Adipicola pacifica* were intermediate between those of the whale tissue and the chemosymbiotic bivalves, and the CDC and SDS were respectively 28.1 ± 2.6 to 59.6% and 41.9 ± 4.1 to 62.2%. The δ^{13}C value of *Osedax japonicus* (−14.6 ± 0.3‰) was similar to that of the whale tissue, and the δ^{15}N (+17.0‰) was higher than the whale tissue. However, its ^{34}S value (−7.6‰) was clearly lower than that of the whale tissue. The CDC of *O. japonicus* was low (up to 6.0 ± 1.4%), but the SDS was 100%. The δ^{13}C values of *Asymmetron inferum* (−22.1 ± 0.8 to −19.2 ± 1.2‰) in the sediment beneath the whale carcasses were lower than those of the whale bones. The δ^{15}N and δ^{34}S values of this species were apparently lower than the whale organic materials. The pattern of the CDC (up to 40.8 ± 3.7%) and SDS (more than 81.8 ± 41.2%) were similar to those of *O. japonicus*. The δ^{13}C, δ^{15}N, and δ^{34}S values of *Protodrilus puniceus* (−21.8 ± 0.8‰, +12.6 ± 0.1‰, and +12.4 ± 0.3‰, respectively) living in the whale bones were slightly lower than those of the whale bones. The CDC and SDS values were similar to those of *Adipicola pacifica*.

4. DISCUSSION

4.1. Nutritional resources for animals in whale-fall ecosystems

Stable isotope ratios, especially of carbon, nitrogen, and sulfur, are often used to estimate nutritional resources for animals in many ecosystems. However, using these isotopes to detect the response to pulse input of the nutritional resources can be challenging. Isotope turnover rates in muscle tissues of marine fish and invertebrates are reported to be at least approximately 2 wk as half-life (cf. Vander Zanden et al. 2015). Thus, the isotopic compositions of the animals collected from Sagami Bay at 2 wk after deployment are likely to reflect the nutritional resources for the animals before whale deployment. The isotopic values of these animals around and beneath the carcass, such as *Simenchelys parasitica*, ophiuroids, and sipunculids, at 2 wk after deployment are likely a background, pre-whale signal. However, these animals had similar δ^{13}C and δ^{34}S values to whale soft tissue and ^{15}N values 3‰ higher than whale soft tissue, implying more general feeding on whale falls prior to coming to this whale fall, or feeding on large fish carcasses.

The isotopic values of the epifauna around the whale carcass at 9 mo after deployment, such as *Bathynomus doederleinii* and Cidaridae gen. sp., are in between whale soft tissue and sedimentary organic matter, which suggest the use of both sources. This is consistent with the visual observations (Aguzzi et al. 2018, Onishi et al. 2018b), and with previous studies from visual observation and isotopic analyses indicating that epifauna in early successional stages at San Diego Trough and Santa Cruz depend primary on whale tissues for their nutrition (Smith & Baco 2003, Smith et al. 2015). The scavengers were the most abundant (>70% in total species; Aguzzi et al. 2018) in the whale-fall community in Sagami Bay. It is thus likely that epifauna in early successional stages of whale-fall communities rely strongly on whale soft tissue, as was also seen in a previous study on the California margin which was based on visual observation and isotopic analyses (Smith & Baco 2003).

Gammarids and cumaceans sampled at 9 mo after deployment did not appear to rely only on the whale tissue because δ^{15}N values of the infauna were lower than the whale soft tissue. According to the CDC and SDS calculations, the relative contribution of chemosynthetic organic matter to gammarid nutrition was ca. 20−35%. The gammarids may rely on

both chemosynthetic organic matter and whale soft tissue in the sediment with different proportions among the individuals. On the other hand, the high CDC and SDS values of cumaceans suggest that they feed primarily on chemosynthetic organic matter. The cumaceans in this study may feed on chemosynthetic bacteria in the sediments beneath the whale carcass. Thus, chemosynthetic organic matter plays an important role in the nutrition of the infauna around the carcass from at least 9 mo after deployment in Sagami Bay.

On the seafloor off Cape Nomamisaki, vigorous microbial sulfate reduction (MSR) and chemosynthetic organic matter production began in sediments beneath the whale carcasses due to the supply of whale bone lipid to the sediments from at least 17 mo after deployment (Fujiwara et al. 2007, Onishi et al. 2018b). There was no variation in the isotopic compositions of each animal in this study between 17 and 78 mo after deployment, implying that the nutritional resource for each species did not change during this 5 yr period.

