



FEATURE ARTICLE

Trophic structure and chemosynthesis contributions to heterotrophic fauna inhabiting an abyssal whale carcass

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ABSTRACT: The trophic structure and role of chemosynthesis remain unexplored in deep-sea whale-fall communities in areas other than the California margin. This gap limits the understanding of these communities and their ecological relationships with other chemosynthetic ecosystems, such as vents and seeps. Here, we studied 3 different whale skeleton microhabitats with hypothesized high, intermediate and low reducing conditions as well as the sediments surrounding an abyssal whale fall (4204 m depth, SW Atlantic Ocean). We analyzed trophic structures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and the contribution of chemosynthetically derived carbon to heterotrophic species. The high and intermediate reducing microhabitats harbored food webs dominated by consumers of chemosynthetic production, similar to those of diffusive areas of hydrothermal vents and seeps. Both the low reducing microhabitat and the sediments harbored food webs with greater trophic complexity, dominated by higher consumers mainly relying on whale and/or photosynthesis-derived organic matter, a type of food web commonly reported in small whale, wood and kelp falls. The main whale-fall ecosystem engineer, the bone-eating worm *Osedax*, appeared to produce unique food web effects not observed in other chemosynthetic habitats. We conclude that whale falls provide the deep sea with a mosaic of microhabitats that supports assemblages with different chemosynthesis reliance levels and trophic structures, similar to those found at vents and seeps. Such a mosaic allows species-rich communities with numerous trophic levels to develop in a very small area of the food-limited deep sea.



Whale carcasses provide the deep sea with a mosaic of microhabitats that support faunal assemblages with different trophic structures and chemosynthesis reliance levels.

Photo: Yoshihiro Fujiwara

KEY WORDS: Deep sea · Whale fall · Trophic structure · Chemosynthesis · *Osedax*

INTRODUCTION

In deep-sea environments such as hydrothermal vents and cold seeps, where reduced compounds are abundant, autochthonous microbial chemosynthetic production supports abundant endemic fau-

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nal communities (Van Dover et al. 2002). At vents and seeps, unlike most of the vast, food-limited deep sea, photosynthetically derived food sources only supplement faunal diets. These food sources have decreasing importance with increasing depth, and the intensity of reducing compound fluxes within habitats affects their use (Levin & Michener 2002, Levesque et al. 2006, Decker & Olu 2012, Bernardino et al. 2012). Among chemosynthetic ecosystems, those derived from cetacean carcasses, the largest organic parcels exported from surface waters, may create conditions that support chemosynthesis for years and even decades (Smith & Baco 2003, Smith 2006, Smith et al. 2015).

Although 'whale falls' are thought to be common along cetacean migratory routes, they were only discovered ca. 30 yr ago and have been much less studied than vents and seeps, especially in basins outside the Northeast Pacific Ocean (Smith et al. 1989, Smith et al. 2015). Carcasses greatly impact small areas of the seafloor (~100 m²), attracting opportunistic and specialized fauna and creating unique island-like habitats considered to be hotspots of biodiversity and sources of evolutionary novelty (Smith et al. 2015). The organic matter in enriched sediments around carcasses and within the lipid-rich skeletons is anaerobically degraded by sulfate-reducing bacteria and archaea, which generate fluxes of reduced compounds as by-products of their metabolism (Deming et al. 1997, Smith & Baco 2003, Goffredi et al. 2008, Treude et al. 2009). The free-living chemosynthetic sulfide-oxidizing bacteria and chemosymbiotic invertebrates that exploit these fluxes also occur in vents, seeps and other organic falls (Smith et al. 1989, Feldman et al. 1998, Fujiwara et al. 2007, Lundsten et al. 2010a,b, Smith et al. 2015). Heterotrophic fauna also colonize whale falls during the 'sulfophilic stage', creating extremely species-rich communities structured in several trophic levels (from 3 to 5) (Baco & Smith 2003, Smith & Baco 2003). Bone fauna assemblages are considered one of the most species-rich hard-substrate habitats in the deep sea (Baco & Smith 2003).

Whale falls have been hypothesized to act as stepping stones for faunal evolution and dispersal among distant nascent seeps and vents (Smith et al. 1989, Smith et al. 2017, Kiel 2017). Some chemosymbiotic seep/vent fauna likely originated in shallow waters and potentially used organic falls to colonize the deep sea (Distel et al. 2000, Jones et al. 2006, Miyazaki et al. 2010, Lorion et al. 2013, Thubaut et al. 2013). Deep-sea chemosynthetic habitats, including whale falls, already face, or are

very likely to face, anthropogenic impacts, such as fisheries and deep-sea mining (Roman et al. 2014, Levin et al. 2016). Therefore, understanding their ecological interactions is crucial for their effective protection (Roman et al. 2014, Levin et al. 2016). The trophic ecology of whale falls has received little attention beyond the deep California margin (Baco & Smith 2003, Smith & Baco 2003). This gap may bias our understanding of these habitats and their ecological links with vents, seeps and other organic falls from other basins. Previous studies report that large whale skeletons in the sulfophilic stage create communities that are similar to those of vents with 3 to 5 trophic levels (Baco & Smith 2003, Smith & Baco 2003). In the Pacific, the low $\delta^{15}\text{N}$ values in the fauna indicate local origins for much of the organic nitrogen (Smith & Baco 2003). In the latter study, chemosymbiotic bivalves (*Vesicomya gigas* and *Idas washingtonia*) dominated macrofaunal abundance and constituted 58% of the molluscan biomass. Species that depend on chemosynthetic production accounted for up to 42% of the community biomass, with just a few species relying on the whale organic material (Smith & Baco 2003). In contrast, small juvenile whale skeletons harbored communities mainly dependent on whale organic matter (Smith & Baco 2003).

