

JOAN MANEL ALFARO LUCAS

**Faunal composition and community structure of the world's deepest whale-fall
community: shedding light on the ecological role of *Osedax* (Polychaeta:
Siboglinidae) and whale-fall biogeography**

Thesis submitted to the Oceanographic
Institute of the University of São Paulo in
partial fulfillment of the requirements for
the degree of Master of Science in
Oceanography

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Oceanographic Institute

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ABSTRACT

This study aims to describe the faunal composition and community structure of the world's deepest whale fall and the first found in the South Atlantic Ocean. This discovery adds very important data on the poorly known whale-fall biogeography and provides new insights on the ecological role of the "bone-eating" worm *Osedax* (Polychaeta: Siboglinidae) on associated biota. The whale fall was serendipitously found in April 24th, 2013 at the base of São Paulo Ridge at 4204 m depth in the SW Atlantic Ocean using the HOV *Shinkai 6500* during the Brazil-Japan joint Iatá-Piúna Oceanographic Expedition on board *RV Yokosuka* (Japan Agency for Marine-Earth Science and Technology, JAMSTEC). Detailed video surveys of the carcass were made using high-definition video cameras. Vertebrae, surrounding sediments and fauna were also collected using the submersible manipulators and a slurp gun. The partial skeleton belonged to an Antarctic Minke whale, *Balaenoptera bonaerensis*, and was composed of nine caudal vertebrae, whose degradation state suggests it was on the bottom for at least 5 years. Most of the 41 invertebrate species found are new to science, with ca. 12% of the genera shared with NE Pacific whale falls, vent and seep ecosystems. This similarity strongly supports the hypothesis that whale falls act as "stepping-stones" for the dispersion of chemosynthetic fauna and that some whale-fall specialists are widespread worldwide at genus level. Detailed investigation of inner bone assemblages indicates that *Osedax* degradation deeply affects their diversity and structure. Bones colonized by *Osedax* support distinct, significantly more diverse and abundant assemblages. This renders *Osedax* the status of ecosystem engineer, creating new microhabitats and enhancing biodiversity in deep-sea whale-fall communities.

Keywords: Deep-sea – Whale fall – Stepping-stone hypothesis – *Osedax* – Biodiversity – Reducing environments.

RESUMO

O presente estudo descreve a composição faunística e a estrutura da comunidade de carcaça de baleia mais profunda do mundo e a primeira a ser encontrada no Oceano Atlântico Sul. A descoberta adiciona dados importantes à quase desconhecida biogeografia destas comunidades, assim como aumenta o conhecimento sobre o papel ecológico dos vermes-zumbis do gênero *Osedax* (Polychaeta: Siboglinidae), especialistas no consumo e degradação de ossos. A carcaça foi fortuitamente descoberta em 24 de Abril de 2013, na base da Dorsal de São Paulo a 4204 m de profundidade (Atlântico Sudoeste), utilizando-se o submersível de pesquisa tripulado *Shinkai 6500* (Agência Japonesa para o Estudo das Ciências do Mar e da Terra e Tecnologia, JAMSTEC na sigla em inglês). Os mergulhos fizeram parte da Expedição Oceanográfica Iatá-Piúna, fruto de um acordo bilateral de cooperação científica em Ciências do Mar entre Brasil e Japão. Durante dois mergulhos foram feitos vídeos detalhados da carcaça através de uma câmera de alta definição. Também foram coletadas vértebras, sedimentos e fauna por meio dos braços manipuladores e de um sugador. O esqueleto parcial pertencia a uma baleia Minke Antártica, *Balaenoptera bonaerensis*, e era composto por nove vértebras, cujo estado de degradação sugere que a baleia estava no assoalho marinho por pelo menos 5 anos. A maioria das 41 espécies de invertebrados encontradas são novas para a ciência e cerca de 12% dos gêneros são compartilhados com ambientes redutores do Pacífico Nordeste, como carcaças, fontes hidrotermais e exsudações frias. Tal semelhança corrobora fortemente a hipótese de que carcaças de baleia atuam como “pedras de dispersão” para a fauna de ambientes quimiossintetizantes e que alguns organismos especialistas de carcaças de baleia possuem distribuição mundial em nível genérico. A investigação detalhada da endofauna dos ossos indicou que a degradação promovida por *Osedax* afeta profundamente sua diversidade e estrutura. Ossos colonizados por *Osedax* sustentam assembleias de organismos significativamente mais diversas e abundantes. Isso faz com que *Osedax* seja considerado um engenheiro ecossistêmico, pois cria novos microhábitats promovendo o aumento da biodiversidade em comunidades de carcaças de baleia no mar profundo.

Palavras-chave: Mar profundo – Carcaças de baleia – Hipótese das pedras de dispersão – *Osedax* – Biodiversidade – Ambientes redutores.

CHAPTER 1

A GIFT FROM ABOVE: WHALE CARCASSES IN THE DEEP

OCEAN

The deep-sea, with an average depth of 4.2 km and an estimated surface of ~430 million km², is the largest environment on Earth (Ramirez-Llodra et al., 2010; Danovaro et al., 2014). It is an extreme environment due to several physical conditions such as low temperatures, high pressures and the complete lack of light below 1000 m depth. Because photosynthesis is restricted to the euphotic zone in the upper 200 m of the water column, the deep-sea has traditionally been considered a heterotrophic ecosystem completely dependent on imported organic matter (Ramirez-Llodra et al., 2010).

However, in 1977 this paradigm was completely challenged with the discovery of a chemosynthesis-based ecosystem in the hydrothermal vent field of the deep Galapagos Rift (Lonsdale, 1977). Soon after other communities were found in cold-water methane seeps in the Florida Escarpment in eastern Gulf of Mexico (Paull et al., 1984). These ecosystems exhibit high primary chemosynthetic productivity allowing the development of high-biomass communities, where autotrophic microorganisms are the primary producers exploiting reduced compounds as sources of energy (Van Dover, 2000). Considered places of evolutionary novelty, endemic lineages of chemosynthetic-symbiont-bearing invertebrates densely dominate these communities, forming highly distinct assemblages of organisms to the otherwise low-abundance background (Van Dover et al., 2002).

Following these discoveries, other cognate communities were found in reducing environments such as those created in dysaerobic basins (Gallardo, 1977) and large organic-falls (Smith et al., 1989). One of the latest findings took place in 1987, when researchers using the *DRSV Alvin* discovered a 20-m long whale carcass harboring a chemosynthetic community at 1240 m depth in Santa Catalina Basin (NE Pacific off California, USA) (Smith et al., 1989).

Whales are the largest species that have ever inhabited our planet. When a whale dies, usually along its migratory route, the carcass is likely to reach the deep-sea bottom rapidly and almost intact due to its large size (Smith and Baco, 2003; Smith, 2006; Smith et al., 2015). Such an organic input develops a specific and complex community consisting of at least three overlapping successional stages driven by facilitation processes (Bennett et al., 1994; Smith et al., 2002, 2015; Smith and Baco, 2003).

During the first stage, the “*mobile-scavenger stage*”, soft tissue is removed by large active necrophages during few months to some years depending on scavenging rates and carcass size (Smith and Baco, 2003). While soft tissue is being consumed, dense assemblages of invertebrates colonize both exposed bones and the organic-enriched sediment around the carcass, characterizing the “*enrichment-opportunist stage*”. Sediment macrofaunal assemblages can achieve 20.000-40.000 individuals per m², the highest macrobenthos density below 1000 m depth ever registered (Smith and Baco, 2003). Nonetheless, species diversity is not high and abundance decreases abruptly few meters from the whale carcass.

Microbial activity is highly enhanced around the carcass causing a rapid decrease in the sediment oxygen content. Assemblages of sulfur-reducing bacteria and methane-oxidizing archaea mediate the anaerobic decomposition of sediment

organic matter (Goffredi et al., 2008; Treude et al., 2009). In addition, anaerobic decomposition of bone organic compounds also occurs as microorganisms penetrate the outermost-dense bone layer to the inner anoxic bone environment (Deming et al., 1997; Treude et al., 2009). The degradation process ultimately leads to the sulfide and methane effluxes that characterize the “*sulfophilic stage*” (Smith et al., 1989; Naganuma et al., 1996; Deming et al., 1997; Goffredi et al., 2008; Treude et al., 2009).