A previous study indicates that chemosynthetic primary production supports the whale-fall animal communities off Cape Nomamisaki (Onishi et al. 2018b). The mytilid mussel *Adipicola crypta* and the sole-myid clam *Solemya pervernicosa* harbor thioautotrophic symbionts in their gill tissues, and these bivalves rely only on the symbionts for their nutrition (Fujiwara et al. 2009, 2010). The isotopic compositions of *A. crypta* and *S. pervernicosa* in this study also supports previous studies (Fujiwara et al. 2009, 2010). Moreover, *A. crypta* migrated into deeper layers each year, apparently to track the peak depth of sulfide concentration (Onishi et al. 2018b). Chemosynthetic organic matter derived from symbionts is likely essential for the survival of the mussel. These bivalves were the most dominant species in this fauna (Fujiwara et al. 2007). Thus, chemosynthetic organic matter was the most important nutritional resource for the whale-fall community.

The other mytilid mussel, *Adipicola pacifica*, has been observed to attach to whale bone surfaces exposed to seawater (Okutani et al. 2003, Fujiwara et al. 2007, Onishi et al. 2018b). *A. pacifica* harbors both thioautotrophic and heterotrophic symbionts (Fujiwara et al. 2010). Therefore, it is expected that *A. pacifica* off Cape Nomamisaki may rely on both chemosynthetic organic matter and other resources derived from their symbionts as their nutritional resources. The intermediate isotopic compositions of *A. pacifica* between thioautotrophic bivalves and the whale organic materials also suggest a mixotro-

phic dietary habit, with the relative contribution of chemosynthetic organic matter to nutrition of 30–60%. They may acquire the whale organic materials in suspended particles via filter-feeding. The increase of the $\delta^{15}N$ values at 29 mo after deployment implies a decrease of the chemosynthetic organic matter contribution into their nutritional resource. The shell length and biomass of *A. pacifica* became smaller each year, suggesting that their habitat conditions were deteriorating (Fujiwara et al. 2007). Thus, chemosynthetic organic matter may be an important nutritional resource for *A. pacifica*.

Bone-eating *Osedax* worms rely on their heterotrophic symbionts to degrade collagen and cholesterol from whale bone (Goffredi et al. 2005, 2007). A recent study on trophic structure in the whale-fall community discovered on São Paulo Ridge suggested that *Osedax* worms on the whale bone rely heavily on whale remains (Alfaro-Lucas et al. 2018). In the present study, the CDC percentage of *O. japonicus* suggests that the worms rely on whale bone. *Osedax* species appear to replace each other based on temporal and spatial niche partitioning (Braby et al. 2007, Lundsten et al. 2010, Higgs et al. 2014), and *Osedax* numbers may decline with the sulfophilic stage (Alfaro-Lucas et al. 2017). It has never been shown that *Osedax* worms derive their nutrition from hydrogen sulfide. The interior of whale bone is often anoxic, and rich in hydrogen sulfide due to active MSR (Smith 1992, Deming et al. 1997). Many aquatic animals can survive in sulfide-rich environments because of metabolic pathways of sulfide tolerance and assimilation (Grieshaber & Völkel 1998). Therefore, *O. japonicus* in the present study may have assimilated the sulfur derived from hydrogen sulfide via sulfide-tolerant metabolism.

The lancelet *Asymmetron inferum* was found in abundance in the sediments beneath the whale carcasses (Nishikawa 2004, Fujiwara et al. 2007). *A. inferum* may assimilate the possible whale organic material as a carbon source and chemosynthetic organic matter as a sulfur source. In these sediments, sulfate-reducing bacteria (SRB) utilize whale lipids to generate hydrogen sulfide (Onishi et al. 2018b). The SRB biomass had a similar $\delta^{13}C$ value to the organic substrate, and a low $\delta^{34}S$ value (Londry & Des Marais 2003, Londry et al. 2004). Therefore, *A. inferum* off Cape Nomamisaki may have fed on the SRB in the sediments. However, their $\delta^{15}N$ values were lower than those of the whale tissues (Fig. 2). It is expected that the $\delta^{15}N$ values of chemosynthetic organic matter are lower than those of the whale remains because chemosynthetic bivalves have low $\delta^{15}N$ val-

ues. Also, the $\delta^{15}N$ values of the sedimentary organic matter may be lower because typical $\delta^{15}N$ values of marine phytoplankton are +3 to +10‰ (Altabet & Francois 1994, Voss et al. 2001), although the $\delta^{15}N$ values of the sedimentary organic matter are not analyzed in this area. Thus, the possibility that chemosynthetic organic matter and sedimentary organic matter serve as nutritional resources for *A. inferum* cannot be excluded.