Here, we present a detailed stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of an abyssal whale-fall community (4204 m depth, off Brazil, SW Atlantic Ocean) at the sulfophilic degradation stage in order to shed light on the reliance of heterotrophic fauna from 3 skeleton microhabitats and sediments on microbial chemosynthetic production. The carcass in this study was a juvenile whale, and previous studies suggested that chemosynthesis may be important, especially in some parts of the skeleton (Sumida et al. 2016, Alfaro-Lucas et al. 2017). Based on visual observations and colonizing fauna, we hypothesized that a gradient in the sulfide concentration would define availability of chemosynthetic production across the skeleton microhabitats, in addition to the fauna from sediments. As reported for vents and seeps, we expected a decrease in faunal reliance on microbial chemosynthetic production from the hypothesized high to low reducing microhabitats. Specifically, in this study, we address the following questions: (1) is free-living microbial chemosynthetic primary production an important food source for heterotrophic species at whale-fall microhabitats; and (2) how does trophic structure vary among different whale-fall microhabitats and across the entire whale-fall community?

MATERIALS AND METHODS

Study site, sample processing and whale-fall microhabitats

We studied a partial natural whale carcass discovered by the submersible HOV (human occupied vehicle) 'Shinkai 6500' during the Brazilian-Japanese Iatá-Piúna expedition, a part of the around-the-world Quelle 2013 project (Japanese Agency for Marine-Earth Science and Technology). The carcass was discovered at 4204 m depth at the base of São Paulo Ridge (28° 31.1191' S, 41° 39.4097' W), off the SE Brazilian margin, SW Atlantic Ocean (Sumida et al. 2016). The juvenile Antarctic minke whale *Balaenoptera bonaerensis* carcass consisted of 9 caudal vertebrae of ca. 11.5 cm in diameter without soft tissues (Sumida et al. 2016) (Fig. 1). During 2 dives, the HOV 'Shinkai 6500' completed detailed video surveys and collected whale bones with its manipulators. The whale-fall location was characterized by a thin layer (<20 cm) of sediments overlying basaltic rocks, which complicated collection of sediment cores (Sumida et al. 2016). Sedimentary fauna were therefore collected immediately below and adjacent (0.5 m) to the carcass using a slurp gun.

Only sediments below the skeleton were dark in color, suggesting high concentrations of sulfide and anoxia (Allison et al. 1991, Sumida et al. 2016). Five of the 9 vertebrae were (1) still joined together by intervertebral discs, (2) significantly eroded only at the spinous and transverse processes, (3) colonized by dense white bacterial mats on their upper parts, (4) stained black at the parts closer to or in contact with the sediment and (5) not colonized by bone-eating *Osedax* worms (Sumida et al. 2016, Alfaro-Lucas et al. 2017). Based on image analysis and the distinct colonizing fauna, we hypothesized that these vertebrae provide 2 microhabitats (Alfaro-Lucas et al. 2017): (1) inferior parts of vertebra close to or in contact with sediments with blackish areas, suggesting high concentrations of sulfide and anoxia, and (2) superior parts entirely exposed to the water column and colonized by dense, white chemoautotrophic bacterial mats (hypothesized to be sulfide oxidizing bacteria), suggesting lesser anoxic and reducing conditions, i.e. a redox interface (Higgs et al. 2011) (Fig. 1A,B). The remaining 4 vertebrae were loose (not joined together by intervertebral discs), and colonized and heavily eroded by a single new species of *Osedax* (Sumida et al. 2016) (Fig. 1C). Although some thin and sparse bacterial mats were present on these bones, *Osedax* apparently prevented the formation of bacterial mats

by decreasing sulfide concentrations by enhancing oxygen penetration to the inner-bone matrices (Alfaro-Lucas et al. 2017) (Fig. 1C). Vertebrae colonized by *Osedax* sp. were associated with different infaunal assemblages from those of non-colonized vertebrae (Alfaro-Lucas et al. 2017).

We analyzed carbon and nitrogen stable isotopes of infaunal species accounting for 97.5, 51.1 and 98.3% of the total faunal abundance of the inferior parts of the vertebrae not colonized by *Osedax* sp., the superior parts of the vertebra not colonized by *Osedax* sp. and the vertebra colonized by *Osedax* sp., respectively (see Table 1). The most abundant and large epifauna of sediments were also analyzed (*Munidopsis* sp. and *Rubyspira* sp.), and the total number of individuals in the community (not percent abundance) was obtained through video analysis (Sumida et al. 2016). We did not estimate abundances of sediment epifauna that were indistinguishable in video analysis, such as *Bathyrurilla* cf. *guaymasensis* and *Bathyfauvelia* sp. (Sumida et al. 2016). For sediment infauna, including nematodes and the new nereidid species *Neanthes shinkai* (Shimabukuro et al. 2017), we did not estimate abundances because we used a slurp gun instead of corers (Sumida et al. 2016) (see Table 1). On board, large infauna from bones (*Osedax* sp.) and epifauna of the sediments (*Neanthes shinkai*, *Bathyfauvelia* sp., *Bathyrurilla* cf. *guaymasensis* and *Rubyspira* sp.) were sorted under a stereomicroscope and preserved in 99.5% non-denatured ethanol. Whale bones and sediments were both preserved in 99.5% non-denatured ethanol for further sorting of the fauna in the laboratory. In order to obtain reliable data for the vertebra infauna while considering superior and inferior microhabitats of vertebrae without *Osedax*, we removed selected rectangular pieces of the upper and lower parts of the vertebrae in the laboratory, using a saw (see detailed procedure in Alfaro-Lucas et al. 2017). Vertebrae colonized by *Osedax* did not exhibit such obvious microhabitats, and several randomly chosen pieces were removed from different parts of the colonizing vertebrae, also using a saw, to sample colonizing infauna (Alfaro-Lucas et al. 2017). All fauna was identified under a stereomicroscope to the lowest possible taxonomic level by expert taxonomists and preserved in 99.5% non-denatured ethanol.