Sulfide and methane are exploited by both free chemoautotrophic microorganisms that form mats on bones and sediments, as well as by chemosynthetic-symbiont-bearing invertebrates (Smith et al., 1989; Smith and Baco, 2003; Fujiwara et al., 2007; Goffredi et al., 2008; Treude et al., 2009; Lundsten et al., 2010a; Amon et al., 2013). This stage presents an astonishing high local species richness as a consequence of the many trophic levels found (from primary production to various levels of heterotrophy) and the variety of physical-chemical substrates (from organic-impacted soft sediments to bones) (Baco and Smith, 2003; Smith and Baco, 2003).

Smith et al. (1989) suggested that whale falls act as “stepping stones” for the dispersion of vent and seep fauna as mechanism for the colonization of new chemosynthetic habitats such as the highly distant hydrothermal vent fields. In fact, whale-fall communities do present faunal overlap with other deep-sea chemosynthetic environments, sharing species and lineages with hydrothermal vents, cold-seeps, dysaerobic basins and other organic-falls such as wood (Smith et al., 1989; Baco et al., 1999; Smith and Baco, 2003; Smith, 2006; Fujiwara et al., 2007; Lundsten et al., 2010a, b). Furthermore, this hypothesis has also evolutionary implications as some of the most abundant symbiont-bearing species evolved from shallow waters to the deep-sea probably using organic-falls in a stepping stone

fashion (Distel et al., 2000; Jones et al., 2006; Miyazaki et al., 2010; Lorion et al., 2012, 2013; Thubaut et al., 2013).

Evidence suggests that for the poorly calcified bones of juvenile whales, which have much lower lipid content, assemblages do not appear to be mainly based in chemoautotrophy (Smith and Baco, 2003). In contrast, adult cetacean vertebrae are especially rich in lipids and proteins. For instance, the skeleton of a 40-ton whale may have about 2000-3000 kg of lipids (Smith and Baco, 2003; Smith, 2006). However, the lipid content is variable among different types of bones and it is predicted that this may affect chemosynthetic fauna distribution along the whale skeleton. This is known as the "*oil-gradient hypothesis*" proposed by Higgs et al., (2011b), which states that the most oil-rich bones (e.g., skull, lumbar and caudal vertebrae) will harbor the bulk of the chemosynthetic fauna in later successional stages due to higher and longer lasting fluxes. In adult bones, the sulfophilic stage may be a long-lasting stage with predicted durations of up to 80 years (Smith, 2006).

After the slow consumption of the bone organic matter by anaerobic heterotrophic microorganisms, suspension feeders may colonize the empty bone mineral matrix taking advantage of the hard substrate. This stage is known as "*reef stage*" and it was predicted in the first ecological succession experiments, but it was only recently observed in a fossil manganese-encrusted bone at the Clarion-Clipperton Zone in the North Pacific Ocean at 4800 m depth (Smith et al., 2015).

Environmental physical-chemical conditions may affect the development of ecological successional stages and their characteristic biodiversity. For instance, the duration of the "*mobile scavenger stage*" is deeply influenced by depth, as large active deep-sea scavengers such as hagfishes and sleeper sharks inhabit shallower depths ranging from 40 to 1200 m depth (Dahlgren et al., 2006; Glover et al., 2010). In

addition, higher temperatures related to shallower waters enhance bacterial metabolic rates accelerating the “*sulfophilic stage*” (Fujiwara et al., 2007). Furthermore, environmental disturbances, such as currents, turbidity flows, organic enrichment and sedimentation rate levels, decrease with depth and have been inversely correlated with community longevity, species diversity and abundance, especially for whale-fall specialists (Braby et al., 2007; Fujiwara et al., 2007; Lundsten et al., 2010b). There is certainly a decrease of specialist taxa in shallow-water whale falls (Glover et al., 2010), as it is seen at other chemosynthetic communities, such as hydrothermal vents (Tarasov et al., 2005).

Many new species with novel adaptations have been described in deep-sea whale falls since their discovery. One of the most striking examples are the bone-eating polychaete worms of the genus *Osedax* (Rouse et al., 2004). *Osedax* males are microscopic and paedomorphic (Worsaae and Rouse, 2010), resembling siboglinid trochophora larvae, and are housed in the trunk and gelatinous tube of *Osedax* females where they form harems (Rouse et al., 2004; however see Rouse et al., 2015). Females lack a mouth and digestive system and penetrate bones using a root-like structure that erode the bone matrix using a proton pump mechanism similar to that of mammalian osteoclast cells (Tresguerres et al., 2013). Evidence suggests that *Osedax* females feed on bone collagen (Goffredi et al., 2005, 2007; Higgs et al., 2010; however see Fujikura et al., 2006) through a symbiosis with aerobic heterotrophic bacteria. These belong mainly to the genus *Oceanospirillales* and are housed inside bacteriocytes in the “root” system (Goffredi et al., 2005; Verna et al., 2010).

Osedax bone erosion rates vary among species ranging from 1 to 6% per year (Higgs et al., 2011a). However, *Osedax* do not equally degrade the entire skeleton since lipid-rich bones are more resistant to bioerosion. The whole process may take several decades in such bones and the resistance caused by lipids lead Higgs et al.,

(2011b) to formulate the “oil-protection hypothesis”. Amon et al. (2013) successfully identified these patterns and found an inverse trend between *Osedax* and bacterial mat abundance, suggesting that competitive exclusion or inappropriate environment may prevent *Osedax* growth and/or settlement on the most lipid-rich bones. Furthermore, lipid-rich bones contain less collagen, which is the main source of nutrition for *Osedax* (Goffredi et al., 2005, 2007; Higgs et al., 2010). Besides, the high lipid content supports larger numbers of microorganisms that generally make the environment more reduced (Higgs et al., 2011b). This may negatively affect *Osedax* which has a high demand for oxygen (Huusgaard et al., 2012).

The above described patterns are not evident for the less calcified and lipid-poorer juvenile whalebones, which *Osedax* is able to completely degrade in sub-decadal time scales (Braby et al., 2007; Lundsten et al., 2010b; Smith et al., 2015). For those juvenile carcasses evidence suggests that *Osedax* feeding activities truncate and shorten the sulfophilic stage, preventing the development of a “reef stage” (Braby et al., 2007; Lundsten et al., 2010b). This led some authors to state that, for juvenile whale-fall communities, bone-eating worms are foundation species that deeply diminish community longevity (Braby et al., 2007; Lundsten et al., 2010b; Smith et al., 2015). Recently, the deep-sea snail *Rubyspira* has been described and is considered another whale-fall specialist (Johnson et al., 2010). Interestingly, the only two species described thus far also base their nutrition in feeding on whalebones (Johnson et al., 2010). Although it is clear that bone-eating specialists play an important role in the overall ecology of whale-fall communities, very little is known about their effects on bone assemblages.

1.1. Thesis aims and objectives

Almost all deep-sea whale-fall studies have been undertaken in the Northeast Pacific Ocean, specifically in the California slope and Monterey Canyon (Smith et al., 1989; Smith and Baco, 2003; Goffredi et al., 2004; Smith, 2006; Braby et al., 2007; Lundsten et al., 2010a, b). In this scenario, the study of whale-fall communities in other ocean basins is necessary to fully understand the evolution, ecology and especially the poorly known biogeography of these deep-sea communities. Besides, even though many of the faunal patterns have already been described, there is still a lack of knowledge on basic ecology such as the effects of bone-eating specialists on the inner bone invertebrate assemblages.

In the present thesis I aimed to characterize the biological community of the first deep-sea whale fall found in the Atlantic Ocean. I will focus on the following specific objectives, which in turn form the chapters of this thesis. First I will identify the faunal composition, determine the age of the carcass and the successional stage of the community. Second I will further explore in more detail the bone epifaunal and infaunal assemblages patterns and the ecological role of the whale-fall specialist *Osedax*. Finally I will integrate and compare my results with the current knowledge of the previously described whale-fall communities in order to explore faunal overlaps with whale falls and other cognate communities of around the world.