The polychaete *Protodrilus puniceus* had intermediate isotopic compositions between chemosynthetic organic matter and whale bone. *P. puniceus* may feed on both chemosynthetic microbes and whale tissues in the bones. As *P. puniceus* inhabits the inside of the whale bone, phytoplanktonic production likely plays a negligible direct role in its nutrition. In the CDC and SDS calculations, the relative contribution of chemosynthetic organic matter was up to 40%. *P. puniceus* also plausibly possesses a sulfide-tolerant metabolism because there is a large amount of hydrogen sulfide in the bone.

4.2. Relationship between available nutrition and faunal composition

Faunal composition is closely linked to microbial activities and geochemical environments because geochemical and microbial conditions influence the available diets and habitats for the animals (Smith & Baco 2003, Alfaro-Lucas et al. 2018). Onishi et al. (2018b) revealed the geochemical environments and microbial processes in the sediment beneath and around the whale carcasses in Sagami Bay and off Cape Nomamisaki. In the present study, we focused on the change of nutritional resources for each animal belonging to the whale-fall communities to reveal the relationship between the geochemical environments and faunal succession.

In Sagami Bay, the supply of whale soft tissue to the sediments beneath and around the carcass began within 2 wk of carcass deployment (Onishi et al. 2018b). As a result of the feeding activities by a dense community of mobile scavengers, fragments of whale soft tissue were scattered over the surrounding sediments. The scattered fragments and fecal pellets of the scavengers (Smith & Baco 2003) could act as a nutritional resource for infauna, such as ophiuroids and sipunculids, 2 wk after deployment. Some infauna such as gammarids and cumaceans begin to utilize the chemosynthetic organic matter as a nutritional resource rather than the whale soft tissue within 9 mo after deployment. Some species of gammarids and cumaceans living in chemosynthesis-based communities acquire their nutrition mainly by grazing from bacterial mats on sediments (Levin & Michener 2002, Levin & Mendoza 2007), although they have broad feeding strategies and are often deposit-feeders or omnivores. It is expected that the animals cannot digest the whale soft tissues and that the chemosynthetic bacteria are more easily digested than the whale soft tissue directly by some macro-infauna. As the gammarids relied not only on the chemosynthetic organic matter but also the whale-derived material, the increases of chemoautotrophic and heterotrophic bacteria in the sediment plausibly supports the macro-infauna. In such conditions, grazers and bacterivores feeding on the bacteria are expected to be dominant in sediments. Thus, an 'enrichment micrograzer stage', which means enrichment of fauna relying on the bacterial biomass, may flourish in the sediments. As micrograzers feeding on the chemoautotrophs are extremely abundant in the sulfophilic stage (Smith & Baco 2003, Glover et al. 2010, Lundsten et al. 2010, Alfaro-Lucas et al. 2018), both the enrichment micrograzer and other stages will be common in the whale fall at Sagami Bay, as has been observed at other juvenile and shallow carcasses (Smith & Baco 2003, Glover et al. 2010, Lundsten et al. 2010).

Vigorous MSR in sediments generated a large amount of hydrogen sulfide and chemosynthetic organic matter (Onishi et al. 2018b). Then, SRB and chemoautotrophs were likely dominant in the microbial community (Goffredi & Orphan 2010). The huge amount of hydrogen sulfide supported a dense chemosynthesis-based animal community around the carcasses off Cape Nomamisaki (Fig. 2c,d). Chemosymbiotic bivalves *Adipicola pacifica*, *Adipicola crypta*, and *S. pervernicosa* relied for their nutrition on chemoautotrophic endosymbionts, although *Adipicola pacifica* also relied on its heterotrophic symbiont. On the other hand, *O. japonicus*, *Asymmetron inferum*, and *P. puniceus*, which lack thioautotrophic symbionts, relied for their nutrition on the whale tissues. In the sulfophilic stage, the most species-rich communities are formed by animals possessing various feeding habits (Baco & Smith 2003, Alfaro-Lucas et al. 2017, 2018). However, all of the animals off Cape Nomamisaki analyzed in this study may assimilate sulfide-sulfur via sulfide-tolerant metabolic pathways. Thus, such animals may be able to easily inhabit whale remains at the sulfophilic stage off Cape Nomamisaki.