Stable isotope analyses, mixing models and species trophic positions

We analyzed stable carbon and nitrogen isotopes of each species in triplicate, except for large or very

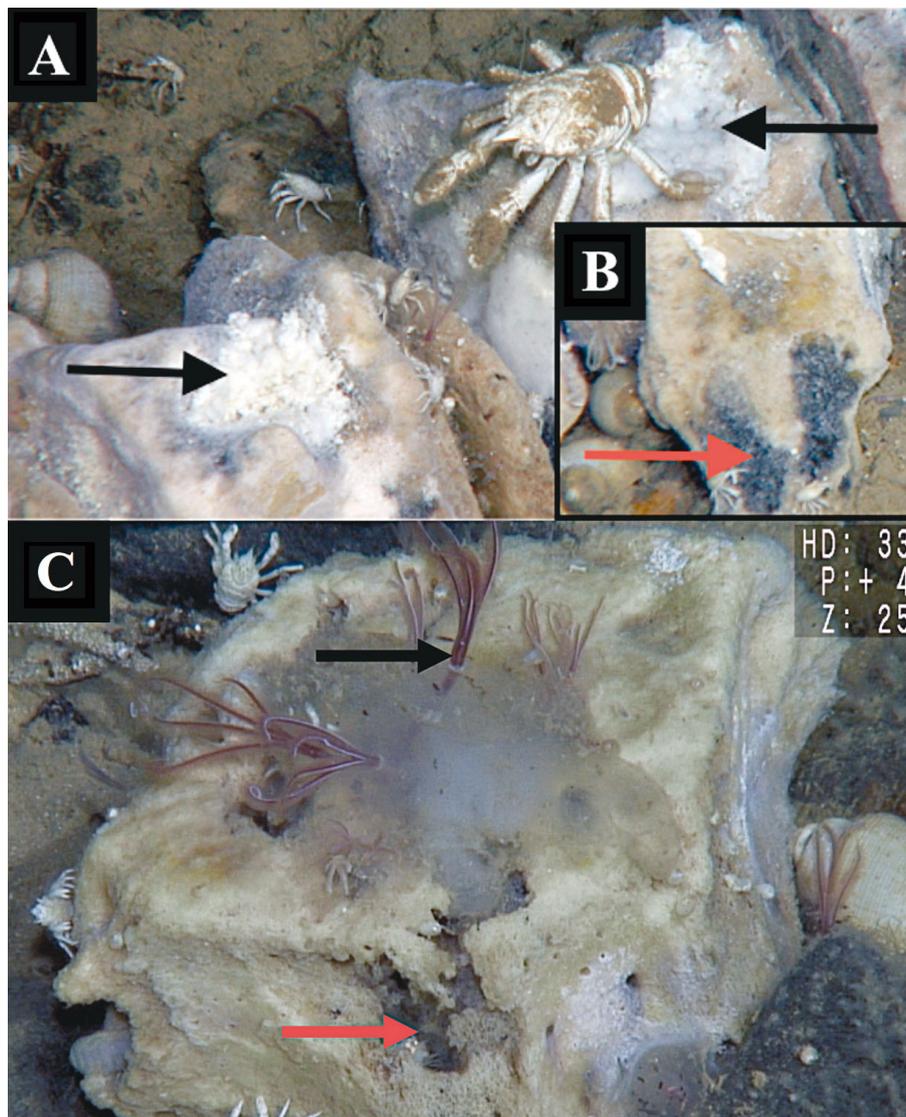


Fig. 1. Three different skeleton microhabitats at the whale-fall community at the base of São Paulo Ridge (4204 m), off Brazil, SW Atlantic Ocean. (A,B) Vertebra not colonized by bone-eating *Osedax* worms, with inferior parts of vertebra in contact with or partially buried in sediments hypothesized to have highly reducing conditions, i.e. dark stains (red arrow), and superior parts entirely exposed to the water column inhabited by dense chemoautotrophic bacterial mats hypothesized to have intermediate reducing conditions (black arrows). (C) Vertebra colonized and bioeroded (red arrow) by *Osedax* sp. (black arrow) hypothesized to have low reducing conditions. Image modified from Sumida et al. (2016)

abundant species, such as *Capitella iatapiuna*, for which we removed 5 samples of pooled individuals from 5 different bones. Six samples belonging to 6 different individuals were sampled for *Neanthes shinkai*. For animals with low densities, such as *Bathyrurilla* cf. *guaymasensis* and *Bathyaufvellia* sp. from the sediments, we analyzed only one sample of each species (Table S1 in the Supplement at www.int-res.com/articles/suppl/m596p001_supp.pdf). We did not remove the gut of very small species, and instead pooled individuals (5–10 individuals for polychaetes;

30–100 individuals for nematodes) in order to attain the minimum biomass required for isotopic analysis. For *Neanthes shinkai*, *Osedax* sp., *Rubyspira* sp., *Munidopsis* sp., *Bathyrurilla* cf. *guaymasensis* and *Bathyaufvellia* sp., we used portions of single individuals for each sample. Given the presence of endosymbiotic bacteria in *Osedax* sp. roots and the possibility of symbiont presence in *Rubyspira* gills (Johnson et al. 2010), we used only parts of the trunk and the foot, respectively, for isotopic analysis. We obtained samples of all species from microhabitats of different

bones whenever possible in order to evaluate the variability between bones (Table S1, in the Supplement). Nematodes were not identified to species level, and we treated samples as a representation of the nematode assemblage for each microhabitat.