1.2. Materials, methods and study site

A partial whale carcass was serendipitously found at 4204 m depth in the Southwest Atlantic Ocean during a *Shinkai 6500* deep-sea manned submersible dive in April 24, 2013. Sampling was carried out during dives 1334 and 1336. This finding is a result of the *Iatá-Piúna* Research Cruise on board *RV Yokosuka*, a collaborative scientific partnership between Brazil and Japan, which is within the framework of the around-the-world project *Quelle 2013* (Quest for the Limit of Life) of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC).

The whale fall was located ca. 700 km from the nearest land on the SE Brazilian coast in São Paulo Ridge (SPR; 28° 31.1191' S, 41° 39.4097' W) (Figure 1). The carcass was situated at the base of a very steep slope (1100 m from the top to the bottom) at 4204 m depth. The surrounding area was characterized by a small amount (only a few centimeters) of sediments overlying basaltic rocks. The area is under the influence of the Antarctic Bottom Water (AABW) (Speer and Zenk, 1993). During the sampling, salinity was 34.7 and the water temperature ranged from 0.4 to 0.6 °C.

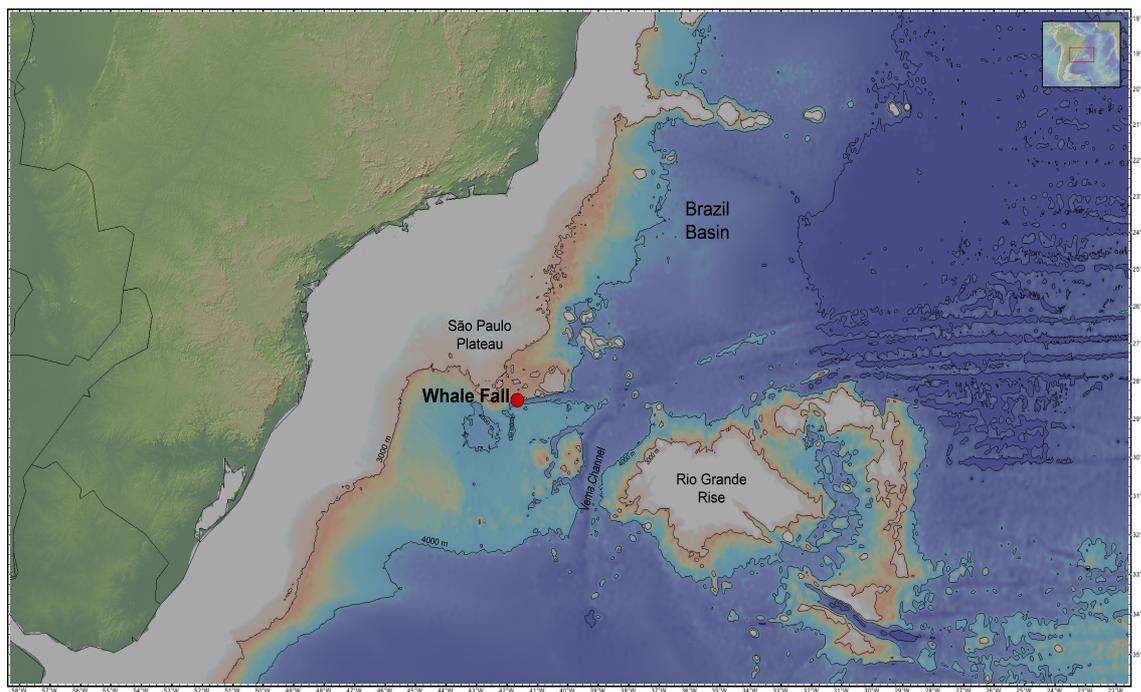


Figure 1. Whale carcass location. The whale fall was found on coordinates $28^{\circ} 31.119' S$, $41^{\circ} 39.4097' W$ (red circle) at 4204 m depth in São Paulo Ridge, SW Atlantic Ocean.

CHAPTER 2

FAUNAL COMPOSITION AND COMMUNITY STRUCTURE OF THE FIRST WHALE-FALL FOUND IN THE DEEP ATLANTIC OCEAN

2.1. Introduction

Whale carcasses in the deep ocean attract a suite of opportunistic and specialist organisms, the latter product of millions of years of evolution that go back to the whales own appearance on earth (Kiel et al., 2010) and other large ocean-going vertebrates before that (Kaim et al., 2008; Kiel, 2008; Vrijenhoek et al., 2009; Kiel et al., 2011). Whale falls can thus be considered as sources of evolutionary novelty and biodiversity in the deep-sea, since they form isles of organic enrichment in an extremely food-poor environment (Smith et al., 2015).

Finding a natural dead whale on the bottom of the ocean is indeed a rare event. During a time span of 26 years only 6 natural whale carcasses were described in the deep-sea (Figure 2). Smith et al. (1989) reported the first time scientists stumbled upon such a habitat back in 1987. Since then most records were from the Pacific Ocean (Naganuma et al., 1996; Smith and Baco, 2003; Goffredi et al., 2004; Lundsten et al., 2010a). Only in 2013 Amon et al. (2013) reported a whale carcass from the Southern Ocean.

During the past few years the knowledge of these communities has increased notoriously. The advancement in the understanding of these poorly known

communities has been mainly due to time-series studies of artificially implanted whale carcasses on the seafloor (Smith and Baco, 2003; Dahlgren et al., 2006; Braby et al., 2007; Fujiwara et al., 2007; Glover et al., 2010; Lundsten et al., 2010b) (Figure 2). Almost all natural and implanted whale-fall community studies are from the deep Northeast Pacific Ocean, specifically from the California slope and Monterey Bay Canyon (Smith et al., 1989; Smith and Baco, 2003; Goffredi et al., 2004; Braby et al., 2007; Lundsten et al., 2010a, 2010b) (Figure 2). The paucity of studies on natural carcasses as well as the scarce data available outside the Northeast Pacific may be hiding relevant information on the biogeography and evolution of both whale-fall fauna and other related chemosynthetic communities.

In this scenario the lack of knowledge of these communities in the Atlantic Ocean is evident. The Atlantic Ocean serves as habitats and migration routes for a number of cetacean species. It also harbors chemosynthetic-based communities, such as vents and cold seeps (Ondréas et al., 2005; Melchert et al., 2008). Therefore, it is hypothesized that Atlantic whale-fall habitats are likely to be historically relevant as they are in the Pacific Ocean (Olu et al., 2010).

The present chapter describes the ecology of the first natural whale-fall community of the deep Atlantic Ocean discovered by the *HOV Shinkai 6500*. This is also deepest whale carcass community described to date worldwide (4204 m depth). Here I present the results of the video surveys and the morphological identification of the fauna. I also discuss the age of the whale and its successional stage.