Microbial biomass and diversity in the sediments strongly influenced the infaunal composition around

the whale carcass. Changes in the sediment microbes influenced the available nutritional resources for the animals around the carcass, which will be an environmental filter for species selection. Thus, faunal succession from the enrichment opportunist stage to the sulfophilic stage can be caused by a shift in the dominant microbial species around carcasses, such as a shift from whale-carcass-utilizing heterotrophs to chemoautotrophs.

5. CONCLUSION

In this study, we examined sperm whale carcasses deployed in 2 locations: a single carcass on the continental slopes in Sagami Bay and 12 carcasses off Cape Nomamisaki. We analyzed the stable carbon, nitrogen, and sulfur isotopic compositions of the animals around the carcasses over time to investigate the nutritional resources during each successional stage. In Sagami Bay, mobile scavengers depended for their nutrition solely on whale soft tissue regardless of the sampling time. On the other hand, the nutritional resources for infaunal species in the sediments around the carcass varied over time. As chemosynthetic organic matter increased in the sediment, the reliance of animals on chemosynthetic organic matter increased. Such changes in faunal feeding habits corresponded with the transition of the geochemical environments in Sagami Bay. On the seafloor off Cape Nomamisaki, vigorous MSR and thioautotrophic primary production nourished the fauna around the carcasses. Thioautotrophic microbial activities continued for at least 5 yr. Fauna in the area consisted of chemosymbiotic bivalves and necrophagous animals with sulfide-tolerant metabolisms. Thus, we conclude that thioautotrophic microbial biomass is an important nutritional resource for infauna. Changes in the microbial composition affected by geochemical conditions may drive faunal succession in whale-fall communities.

LITERATURE CITED

Aguzzi J, Fanelli E, Ciuffardi T, Schirone A and others (2018) Faunal activity rhythms influencing early community succession of an implanted whale carcass offshore Sagami Bay, Japan. Sci Rep 8:11163

Alfaro-Lucas JM, Shimabukuro M, Ferreira GD, Kitazato H, Fujiwara Y, Sumida PYG (2017) Bone-eating *Osedax* worms (Annelida: Siboglinidae) regulate biodiversity of deep-sea whale-fall communities. Deep Sea Res II 146: 4–12

Alfaro-Lucas JM, Shimabukuro M, Ogata IV, Fujiwara Y, Sumida PYG (2018) Trophic structure and chemosynthesis contributions to heterotrophic fauna inhabiting an abyssal whale carcass. Mar Ecol Prog Ser 596:1–12

Allison PA, Smith CR, Kukert H, Deming JW, Bennett BA (1991) Deep-water taphonomy of vertebrate carcasses: a whale skeleton in the bathyal Santa Barbara Basin. Paleobiology 17:78–89

Altabet MA, Francois R (1994) Sedimentary nitrogen isotopic ratio as a recorder for surface ocean nitrate utilization. Glob Biogeochem Cycles 8:103–116

Baco AR, Smith CR (2003) High species richness in deep-sea chemoautotrophic whale skeleton communities. Mar Ecol Prog Ser 260:109–114

Bennett BA, Smith CR, Glaser B, Maybaum HL (1994) Faunal community structure of a chemoautotrophic assemblage on whale bones in the deep northeast Pacific Ocean. Mar Ecol Prog Ser 108:205–223

Braby CE, Rouse GW, Johnson SB, Jones WJ, Vrijenhoek RC (2007) Bathymetric and temporal variation among *Osedax* boneworms and associated megafauna on whale-falls in Monterey Bay, California. Deep Sea Res I 54:1773–1791

Deming JW, Reysenbach AL, Macko SA, Smith CR (1997) Evidence for the microbial basis of chemoautotrophic invertebrate community at a whale fall on the deep seafloor. Microsc Res Tech 37:162–170

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Distel DL, Baco AR, Chuang E, Morrill W, Cavanaugh CM, Smith CR (2000) Do mussels take wooden steps to deep-sea vents? Nature 403:725–726

Fry B, Sherr EB (1984) ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib Mar Sci 27:13–47

Fry B, Gest H, Hayes JM (1983) Sulfur isotopic compositions of deep-sea hydrothermal vent animals. Nature 306: 51–52

Fujiwara Y, Kawato M, Yamamoto T, Yamanaka T and others (2007) Three-year investigations into sperm whale-fall ecosystems in Japan. Mar Ecol 28:219–232