Individuals or groups of individuals were rinsed in Milli-Q water, frozen, freeze-dried, weighed and encapsulated in tin boats. Samples were then transferred to a Costech Instruments Elemental Combustion System coupled to a Thermo Scientific Delta V Advantage Isotope Ratio Mass Spectrometer (EA-IRMS) to measure stable isotopic composition. Carbon and nitrogen isotope results are expressed in delta notation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{reference}}} - 1 \right) \right] \times 1000 \quad (1)$$

where $X = {}^{13}\text{C}$ or ${}^{15}\text{N}$ and $R_{\text{sample}} = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$ (Peterson & Fry 1987). We used Vienna Peedee Belemnite ($R_{\text{VPDB}} = 0.01118$) as a reference ($R_{\text{reference}}$) for carbon, and atmospheric N_2 as a reference for nitrogen. Analytical precision based on the standard deviation of internal standard replicates was 0.05% for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. We considered the effects of ethanol preservation to be negligible for the $\delta^{15}\text{N}$ analyses of the species (Fanelli et al. 2010, Lau et al. 2012). Although ethanol may potentially affect $\delta^{13}\text{C}$ values, its effects are variable, taxon specific and usually more important in mollusks than in polychaetes or crustaceans, which limits the use of standard corrections. Therefore, we applied no correction factor (Fanelli et al. 2010, Lau et al. 2012), and analyzed only one mollusk in this study (*Rubyspira* sp.).

Chemosynthetic bacterial mats, whale vertebrae and sedimentary organic matter of photosynthetic origin (SOM) were used to estimate the dependence of consumers on chemosynthetic primary production. We estimated the percentage of the chemosynthetically derived carbon (CDC) of species with an isotope mixing model assuming no trophic shift for $\delta^{13}\text{C}$ (Levin & Michener 2002):

$$\text{CDC} = \frac{(\delta^{13}\text{C}_{\text{sp}} - \delta^{13}\text{C}_{\text{SOM}})}{(\delta^{13}\text{C}_{\text{chemo bacteria}} - \delta^{13}\text{C}_{\text{SOM}})} \times 100 \quad (2)$$

where $\delta^{13}\text{C}_{\text{sp}}$, $\delta^{13}\text{C}_{\text{SOM}}$ and $\delta^{13}\text{C}_{\text{chemo bacteria}}$ are the mean carbon isotopic values of species, SOM and chemosynthetic bacterial mats, respectively. Given that we could not collect chemosynthetic bacterial mats for this community, we used mats collected from the thorax vertebrae of a humpback whale *Megaptera novaeangliae* implanted in the same region (at 28° 1.7' S, 43° 31.8' W) and water mass at 3300 m depth (Antarctic Bottom Water, 0.9°C and salinity of

34.7; Orsi et al. 1999). We used the isotope composition of SOM from North São Paulo Plateau sediments sampled in the same expedition (2692–2706 m depth, 2.51 to 2.63°C and salinity of 34.90 to 34.92) (Fujikura et al. 2017). For species that also consume whale organic matter, this model may overestimate CDC percentages (CDC_{max} , see Table 1) (Levin & Michener 2002, Thurber et al. 2010). To address this issue, we replaced $\delta^{13}\text{C}_{\text{SOM}}$ in the model with the $\delta^{13}\text{C}$ of whale bones ($\delta^{13}\text{C}_{\text{whale}}$), thus providing a conservative estimate of CDC (CDC_{min} , see Table 1). We analyzed 3 distinct inner bone matrices completely depleted of lipids in order to obtain the vertebrae $\delta^{13}\text{C}_{\text{whale}}$ and $\delta^{15}\text{N}_{\text{whale}}$. Because bone lipids were partially dissolved in 99.5% non-denatured ethanol, we decided to analyze the isotopic composition of vertebra matrices depleted of lipids, which are rich in organic compounds such as collagen, in order to obtain a reliable isotopic composition of whale organic matter.

For the different microhabitats and the overall community, we used the method proposed in Post (2002) to estimate the trophic position (TP) of each species:

$$\text{TP} = \lambda + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}}) / \Delta n \quad (3)$$

where $\delta^{15}\text{N}_{\text{chemo bacteria}}$ was used as a $\delta^{15}\text{N}_{\text{base}}$ (see Table 1). λ is the trophic position of the organisms used to estimate $\delta^{15}\text{N}_{\text{base}}$, which is represented by chemosynthetic bacteria in our study, so $\lambda = 1$. $\delta^{15}\text{N}_{\text{consumer}}$ denotes the mean $\delta^{15}\text{N}$ of the species, and Δn denotes the enrichment in $\delta^{15}\text{N}$ per trophic level, which was set at 3.4‰ (DeNiro & Epstein 1981, Minagawa & Wada 1984, Post 2002).

CDC_{max} and TP values were compared for *Capitella iatapiuna* inhabiting the 3 putative skeleton microhabitats. We excluded samples from superior parts of vertebra not colonized by *Osedax* because they were obtained from the same bone and were not considered independent (Table S1). We tested normality and homogeneity of variance using Shapiro-Wilk and homogeneity of variance tests, respectively. All statistical analyses were performed in R (R Core Team 2014).