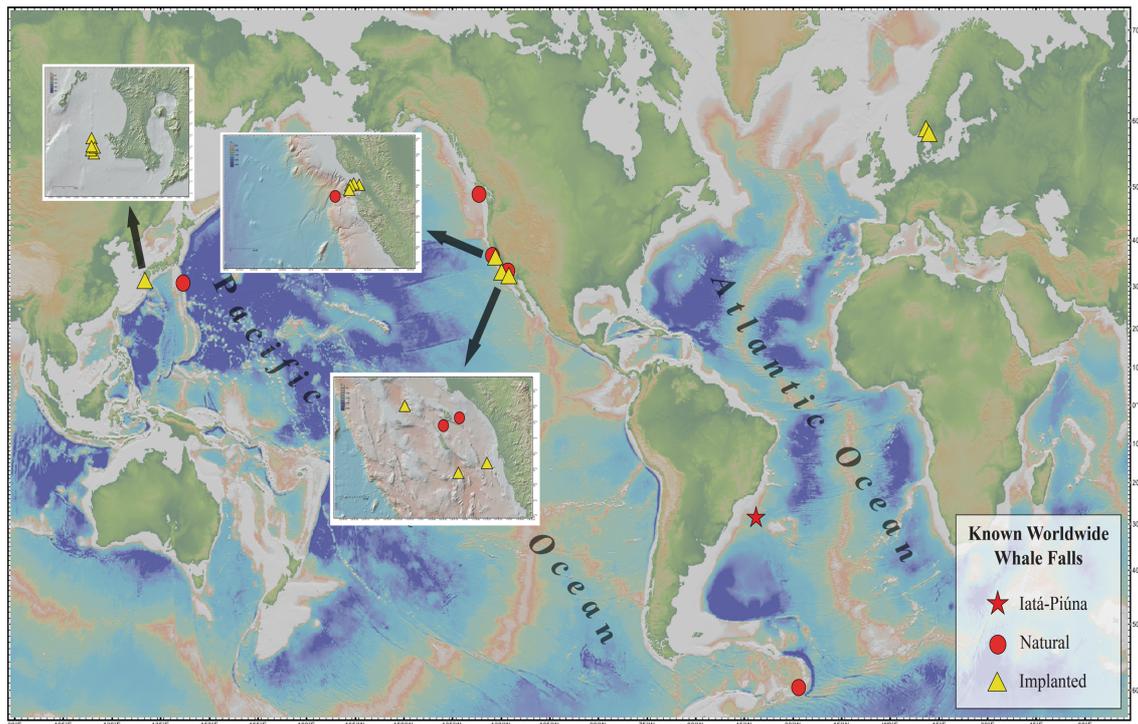


Figure 2. Map of deep-sea whale-fall communities in the World's oceans. Known deep-sea natural (red circles) and implanted (yellow triangles) whale carcasses. The available knowledge of Atlantic whale falls thus far is from experiments in relative shallow waters in the North Sea (Dahlgren et al., 2006; Glover et al., 2010). The carcass studied in the present thesis is highlighted with a red star and named with the project name.

2.2. Materials and methods

Detailed video surveys were made during 2 *Shinkai 6500* dives, including whole community surveys and fauna close-ups. All epifaunal organisms larger than ca. 5 mm were identified and counted in videos. Videos were also used to verify faunal distribution on the sediments and whale skeleton.

After video surveys, whalebones were collected using the submersible manipulators and the fauna surrounding the area was retrieved using a slurp gun and maintained in local cold seawater during submersible ascent. Upon arrival on

deck, bones were immediately transferred to a cold room at a constant temperature of 1°C. Bone and sediment epifauna and infauna were sorted manually and under stereomicroscope. Samples were taken for morphological identification and molecular analyses (99.5% non-denatured ethanol and deep-frozen at -196°C, respectively). Remaining bones and sediments were preserved in 99.5% ethanol for further sorting at the Benthic Dynamics Lab of the Oceanographic Institute of the University of São Paulo.

2.3. Results

Whale carcass description

Mitochondrial COI analysis revealed that the carcass was from an Antarctic minke whale (*Balaenoptera bonaerensis*) (99% identity, Y. Fujiwara pers. comm., Japan Agency for Marine-Earth Science and Technology). The carcass was composed of nine small vertebrae, seven of which were standing side by side. Among those, five vertebrae were still joined by intervertebral discs (vertebrae 1-5) (Figure 3). Additionally, five intervertebral discs were scattered around the skeleton. No soft tissues were present in the bones, which were all exposed to the overlying water (i.e., not covered by sediment). All vertebrae were similar in shape and dimensions (ca. 11.5 cm in diameter), presumably belonging to the caudal portion of the animal (M.C.O. Santos pers. comm., University of São Paulo). The sediment underneath bones and discs was dark in color suggesting iron sulfide formation, highlighting bacterial sulfur-reducing activity and anoxia.

Vertebral processes of vertebrae 1-7 were slightly leaning to the right of the animal and were in contact with the substratum. Those were more preserved than

the left side processes that were away from the sediment and very degraded (eroded bone matrix). Although vertebrae 6 and 7 were still joined together, they were not joined to the other bones by intervertebral discs (Figure 3A). Both of them had their left vertebral processes completely degraded. The right vertebral process of vertebra 7 was also completely degraded. Vertebrae 8 and 9 were lying on their sides. The former did not present vertebral processes, whereas vertebra 9 was highly degraded and only some parts of the outer surface of the bone remained.

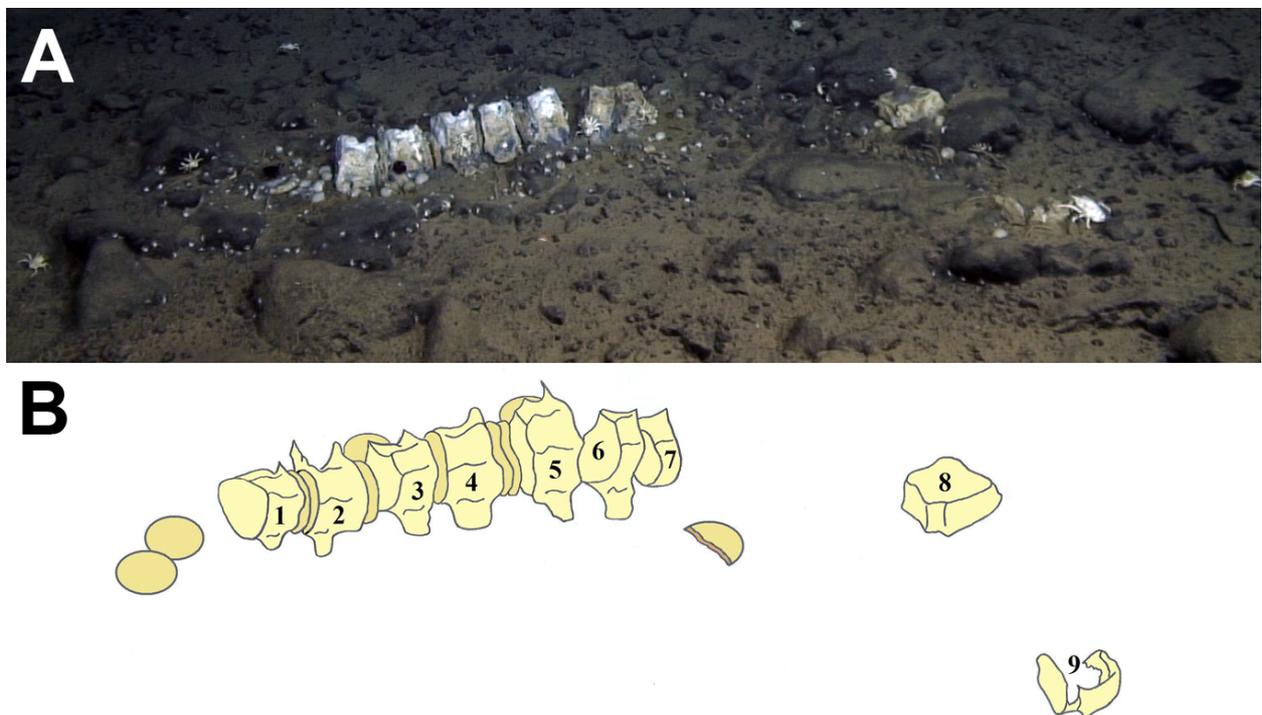


Figure 3. Whale-fall community found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean. A: Partial Antarctic minke whale skeleton found at 4204 m in the Southwest Atlantic Ocean with manned submersible Shinkai 6500. B: Schematic view of the whale skeleton reconstructed from Shinkai 6500 videos. The nine vertebrae are numbered and shown in pale yellow color, while the round intervertebral discs are darker. Vertebrae were numbered from the posterior end of the animal towards the head.

Qualitative and quantitative analysis of the macrofauna assemblage and species distribution

Five phyla were recovered from the study area comprising at least 41 species (Table 1). Nematoda occurred in large numbers both inside bones and in the surrounding sediments and may be composed of more than one species. However nematodes will be treated in detail in the next chapter.

Polychaeta was the most speciose taxon on all substrates (i.e., whale bones and soft sediments), with at least 28 species ($\approx 68\%$), most of which are probably new to science. Among these, was a new species *Osedax* (Figs 4C, D). At least eight morphotypes of the dorvilleid *Ophryotrocha* and a new species of *Capitella* (*Capitella iatapiuna* (Silva et al., *in press*)) boring into the bones were found during the present study, with the latter also found inhabiting the surrounding sediments. Three species of polynoid polychaetes, indistinguishable in video analyses, occurred on the surface of bones and sediments (Figs 4E, 5H), with a higher abundance on the former. Interestingly, antagonistic behavior could be observed in videos, where two polynoids were fighting, possibly for space or food resources. Five species of Hesionidae (*Hesiocaeca* sp. nov., *Microphthalmus* sp. nov., *Pleijelius* sp. nov. 1 and 2 and *Vrijenhoekia* sp. nov.) (Fig. 5I) and two species of cirratulids (*Raphidrilus* and *Tharyx*) were also present in both sediment and bones, except for both species of *Pleijelius* and *Raphidrilus*, which were only found on bones. Another important species occurring in bones was the chrysopetalid *Vigtorniella*.