Fujiwara Y, Okutani T, Yamanaka T, Kawato M and others (2009) *Solemya pervernicosa* lives in sediment underneath submerged whale carcasses: its biological significance. Venus (Tokyo) 68:27–37

Fujiwara Y, Kawato M, Noda C, Kinoshita G and others (2010) Extracellular and mixotrophic symbiosis in the whale-fall mussel *Adipicola pacifica*: a trend in evolution

from extra- to intracellular symbiosis. PLOS ONE 5: e11808

Gearing JN, Gearing PJ, Rudnick DT, Requejo AG, Hutchins MJ (1984) Isotopic variability of organic carbon in a phytoplankton-based, temperature estuary. Geochim Cosmochim Acta 48:1089–1098

Glover AG, Higgs ND, Bagley PM, Carlsson R and others (2010) A live video observatory reveals temporal processes at a shelf-depth whale-fall. Cah Biol Mar 51:1–7

Goffredi SK, Orphan VJ (2010) Bacterial community shifts in taxa and diversity in response to localized organic loading in the deep sea. Environ Microbiol 12:344–363

Goffredi SK, Orphan VJ, Rouse GW, Jahnke L and others (2005) Evolutionary innovation: a bone-eating marine symbiosis. Environ Microbiol 7:1369–1378

Goffredi SK, Johnson SB, Vrijenhoek RC (2007) Genetic diversity and potential function of microbial symbionts associated with newly-discovered species of *Osedax* polychaete worms. Appl Environ Microbiol 73: 2314–2323

Grieshaber MK, Völkel S (1998) Animal adaptations for tolerance and exploitation of poisonous sulfide. Annu Rev Physiol 60:33–53

Higgs ND, Glover AG, Dahlgren TG, Smith CR and others (2014) The morphological diversity of *Osedax* worm borings (Annelida: Siboglinidae). J Mar Biol Assoc UK 94: 1429–1439

Jones WJ, Won YJ, Maas PY, Smith PJ, Luts RA, Vrijenhoek RC (2006) Evolution of habitat use by deep-sea mussels. Mar Biol 148:841–851

Kaplan IR, Rittenberg SC (1964) Microbiological fractionation of sulphur isotopes. J Gen Microbiol 34:195–212

Kiel S (2017) Reply to Smith et al.: Network analysis reveals connectivity pattern in the continuum of reducing ecosystems. Proc R Soc B 284:20171644

Levin LA, Mendoza GF (2007) Community structure and nutrition of deep methane-seep macrobenthos from the North Pacific (Aleutian) Margin and the Gulf of Mexico (Florida Escarpment). Mar Ecol 28:131–151

Levin LA, Michener RH (2002) Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: the lightness of being at Pacific methane seeps. Limnol Oceanogr 47:1336–1345

Londry KL, Des Marais DJ (2003) Stable carbon isotope fractionation by sulfate-reducing bacteria. Appl Environ Microbiol 69:2942–2949

Londry KL, Jahnke LL, Des Marais DJ (2004) Stable carbon isotope ratios of lipid biomarkers of sulfate-reducing bacteria. Appl Environ Microbiol 70:745–751

Lorion J, Kiel S, Faure B, Kawato M and others (2013) Adaptive radiation of chemosynthetic deep-sea mussels. Proc R Soc B 280:20131243

Lundsten L, Paull CK, Schlining KL, McGann M, Ussler W III (2010) Biological characterization of a whale-fall near Vancouver Island, British Columbia, Canada. Deep Sea Res I 57:918–922

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. Geochim Cosmochim Acta 48:1135–1140

Miyazaki JI, de Oliveira Martins L, Fujita Y, Matsumoto H, Fujiwara Y (2010) Evolutionary process of deep-sea *Bathymodiolus* mussels. PLOS ONE 5:e10363

Mizota C, Yamanaka T (2003) Strategic adaptation of a deep-sea, chemosynthesis-based animal community: an evaluation based on soft body part carbon, nitrogen and sulfur isotopic signatures. Jpn J Benthol 58:56–59 (in Japanese with English abstract)

Mizota C, Shimoyama S, Yamanaka T (1999) An isotopic characterization of sulfur uptake by benthic animals from Tsuyazaki inlet, northern Kyushu. Benthos Res 54:81–85