RESULTS

Nematode samples from inferior parts of the bones not colonized by *Osedax* (mean = $-30.13 \pm 0.28\text{‰}$) contained the most depleted mean $\delta^{13}\text{C}$ values of fauna, whereas the polynoid *Bathykurila* cf. *guaymasensis* from sediments contained the most enriched value (-19.7‰ ; Fig. 2A,C, Table 1). The dorvilleid

Ophryotrocha sp. 1 from the vertebrae bioeroded by *Osedax* was most depleted in $\delta^{15}\text{N}$ (mean = $4.87 \pm 1\%$), whereas *Osedax* was most enriched (mean = $12.84 \pm 0.16\%$; Fig. 2B, Table 1). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ standard deviations were generally low among the samples, except for the galatheid crab *Munidopsis* sp. from sediments, *Pleijelius* sp., and nematodes from the bones bioeroded by *Osedax* (Fig. 2B,C, Table 1). The isotopic values of food sources were clearly distinct (Fig. 2, Table 1). The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of chemosynthetic bacterial mats were $-30.47 \pm 0.27\%$ and $2.94 \pm 2.62\%$, respectively. The isotopic composition of the organic matter of the whale vertebrae was $-24.04 \pm 0.47\%$ and $7.36 \pm 0.24\%$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

The maximum species CDC percentages ranged from $96.45 \pm 2.96\%$ in nematodes from inferior parts of the vertebrae without *Osedax* to $2.93 \pm 1.17\%$ in the gastropod *Rubyspira* sp. from sediments. Minimum values varied from $94.72 \pm 4.41\%$ in nematodes

from inferior parts of the vertebrae without *Osedax* to 0% in many species (Table 1). Most species from the vertebrae bioeroded by *Osedax* and sediments had zero CDC percentages (Table 1). The $\delta^{13}\text{C}$ values in the polynoid *Bathykurila* cf. *guaymasensis* from sediments were greater than the SOM value; we did not calculate its CDC percentages (Fig. 2B,C, Table 1). TP values varied from 1.7 to 3.83, with values for most species between 2 and 3 (Table 1).

The capitellid *Capitella iatapiuna* and nematodes were the dominant fauna in the 3 hypothesized microhabitats (Table 1). Nematode CDCs from highly reducing environments were ca. 5–6 times higher than those from low reducing environments and sediments. Moreover, significantly higher CDC percentages in *C. iatapiuna* occurred in putative high reducing compared to low reducing microhabitats ($t = 8.83$, $df = 5$, $p = 0.0003$). Despite these values, we found no significant differences in their TP ($t = 0.97$, $df = 5$, $p = 0.37$) (Table 1).

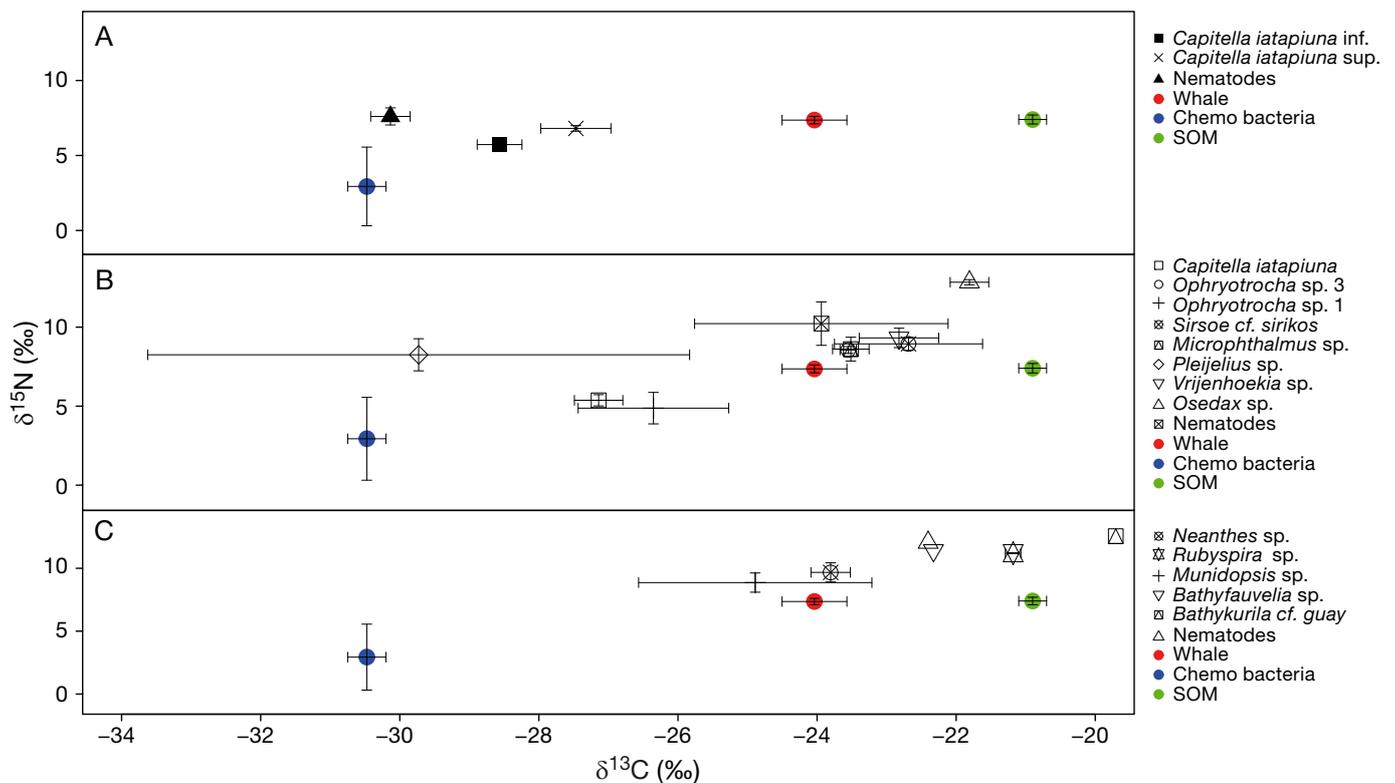


Fig. 2. Mean and standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios of species and food sources in different whale-fall microhabitat assemblages. (A) Species from the hypothesized high (*Capitella iatapiuna* inf. and nematodes) and intermediate (*Capitella iatapiuna* sup.) reducing microhabitats. (B) Species of the putative low reducing skeleton microhabitat. Note that *Ophryotrocha* sp. 3 and *Microphthalmus* sp., and *Sirsoe* cf. *sirikos* and *Vrijenhoekia* sp. values, respectively, show considerable overlap. (C) Species from the sediments. Values for *Bathykurila* cf. *guaymasensis*, *Bathyfauvelia* sp. and nematodes were obtained from 1 sample each. Food sources, i.e. chemosynthetic bacterial mats (chemo bacteria), whale matter and sedimentary organic matter of photosynthetic origin (SOM) are indicated with blue, red and green filled circles, respectively. *Capitella iatapiuna* inf. and *C. iatapiuna* sup. refer to the species in the high and intermediate reducing microhabitats, respectively