Some polychaetes were found exclusively in sediments surrounding the bones. Among them, one species of ampharetid resembling the genus *Grassleia* (Figs 4C, D, 5G) and a new species of eyeless nereid from the genus *Neanthes* were abundant (Figs 4F, 5F). Ampharetids dwelt in tubes that were widespread in sediments close to the bones and were only less abundant in videos than *Osedax* sp.

nov. (Table 1) (Figs 4C, D). *Neanthes* sp. nov. could not be counted in videos, however it was observed in videos in the anoxic sediment under bones and intervertebral discs coming out the sediment and climbing the bones, without totally leaving its burrow (Fig. 4F).

Table 1. *Species collected at the SW Atlantic whale fall site at 4204 m depth. Each species is assigned to a location within the habitat. * Only for organisms that could be counted in videos. **Includes eight different species. *** Includes all three polynoid species collected. n.d. = not determined.*

Phylum	Class	Order	Family	Species or tag name	Number of Individuals *	Location
Cnidaria	Anthozoa	Actiniaria	n.d.	Cnidaria sp.	n.d.	Rocks
Annelida	Polychaeta	Aciculata	Dorvilleidae	<i>Ophryotrocha</i> spp.**	n.d.	Bone/Sediment
Annelida	Polychaeta	Aciculata	Nereididae	<i>Neanthes</i> sp. nov.	n.d.	Sediment
Annelida	Polychaeta	Canalipalpata	Ampharetidae	cf. <i>Grassleia</i> sp.	40	Sediment
Annelida	Polychaeta	Canalipalpata	Chaetopteridae	<i>Spiochaetopterus</i> sp.	n.d.	Sediment
Annelida	Polychaeta	Canalipalpata	Cirratulidae	<i>Raphidrilus</i> sp.	n.d.	Bone
Annelida	Polychaeta	Canalipalpata	Cirratulidae	<i>Tharyx</i> sp.	n.d.	Sediment
Annelida	Polychaeta	Canalipalpata	Spionidae	<i>Lindaspio</i> sp. nov.	n.d.	Bone
Annelida	Polychaeta	Canalipalpata	Spionidae	<i>Prionospio</i> sp.	n.d.	Sediment
Annelida	Polychaeta	Capitellida	Capitellidae	<i>Capitella iatapiuna</i>	n.d.	Bone/Sediment

Annelida	Polychaeta	Phyllodocida	Hesionidae	<i>Hesioacaeca</i> sp. nov.	n.d.	Bone/Sediment
Annelida	Polychaeta	Phyllodocida	Hesionidae	<i>Microphthalmus</i> sp. nov.	n.d.	Bone/Sediment
Annelida	Polychaeta	Phyllodocida	Hesionidae	<i>Pleijelius</i> sp. nov.1	n.d.	Bone
Annelida	Polychaeta	Phyllodocida	Hesionidae	<i>Pleijelius</i> sp. nov.2	n.d.	Bone
Annelida	Polychaeta	Phyllodocida	Hesionidae	<i>Vrijenhoekia</i> sp. nov.	n.d.	Bone/Sediment
Annelida	Polychaeta	Phyllodocida	Polynoidae	Polynoidae	18***	Bone/sediment
Annelida	Polychaeta	Phyllodocida	Polynoidae	Polynoidae sp.	n.d.	Bone/sediment
Annelida	Polychaeta	Phyllodocida	Polynoidae	<i>Bathykurila</i> sp.	n.d.	Bone/sediment
Annelida	Polychaeta	Phyllodocida	Polynoidae	<i>Bathyfauvelia</i> sp.	n.d.	Bone/sediment
Annelida	Polychaeta	Phyllodocida	Sigalionidae	Sigalionidae	n.d.	Sediment
Annelida	Polychaeta	Phyllodocida	Sphaerodoridae	<i>Sphaerodoropsis</i> sp. nov.	n.d.	Sediment
Annelida	Polychaeta	Phyllodocida	Chrysopetalida e	<i>Vigtorniella</i> sp.	n.d.	Bone
Annelida	Polychaeta	Sabellida	Siboglinidae	<i>Osedax</i> sp. nov.	98	Bone
Arthropoda	Malacostraca	Amphipoda	Uristidae	<i>Stephonyx</i> sp.	17	Bone/sediment
Arthropoda	Malacostraca	Isopoda	n.d.	<i>Epicaridea</i> sp.	n.d.	Sediment
Arthropoda	Malacostraca	Decapoda	Munidopsidae	<i>Munidopsis</i> spp.	295	Bone/sediment
Arthropoda	Maxillopoda	n.d.	n.d.	Copepoda sp.1	n.d.	Parasitic on <i>Osedax</i>
Arthropoda	Maxillopoda	Harpacticoida	n.d.	Copepoda sp.2	n.d.	Bone
Arthropoda	Maxillopoda	Cyclopoida	n.d.	Copepoda sp.3	n.d.	Bone
Nematoda	Chromadore a	Monhysterida	Xyalidae	<i>Theristus</i> sp.	n.d.	Bone
Mollusca	Bivalvia	Nuculanoida	Mallettiidae	<i>Malletia</i> sp.	n.d.	Sediment

Mollusca	Gastropoda	unassigned	unassigned	<i>Rubyspira</i> sp. nov.	52	Sediment
Mollusca	Gastropoda	Neogastropoda	Raphitomidae	Raphitomid sp.	20	Bone epifaunal
Echinodermata	Echinoidea	indet.	indet.	Echinoidea sp.	2	Bone epifaunal
Echinodermata	Ophiuroidea	indet.	indet.	Ophiuroidea sp.	n.d.	Sediment

Among mollusks, a new species of the gastropod *Rubyspira* was present in large numbers (Figs 4B, D, F, 5D) and individuals were quite large in size, attaining up to 3-4 cm in length. Another gastropod was a small species of the family Raphitomidae found on the surface of bones (Fig. 4B). Several large empty shells of *Rubyspira* were found around the skeleton, but no empty shells of the small raphitomid gastropod.

Seven species of crustaceans occurred on bones and surrounding sediments (Table 1), including a species of copepod parasitic on *Osedax*. *Munidopsis* spp. were found in large numbers being widely distributed up to 1 m away from the carcass (Figs 3, 4E, 5B). We found two morphotypes of *Munidopsis*, one large and one small, which probably represent different species. A total of 295 individuals of both species were counted in videos (Table 1) and observations suggest these organisms feed on bacterial mats. However, some of the galatheid crabs were also seen processing sediments in their mouthparts. One ovigerous female of the large morphotype was collected, which suggests that at least one of the galatheid species is reproducing on site. The amphipod *Stephonix* sp. (Figs 4D, 5C) occurred mainly on bones, frequently coming in and out of cracks and holes in degraded areas probably produced by *Osedax*.

A small species of anemone (polyps ca. 1-2 mm in diameter) was observed forming extensive carpets of thousands of polyps on rocks around the carcass (Figs 4B, 5A). It was probably the most abundant epifaunal organism, with close-up photographs suggesting a density of ca. 10 ind. cm⁻². However, these anemones could not be counted since they could not be resolved in video analyses due to their small size. This anemone was not observed on rocks far from bones.