Nelson DC, Fisher CR (1995) Chemoautotrophic and methanotrophic endosymbiotic bacteria at deep-sea vents and seeps. In: Karl DM (ed) The microbiology of deep-sea hydrothermal vents. CRC Press, Boca Raton, FL, p 125–167

Nishikawa T (2004) A new deep-water lancelet (Cephalochordata) from off Cape Nomamisaki, SW Japan, with a proposal of the revised system recovering the genus *Asymmetron*. Zool Sci 21:1131–1136

Okutani T, Fujiwara Y, Fujikura K, Miyake H, Kawato M (2003) A mass aggregation of the mussel *Adipicola pacifica* (Bivalvia: Mytilidae) on submerged whale bones. Venus (Tokyo) 63:61–64

Onishi Y, Yamanaka T, Okumura T, Kawagucci S, Watanabe HK, Ohara Y (2018a) Evaluation of nutrient and energy sources of the deepest known serpentinite-hosted ecosystem using stable carbon, nitrogen, and sulfur isotopes. PLOS ONE 13:e0199000

Onishi Y, Shimamura S, Yamanaka T, Nakayama R and others (2018b) Variation of geochemical environments associated with whale-fall biomass mineralization processes in the sediment during the mobile scavenger, enrichment opportunist, and sulfophilic stages. Mar Biol 165:141

Rau GH, Sweeney RE, Kaplan IP (1982) Planktonic ^{13}C:^{12}C ratio change with latitude: differences between northern and southern oceans. Deep-Sea Res A 29:1035–1039

Rees CE, Jenkins WJ, Monster J (1978) The sulphur isotopic composition of ocean water sulphate. Geochim Cosmochim Acta 42:377–381

Ruby EG, Jannasch HW, Deuser WG (1987) Fractionation of stable carbon isotopes during chemoautotrophic growth of sulfur-oxidizing bacteria. Appl Environ Microbiol 53: 1940–1943

Sim MS, Bosak T, Ono S (2011) Large sulfur isotope fractionation does not require disproportionation. Science 333: 74–77

Smith CR (1992) Whale falls: chemosynthesis on the deep seafloor. Oceanus 35:74–78

Smith CR, Baco AR (2003) Ecology of whale falls at the deep-sea floor. Oceanogr Mar Biol Annu Rev 41:311–354

Smith CR, Kukert H, Wheatcroft RA, Jumars PA, Deming JW (1989) Vent fauna on whale remains. Nature 341:27–28

Smith CR, Baco AR, Glover AG (2002) Faunal succession on replicate deep-sea whale falls: time scales and vent-seep affinities. Cah Biol Mar 43:293–297

Smith CR, Glover AG, Treude T, Higgs ND, Amon DJ (2015) Whale-fall ecosystems: recent insights into ecology, paleoecology, and evolution. Annu Rev Mar Sci 7: 571–596

Smith CR, Amon DJ, Higgs HD, Glover AG, Young EL (2017) Data are inadequate to test whale falls as chemosynthetic stepping-stones using network analysis: faunal overlaps do support a stepping-stone role. Proc R Soc B 284:20171281

Thubaut J, Puillandre N, Faure B, Cruaud C, Samadi S (2013) The contrasted evolutionary fates of deep-sea chemosynthetic mussels (Bivalvia, Bathymodiolinae). Ecol Evol 3:4748–4766

Treude T, Smith CR, Wenzhöfer F, Carney E and others (2009) Biogeochemistry of a deep-sea whale fall: sulfate reduction, sulfide efflux and methanogenesis. Mar Ecol Prog Ser 382:1–21

Vander Zanden MJ, Clayton MK, Moody EK, Solomon CT, Weidel BC (2015) Stable isotope turnover and half-life in animal tissues: a literature synthesis. PLOS ONE 10: e0116182

Voss M, Dippner JW, Montoya JP (2001) Nitrogen isotopic patterns in the oxygen-deficient waters of the Eastern Tropical North Pacific Ocean. Deep Sea Res I 48: 1905–1921

Yamanaka T, Mizota C (2001) Sulfur nutrition of gastropods and bivalves relevant to the mangrove forests: a case study from central Sumatra, Indonesia. Venus (Tokyo) 60:71–78

Yamanaka T, Shimamura S, Nagashio H, Yamagami S and others (2015) 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: variation in energy source and importance of subsurface microbial processes in the sediment-hosted systems. In: Ishibashi JI, Okino K, Sunamura M (eds) Subseafloor biosphere linked to hydrothermal systems: TAIGA concept. Springer, Tokyo, p 105–129