Table 1. Abundance, feeding mode (FM), number of analyzed samples (n), mean (\bar{x}) and standard deviation (SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios (‰), percentage of chemosynthetically derived carbon (CDC) and mean and standard deviation of trophic position (TP) of whale-fall species and food source isotopic values. BG: bacterial grazer; BS: bone specialist; OP: opportunist; ? : indeterminate. Condition, abundance and feeding mode data were obtained from analyses and literature cited in Sumida et al. (2016) and Alfaro-Lucas et al. (2017). Abundances are expressed as percentage of the total faunal abundance of the sampled microhabitat or, in the case of sediment fauna and *Osedax*, as the number of individuals counted in video analysis. Values were rounded to 2 decimal places

Microhabitat	Hypothesized condition	Species	Abundance	FM	n	Stable isotopes			CDC (%)			TP				
						\bar{x} $\delta^{13}\text{C}$	SD $\delta^{13}\text{C}$	\bar{x} $\delta^{15}\text{N}$	SD $\delta^{15}\text{N}$	Max.	SD	Min.	SD	\bar{x}	SD	
Inferior parts of vertebrae	High reducing	<i>Capitella iatapiuna</i>	15.9 %	BG	5	-28.56	0.32	5.73	0.38	80.06	3.36	70.33	5	1.8	0.11	
		Nematodes	81.6 %	?	3	-30.13	0.28	7.6	0.57	96.45	2.96	94.72	4.41	2.33	0.16	
Superior parts of vertebrae	Intermediate reducing	<i>Capitella iatapiuna</i>	51.1 %	BG	3	-27.47	0.5	6.81	0.19	68.6	5.26	53.28	7.83	2.11	0.05	
Vertebrae bioeroded by <i>Osedax</i>	Low reducing	<i>Capitella iatapiuna</i>	38.9 %	BG	3	-27.14	0.35	5.37	0.36	65.19	3.63	48.2	5.4	1.7	0.1	
		Nematodes	52.2 %	?	3	-23.94	1.82	10.22	1.37	31.73	19.03	0	-	3.08	0.39	
		<i>Ophryotrocha</i> sp. 1	2.72 %	BG	3	-26.35	1.08	4.87	1	56.95	11.31	35.94	16.83	1.55	0.28	
		<i>Ophryotrocha</i> sp. 3	1.7 %	BG	3	-23.55	0.12	8.55	0.22	27.71	1.23	0	-	2.6	0.06	
		<i>Sirsoe</i> cf. <i>sirikos</i>	0.67 %	?	3	-22.68	1.06	8.94	0.41	18.64	11.12	0	-	2.72	0.12	
		<i>Microphthalmus</i> sp.	0.81 %	?	3	-23.51	0.26	8.61	0.75	27.31	2.74	0	-	2.62	0.21	
		<i>Pleijelus</i> sp.	0.92 %	?	3	-29.73	3.9	8.25	1.02	92.21	40.7	88.41	60.6	2.52	0.29	
		<i>Vrijenhoekia</i> sp.	0.4 %	?	3	-22.82	0.57	9.39	0.61	20.07	6	0	-	2.82	0.17	
		<i>Osedax</i> sp.	98 ind.	BS	3	-21.81	0.28	12.84	0.16	9.49	2.9	0	-	3.83	0.05	
		Sediments	Heterogeneous	<i>Neanthes shinkai</i>	-	BG	6	-23.8	0.28	9.67	0.761	30.34	3	0	-	2.92
<i>Munidopsis</i> sp.	295 ind.			OP	3	-24.89	1.68	8.86	0.76	41.66	17.5	17.03	20.95	2.69	0.22	
<i>Rubyspira</i> sp.	52 ind.			BS	3	-21.18	0.19	11.2	0.04	2.93	1.17	0	-	3.36	0.01	
<i>Bathyporeia</i> cf. <i>guaymasensis</i>	-			BG	1	-19.7	-	12.55	-	-	-	-	-	-	3.75	-
<i>Bathyporeia</i> sp.	-			BG	1	-22.33	-	11.42	-	14.94	0	0	-	-	3.42	-
Nematodes	-			?	1	-22.4	-	12.05	-	15.67	0	0	-	-	3.6	-
Food sources		Chemosynthetic bacteria	-	-	6	-30.47	0.27	2.94	2.62	-	-	-	-	-	-	
		Whale	-	-	3	-24.04	0.47	7.36	0.24	-	-	-	-	-	-	
		Sedimentary organic matter ^a	-	-	6	-20.9	0.2	7.4	0.3	-	-	-	-	-	-	
			-	-	6	-20.9	0.2	7.4	0.3	-	-	-	-	-	-	

^aSedimentary organic matter obtained from Fujikura et al. (2017)

DISCUSSION

To date, the relative importance of chemosynthesis to late successional food webs has only been explored on whale falls from the California margin (Baco & Smith 2003, Smith & Baco 2003). The faunal communities of large whale falls depend predominantly on chemosynthesis-based food webs similar to those of some vents (Smith & Baco 2003). Our study confirms the importance of organic matter of chemosynthetic origin as a food source in our juvenile whale carcass. We further show that the skeleton microhabitats in different reducing conditions exhibit varying levels of dependence on microbial chemosynthetic primary production with contrasting food web structures, such as those observed in vents and seeps (Levin & Michener 2002, Levesque et al. 2006, Bernardino et al. 2012, Decker & Olu 2012). This finding indicates that whale falls form a mosaic of different microhabitats that harbor distinct faunal assemblages in terms of species, dependence on chemosynthetic production, and trophic structure, which helps to explain the high biodiversity found in these restricted deep-sea habitats.