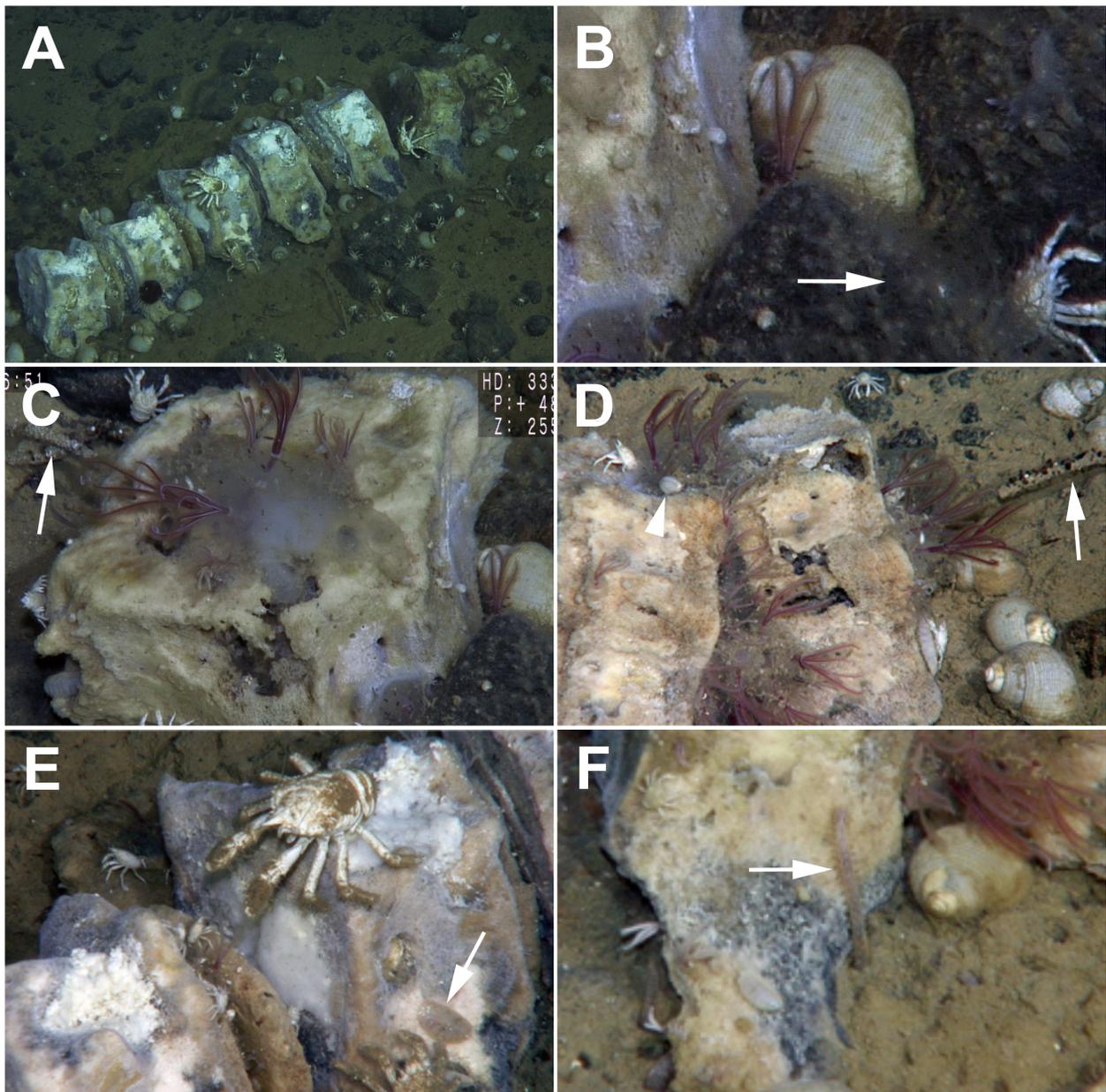


Figure 4. Details of the whale-fall community collected at the 4204 m depth whale fall in the São Paulo Ridge, Southwest Atlantic Ocean. **A.** General view of the SW Atlantic whale carcass vertebrae 1-7. Note the abundant fauna and the bacterial mats in bones 1-5. A dark echinoid can be seen on top of vertebra 2; **B.** Black rocks around the whale fall were heavily colonized by cnidarians (arrow) forming dense carpets (up to 10 ind. cm⁻²). The large gastropod *Rubyspira* sp. nov. lies behind the *Osedax* sp. nov. palps and the small unidentified raphitomid gastropods are attached to the bone; **C.** Red palps and gelatinous tube of several *Osedax* sp. nov. Note also the ampharetid polychaete tubes (arrow); **D.** Clusters of *Osedax* sp. nov. On the surrounding sediment, *Rubyspira* sp. nov. and a tube of an ampharetid polychaete (arrow). A small amphipod lies on the top of the bone (arrowhead); **E.** Dense bacterial mats covering the vertebrae. Here we can see the small and the large *Munidopsis* and a polynoid polychaete (arrow); **F.** *Neanthes* sp. nov. climbing the bone surface (arrow).

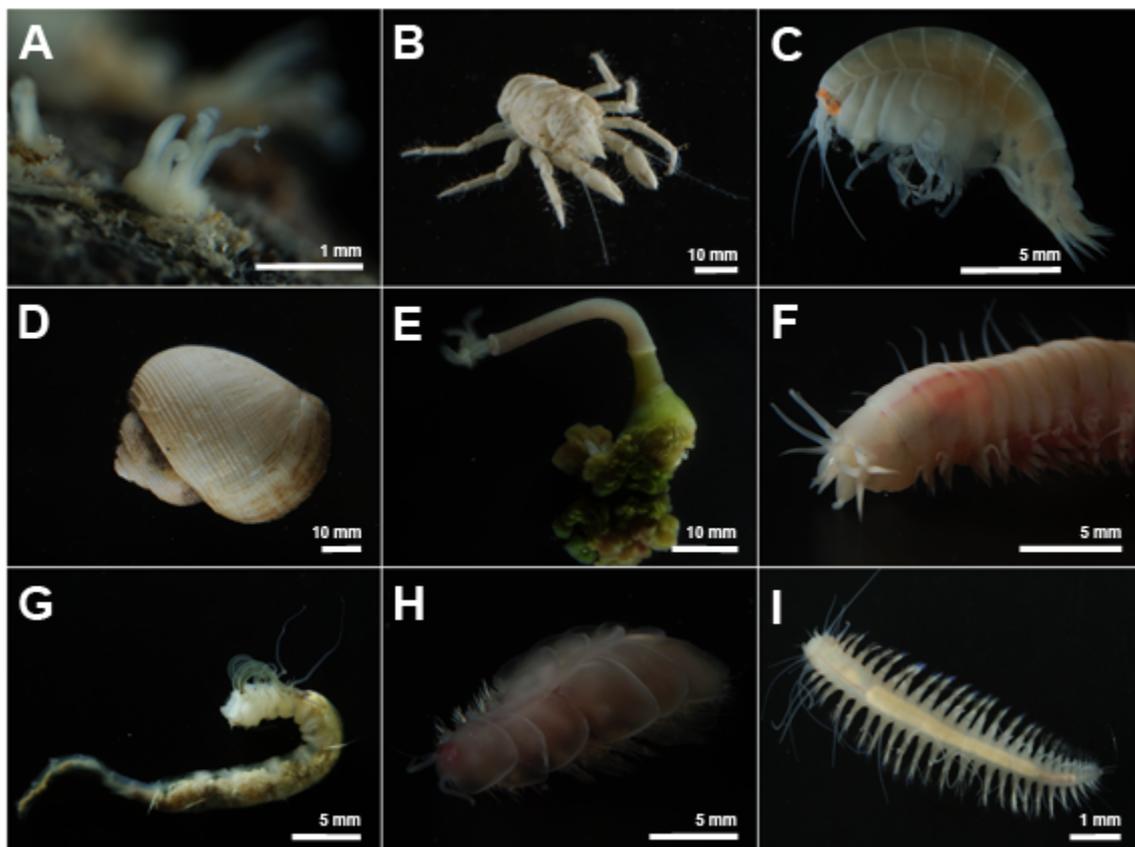


Figure 5. *Some of the most abundant organisms collected at the 4204 m depth whale fall in the São Paulo Ridge, Southwest Atlantic Ocean. A) Unidentified sea anemone inhabiting the rocks surrounding the whale skeleton; B) Large Munidopsis sp.; C) The amphipod Stephonix sp.; D) Rubyspira sp. nov.; E) Osedax sp. nov.; F) Neanthes sp. nov.; G) cf. Grassleia sp.; H) Bathykurila sp. nov.; I) Vrijenhoekia sp. nov.*

2.4. Discussion

Age of the whale-fall community

It is important to stress that community characteristics change accordingly to whale fall successional stages and that carcass persistence times vary according to the age of the whale and habitat attributes (Smith et al., 2002, 2015; Smith and Baco, 2003; Lundsten et al., 2010b).

The small vertebrae size and some typical juvenile characteristics, such as intervertebral discs not fused to vertebrae, relatively low calcification degree and lipid content (vertebrae not completely filled by lipids), suggest that this carcass belonged to a juvenile whale. The community composition was probably composed of organisms typical of two stages, i.e., the enrichment opportunist and sulfophilic stages (sensu Smith et al., 2002). However, it is also important to mention that the carcass belonged to a small whale and only the hind part of it was found, which it may be insufficient to create an intense sulfophilic stage for a long period (Smith and Baco, 2003; Lundsten et al., 2010b; Smith et al., 2015).

Although studies in the Californian slope predicted whale-fall community ages for adult whale carcasses of about 80 years (Smith, 2006), other studies observed that complete degradation of juvenile whale-fall might occur in less than

10 years (Braby et al., 2007; Lundsten et al., 2010b). However, Braby et al. (2007) and Lundsten et al. (2010b) studies were done along a deep-sea canyon, where physical and biological conditions may differ from the surround deep-sea. Physical characteristics, for instance, may play an important role on the degradation state of whale falls. High hydrostatic pressure and low temperatures may hinder carcass decomposition by limiting microbial activity (Goffredi et al., 2004; Fujiwara et al., 2007). It may also limit the bathymetric distribution of mobile scavengers (e.g., hagfishes, sleeper sharks) and therefore influence the duration of early successional stages (Dahlgren et al., 2006; Glover et al., 2010).

As we go deeper, physical disturbances decrease, which seems to facilitate increases in species diversity (Braby et al., 2007; Lundsten et al., 2010b) and the presence of bone specialists along time (Glover et al., 2010; Lundsten et al., 2010b). This has deep implications as some bone specialists, such as *Osedax* and *Rubyspira*, may limit the duration of the community through the degradation of bones (Braby et al., 2007; Johnson et al., 2010; Lundsten et al., 2010b). For instance, bone erosion rates of small *Osedax* species are on the order of 1-6% per year (Higgs et al., 2010, 2011a), but it can be higher depending on the abundance and species identity (Higgs et al., 2011a). Assuming that the relatively large *Osedax* sp. nov. degrades at least at a rate of 6% per year and taking into account the most degraded vertebra (vertebra 9, see above), I roughly estimate that *Osedax* colonization began at 7 years before we found the carcass. This estimate is also corroborated by the presence of large individuals of the genus *Rubyspira*, which seems to need the presence of *Osedax* to facilitate access to the bones and it was only reported 2 and 5 years after in a recently arrived and an implanted carcass, respectively (Johnson et al., 2010). In addition, taking into account the physical-chemical characteristics of the study area (4204 m; 0.4-0.6°C), the age of the whale (juvenile) and the community fauna composition (large *Osedax* sp. nov.

and *Rubyspira* sp. nov.) and its successional stage (mainly in a sulfophilic stage), I conservatively estimate that this whale-fall community is at least 5 years old.

Whale-fall fauna and successional stage

The whale-fall fauna obtained in the present study are mostly new to science. Many of the collected genera are commonly found in reducing environments and their bathymetric and latitudinal ranges were considerably expanded by the present work. To the best of my knowledge, the specialist whale-fall gastropod *Rubyspira* sp. nov., the polynoid polychaetes *Bathyfauvelia* sp. and *Bathykurila* sp., the ampharetid polychaete *Grassleia* sp. and the hesionid polychaete *Vrijenhoekia* sp. nov. comprise genera with distribution hitherto restricted to the Pacific (Glover et al., 2005a; Pleijel et al., 2008; Johnson et al., 2010; Reuscher et al., 2012). The genus *Grassleia*, for instance, occurs only in NE Pacific hydrothermal vents and seeps (Reuscher et al., 2012), whereas the genus *Bathykurila* occurs in NE Pacific hydrothermal vents and whale falls (Glover et al., 2005). Furthermore, for the genera *Rubyspira* and *Vrijenhoekia* this is the first record anywhere outside the Monterey canyon. They also represent only the third and second species found in these genera, respectively, and are the deepest living ones expanding their bathymetric ranges by more than 1300 m (Johnson et al., 2010; Reuscher et al., 2012). The polynoid polychaete *Bathyfauvelia* is also registered for the first time on a chemosynthetic-related habitat in the present work. Other genera collected here were also found in other cognate communities in the Atlantic Ocean. This is the case of the new the hesionid polychaetes *Hesiocaeca* sp. nov. and *Pleijelius* sp. nov. 1 and 2 and the spionid polychaete *Lindaspio* sp. nov. These genera were previously recorded in a NW Atlantic methane hydrate (Desbruyères and Toulmond, 1998), a NW Atlantic wood-fall experiment (Salazar-

Vallejo and Orensanz, 2006) and in a SE Atlantic oil field (Bellan et al., 2003), respectively.

Many organisms found in the present study were more similar to those found in other carcasses. In general, polychaetes were the dominant fauna similar to what was registered in other whale fall communities (Bennett et al., 1994; Smith and Baco, 2003; Goffredi et al., 2004; Braby et al., 2007). One of these polychaetes was *Osedax* sp. nov. (Figure 5E), which is the deepest living species of this genus found to date, increasing the genus bathymetric range by more than 1300 m (Goffredi et al., 2004; Rouse et al., 2004). This is also the first record of *Osedax* for the deep Atlantic Ocean, since *O. mucofloris* was found only in shallow waters of the North Sea (Glover et al., 2005b). The dorvilleid genus *Ophryotrocha* were composed of 8 morphotypes in the present study. This genus is usually abundant during both the enrichment-opportunistic and sulfophilic stages (Bennett et al., 1994; Smith and Baco, 2003; Dahlgren et al., 2006; Wiklund et al., 2009a), as are the crysopetalid polychaete *Vigtoriniella* (Dahlgren et al., 2006; Wiklund et al., 2009b), and the galatheid *Munidopsis* and amphipods (Bennett et al., 1994; Naganuma et al., 1996; Smith and Baco, 2003; Goffredi et al., 2004; Braby et al., 2007; Lundsten et al., 2010b).

Interestingly, this is the first and second report for the typical shallow-water inhabitants genus *Microphthalmus* and *Capitella* in a deep-sea whale-fall community, respectively (Amon et al., *in press*). *Capitella*, for instance, is commonly found in organic-rich environments and it was traditionally considered a major taxonomic difference between deep-sea whale falls and their shallow-water counterparts (Smith et al., 2015).

Except for a possible reliance on symbiotic bacteria by *Rubyspira* sp. nov. (Johnson et al., 2010), no chemoautotrophic-symbiotic-bearing organisms have been

found. This may be related to the small size of the carcass. A span of less than 2 meters run from vertebrae 1 to 9, meaning that not much organic matter impacted the seafloor. This could explain the absence of bacterial mats on surrounding sediments (sulfide-impacted areas were only observed below bones). Moreover, the carcass belonged to a juvenile whale (see discussion above), which is not thought to support a predominantly chemoautotrophic community but only a sulfide tolerant one (Smith and Baco, 2003; Lundsten et al., 2010b). Furthermore, colonization by chemoautotrophic fauna groups is normally delayed in relation to other fauna (Feldman et al., 1998; Braby et al., 2007; Lundsten et al., 2010b). Despite this and owing to the numerous organisms typical of chemosynthetic habitats, i.e, white dense bacterial mats, *Ophryotrocha* spp., *Bathykurila* sp., *Grassleia* sp., *Vrijenohekia* sp. nov., *Hesiocaeca* sp. nov., *Vigtorniella* sp. and *Lindaspio* sp. nov., we conclude that this whale-fall community was mainly in a sulfophilic stage when discovered.