Although the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in chemosynthetic bacterial mats in the present study were lower than those of Smith & Baco (2003) from whale falls off California, they coincided with studies conducted in other related habitats (e.g. Colaço et al. 2002, Levin & Michener 2002). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the whale vertebrae clearly matched those reported for other species such as southern right whales *Eubalaena australis* and Adélie penguins *Pygoscelis adeliae* (e.g. Best & Schell 1996, Cherel 2008) that, like Antarctic minke whales, inhabit the South Atlantic/Southern Ocean and feed primarily on euphausiids. The SOM isotopic values obtained by Fujikura et al. (2017) also correspond to the SOM values of photosynthetic origin from the abyssal South Atlantic Ocean reported for other basins (e.g. see Table 1 in Holmes et al. 1996). The food source isotopic values used in our CDC and trophic position estimates were therefore reliable.

In the putative high reducing bone microhabitat, high CDC percentages ($96.45 \pm 2.96\%$ to $94.72 \pm 4.41\%$ and $80.06 \pm 3.36\%$ to $70.33 \pm 5\%$, respectively) and low mean TPs (2.33 ± 0.16 and 1.8 ± 0.11 , respectively) characterized nematodes (81.6% of total faunal abundance) and the annelid *Capitella iatapiuna* (15.9% of total faunal abundance), indicating that they are mainly primary consumers of chemosynthetic bacteria (Table 1). Thus, our results suggest that 97.5% of the fauna in this microhabitat relies on chemosynthetic carbon. We found similar results in

the bone microhabitats postulated to have intermediate degrees of reduction, where *C. iatapiuna* (51.1% of total faunal abundance) exhibited CDC percentages of $68.6 \pm 5.26\%$ to $53.28 \pm 7.83\%$ and a TP of 2.12 ± 0.05 (Table 1). Our results confirm those from larger whale falls from the California margin measured during the sulfophilic stage, where organisms reliant on chemosynthetic carbon dominated in terms of abundance and biomass (Smith & Baco 2003). Up to 60% of the weight of some types of whale bones, such as the caudal vertebrae studied here, may be composed of lipids with abundant proteins (Smith & Baco 2003, Higgs et al. 2011). Anoxic conditions or very low O_2 levels reported within and on the surface of bones (Huusgaard et al. 2012) suggest anoxic inner bone matrices. The chemical energy of lipids and proteins may only become available through anaerobic microbial decomposition, which produces sulfides and fuels chemosynthetic primary production (Deming et al. 1997, Smith & Baco 2003, Treude et al. 2009). Chemosynthetic bacteria require a redox interface rather than deep, anaerobic inner-bone matrices, which increases their accessibility to metazoans. Given that we found no chemosymbiotic species in our whale fall, we suggest that free-living chemosynthetic bacteria (or the primary consumers of these bacteria) were the primary food source in these 2 microhabitats. These patterns are analogous to those described in diffusive areas of hydrothermal vents and seeps, where heterotrophic fauna mainly feed on free-living chemosynthetic bacteria or on primary microbial consumers (Van Dover & Fry 1994, Bergquist et al. 2007, Govenar 2012, Portail et al. 2016).

In the putative low reducing skeleton microhabitat, species that did not primarily rely on CDC (from $31.73 \pm 19.03\%$ to 0%) and with higher TPs (from 3.83 ± 0.05 to 2.6 ± 0.06), namely the nematodes, *Ophryotrocha* sp. 3, *Sirsoe* cf. *sirikos*, *Microphthalmus* sp., *Vrijenhoekia* sp. and *Osedax* sp., were more abundant (55.8% of total faunal abundance) than the consumers predominantly relying on microbial chemosynthetic production (Table 1). Even the main chemosynthetic consumer common to the 3 different skeleton microhabitats, the annelid *C. iatapiuna*, had a significantly lower CDC contribution in the putative low reducing microhabitat than in the higher one. These results agree with previous findings in other less sulfophilic organic falls, such as small whale skeletons, kelp and wood falls, where heterotrophic fauna mainly exploit whale, kelp and wood organic matter rather than CDC (Smith & Baco 2003, Bernardino et al. 2010). Furthermore, some hesionid species such as *Microphthalmus* sp. and *Vrijenhoekia*

sp., which are presumably omnivores, occurred only in the less reduced microhabitat (Alfaro-Lucas et al. 2017). A similar increase in food web complexity was reported in vent and seep environments with low fluid fluxes, where fauna benefit from more basal food sources (Portail et al. 2016). The engineering activity of the bone-eating worm *Osedax* sp. may cause food web differences by physically degrading the skeleton, potentially altering the anoxic inner bone environment (Alfaro-Lucas et al. 2017). These effects may, however, be much more dramatic in juvenile whale skeletons, such as in our study, which apparently experience greater amounts of *Osedax* bioerosion than well-calcified, lipid-rich adult skeletons (Smith et al. 2015). The effects of *Osedax* in whale fall communities thus greatly differs from the effects of the main physical ecosystem engineers in other similar habitats where the engineer species promote chemosynthesis (e.g. *Xylophaga* spp. in wood falls and vesicomysd clams in vents/seeps; Bienhold et al. 2013, Portail et al. 2016) or increase the area of influence of reduced compound fluxes and thus access to chemosynthetic food sources (Johnson et al. 1988, Govenar 2012).

Importantly, the primary chemosynthetic consumer, the dorvilleid *Ophryotrocha* sp. 1 (TP = 1.55 ± 0.28 ; CDC = 56.95 ± 11.31 to 35.94 ± 16.83), co-occurs with the congeneric *Ophryotrocha* sp. 3, which had lower percentages of CDC ($27.71 \pm 1.23\%$ to 0%) and a much higher mean TP (2.6 ± 0.06 ; Table 1), suggesting a primarily opportunist omnivore that also consumes whale organic matter. This whale-fall community harbored 8 different species of *Ophryotrocha* (Sumida et al. 2016), and the most abundant species were co-occurring sp. 1 and 3 (Alfaro-Lucas et al. 2017). Abundant and numerous species of *Ophryotrocha* usually inhabit whale-fall communities (Smith & Baco 2003, Wiklund et al. 2009, 2012, Taboada et al. 2013, Ravara et al. 2015). In cold seeps, where many species of this genus also co-occur, trophic niche partitioning may partially facilitate coexistence (Levin et al. 2003, 2013), as also hypothesized for whale falls (Thornhill et al. 2012, Ravara et al. 2015). Similarly, the hesionid *Pleijelius* sp., a higher consumer of chemosynthetic production, showed a marked difference in CDC compared to co-occurring and presumably omnivorous confamilial species *Sirsoe* cf. *sirikos*, *Microphthalmus* sp. and *Vrijenhoekia* sp. (Table 1). Our results suggest that trophic partitioning commonly occurs among these congeneric and confamilial species. However, more research is needed to determine whether trophic niche partitioning allows these species to co-exist (Connell 1980).

Our results suggest a reduced importance of chemosynthesis for sediment-dwelling species, although some species apparently relied on CDC, such as the annelids *Neanthes shinkai*, *Bathyaufvelia* sp. and *Bathykurila* cf. *guaymasensis*, and the squat lobster *Munidopsis* sp. (Table 1). This reliance is especially true for *Bathykurila* cf. *guaymasensis*, an organism known to inhabit vents and whale falls that has long been thought to rely on chemosynthetic bacteria (Glover et al. 2005; however, see Portail et al. 2016). However, the enriched $\delta^{13}\text{C}$ values of *Bathykurila* cf. *guaymasensis* (-19.7%) compared to SOM ($-20.9 \pm 0.2\%$), its high TP (3.75), and the low CDC percentage (14.94 to 0%) and TP (3.42) of *Bathyaufvelia* sp., in combination with observations of these polynoids attacking other congeneric/familial individuals in this community (see supplementary video in Sumida et al. 2016), suggest that these species prey upon lower consumers. In fact, polynoids are frequently predators in other related communities, such as vents, although with highly variable patterns of nutrition (e.g. Tunnicliffe 1991, Van Dover 2002, Govenar 2012, Portail et al. 2016). Because of these traits and given that we analyzed only one isotopic sample per polynoid species (Table 1), our results must be interpreted with caution. The low reliance of the sedimentary fauna on CDC may relate to the small size of the carcass and to the fact that it was a juvenile whale, limiting microbial chemosynthetic activity in sediments (Smith & Baco 2003). Furthermore, we estimated this carcass to be 5 to 10 yr old (Sumida et al. 2016), which is probably sufficient time for the degradation of much of the whale organic matter in sediments (Smith et al. 1998, Smith & Baco 2003). These factors likely explain reasons for the absence of chemosymbiotic fauna in this community (Smith & Baco 2003, Cunha et al. 2013). Less calcified and less lipid-rich skeletons of juvenile cetaceans may create predominantly non-chemosynthetic habitats (Smith & Baco 2003, Lundsten et al. 2010b). Although we show that the carcass did support chemosynthesis, the low whale biomass (only the hind part of the carcass with 9 vertebrae) may have created a very localized reducing environment, hindering colonization by chemosymbiotic fauna (Sumida et al. 2016), but nonetheless supporting heterotrophs.

This small whale-fall community harbored between 3 and 4 trophic levels in total, with 2 main nutritional sources at the base of the food web. These results confirm those of another study of juvenile carcass communities, which also reported 3 trophic levels rather than the 5 that are common in larger adult carcasses (Smith & Baco 2003). Whale bone consumers

occupied one end of the food web; the bone-eating worm *Osedax* sp. exploited the skeleton, as likely did *Rubyspira* sp. and nematodes from the sediments and the putative low reducing skeleton microhabitats, because all had low CDC values and TPs higher than 3 (Table 1). Heterotrophic bacteria, especially sulfate reducers, may play a key role in the development of the other 'end' of the food web as chemosynthetic bacteria (primary producers) exploit the reduced inorganic compounds generated as a by-product of their metabolism (Table 1). As reported in vents, seeps and large organic falls (Smith & Baco 2003, Portail et al. 2016), lower $\delta^{15}\text{N}$ values suggest important local sources other than the whale organic matter and water column nitrates for primary producers. *Capitella iatapiuna* and nematodes of the putative highly reducing microhabitats mainly rely on these chemosynthetic bacteria, as does *Ophryotrocha* sp. 1 in low reducing microhabitats. These organisms form a group of primary consumers of microbial chemosynthetic production with low TPs ranging from ca. 1.5 to 2.33 (Table 1). High CDC values but a TP higher than those of the aforementioned species suggest that *Pleijelius* sp. is a higher consumer that also depends on chemosynthetic carbon (Table 1). We consider species with TPs ranging from ca. 2.6 to 3 with intermediate/low CDC percentage, such as hesionids, *Neanthes shinkai* and *Munidopsis* sp., as omnivores or scavengers. The CDC values and TPs in both polynoids *Bathykurila* cf. *guaymasensis* and *Bathyfauvelia* sp. were similar to those of whale consumers such as *Osedax* sp. and *Rubyspira* sp., but our observations suggest predatory behaviors.

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