CHAPTER 3

THE DARK SIDE OF DEEP-SEA WHALE-FALL COMMUNITIES: RICH BONE-HIDDEN ASSEMBLAGES AND THE ECOLOGICAL ROLE OF *OSEDAX*

3.1. Introduction

The arrival of a whale carcass in the deep-sea attract diverse assemblages of generalist and specialist whale-fall fauna, which exploit these massive organic inputs differently in space and time (Bennett et al., 1994; Smith et al., 1998, 2002, 2015; Smith and Baco, 2003; Fujiwara et al., 2007; Lundsten et al., 2010b). Thus, for such a food-poor environment these carcasses constitute remarkable and complex oases of life and are considered sources of evolutionary novelty (Baco and Smith, 2003; Smith et al., 2015).

Whale falls are significant intense organic inputs locally and the arrival of a 40-t whale carcass on the seafloor represents ~2000 years worth of phytodetritus (Smith and Baco, 2003). However, while soft tissues are rapidly incorporated into the deep-sea food webs (from several months to few years), skeletons are much more difficult to exploit due to the mineral matrix of apatite (Smith and Baco, 2003; Braby et al., 2007; Fujiwara et al., 2007; Lundsten et al., 2010b). Nonetheless, whalebones are extremely valuable resources that have enormous amounts of high-energy compounds, such as proteins and lipids (Higgs et al., 2011b).

Anaerobic sulfate reducing bacteria degrade the inner bone organic matter for even longer periods than those on impacted sediments around the carcass. The

degradation inside bones occurs at slow rates and produces effluxes of reduced compounds (Deming et al., 1997; Goffredi et al., 2008; Treude et al., 2009; Goffredi and Orphan, 2010; Smith et al., 2015). These compounds are exploited by chemosynthetic-symbiont-bearing fauna common in other cognate communities, which use these island-like habitats as stepping-stones for their dispersion (Smith et al., 1989; Tunnicliffe and Juniper, 1990; Feldman et al., 1998; Smith and Baco, 2003; Smith, 2006; Fujiwara et al., 2007; Lundsten et al., 2010a; Smith et al., 2015). Furthermore, whale skeletons represent hard physical structures in an otherwise soft-bottom background and coupled with diverse trophic pathways (i.e., from heterotrophy to chemosynthesis), which make them one of the most species-rich hard substrates in the deep-sea (Smith and Baco, 2003).

Although sulfate-reducing bacteria are key for the availability of the bone inner resources, some specialist metazoan taxa present unique adaptations to exploit them directly. One of the most striking examples is the “bone-eating” siboglinid genus *Osedax* (Rouse et al., 2004). *Osedax* males are microscopic and paedomorphic resembling trocophore larvae (Worsaae and Rouse, 2010) and are housed in the trunk and gelatinous tube of macroscopic *Osedax* females (Rouse et al., 2004). Females bore into bones using a root-like structure that erode the mineral matrix by an acidification process (Rouse et al., 2004; Tresguerres et al., 2013). Lacking a digestive system evidence suggest that *Osedax* feeds on bone collagen (Goffredi et al. 2005; Goffredi et al. 2007; Higgs et al. 2010; however see Fujikura et al. 2006) through a symbiosis with heterotrophic bacteria (Rouse et al., 2004).

Since the discovery of the genus, 31 new putative *Osedax* species have been found in all oceans ranging from 21 to 4204 m depth (Smith et al., 2015). This highlights their main role in the cycling of the largest organic inputs that reach the deep-sea worldwide. Bone-eating worms are able to completely degrade juvenile

whale carcasses in sub-decadal time scales (Lundsten et al., 2010b). Even for the highly calcified and lipid-rich adult whale skeletons, which could last up to ~80 years on the seafloor (Smith, 2006), *Osedax* is able to feed on them causing evident degradation (Smith et al., 2015).

Strikingly, apart from the bone degradation process itself, very little is known about the ecological effects of *Osedax* on bone assemblages (Higgs et al., 2010, 2011a). Evidence suggests that *Osedax* degradation deeply affects ecological community succession, shortening and truncating the sulfophilic stage in juvenile whale carcasses but not in adult ones, despite causing evident erosion (Braby et al., 2007; Lundsten et al., 2010b; Higgs et al., 2011b; Smith et al., 2015). However, there is no available information of how *Osedax* affects faunal assemblages and biodiversity by degrading bones, especially for the poorly known bone infaunal assemblages.

In this chapter I aim to describe the epifaunal and infaunal community structure along the skeleton of a natural juvenile whale carcass in relation to *Osedax* presence in order to shed light on the bone ecological succession process. The contrasting faunal patterns found, with *Osedax* colonized and non-colonized vertebrae, allowed me to specifically explore: (a) whether bones colonized by *Osedax* present different epifaunal and infaunal assemblages and (b) how their different biodiversity components, i.e., species richness and abundance, are affected by the *Osedax* degradation process.

3.2. Methods

Field collection and epifaunal assemblage analyses

Only nine caudal vertebrae formed the carcass and five of them were still together and joined by intervertebral discs. Interestingly, no *Osedax* colonized the 5 joined vertebrae, but they were abundant on the remaining 4 (Figures 4C, D). Detailed video surveys were made during two dives (dives 1334 and 1336) of the HOV *Shinkai 6500*, including whole community surveys and fauna close-ups. All epifaunal organisms larger than ca. 5 mm were identified and counted in videos. Videos were also used to verify faunal distribution patterns along the nine vertebrae. Only video footage taken prior to the sampling of vertebrae and sediments were used in video analyses to guarantee community was not disturbed. Images were processed using the computer program ImageJ (Schneider et al., 2012).

Vertebrae processing and infaunal assemblage analyses

Vertebrae were collected using *Shinkai 6500* manipulators. Vertebrae not colonized by *Osedax* were individually preserved in 99.5% ethanol. Colonized vertebrae were maintained in cold seawater (ca. 1°C) and sorted under stereomicroscope to remove *Osedax* within 2 days of collection on board R/V *Yokosuka* (JAMSTEC). After the sorting process all vertebrae were also preserved in 99.5% ethanol.

Four out of five vertebrae not colonized by *Osedax* (vertebrae 1, 2, 3 and 5, see Figure 6 and Table 2) were sorted for infaunal assemblage analyses. In order to obtain reliable quantitative data while considering microenvironments five rectangular pieces were removed from each of these four vertebrae using a saw.

Pieces were taken from: (a) the superior part of the vertebrae (part in contact with seawater and colonized by dense white bacterial mats); (b) the inferior part of the vertebrae (blackened part in contact with or partially buried in sediment); (c) the two lateral bone faces (parts between superior and inferior parts); and (d) the innermost part of the bones (i.e., the innermost bone matrix). The volume of each bone piece was measured (Table 2). Similarly, three out of four vertebrae colonized by *Osedax* (vertebrae 6, 7 and 8, see Figure 6 and Table 2) were also sorted for infaunal assemblage analyses. However, since these vertebrae did not exhibit evident microenvironments and were previously sorted for *Osedax* on board *R/V Yokosuka*, we could not clearly identify the different parts of the bones as done above for *Osedax* uncolonized bones. Consequently, it was not possible to repeat the same sampling scheme detailed above. Instead, random-chosen pieces were sorted and their volumes measured (Table 2). Organisms inhabiting bone matrices were removed under stereomicroscope and identified to the lowest possible taxonomic level or morphotyped when species identification was not possible.

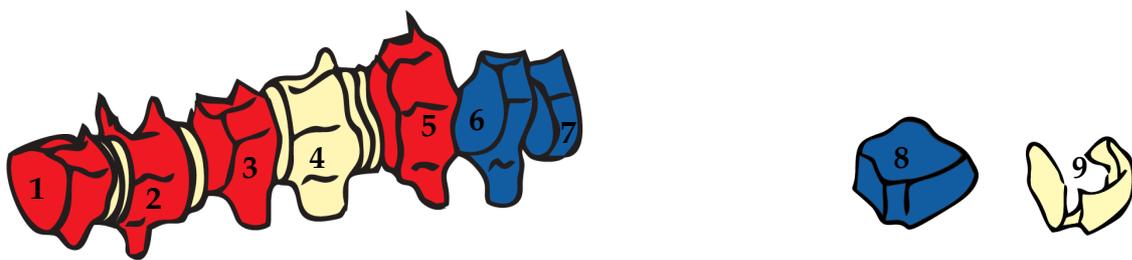


Figure 6. Scheme of the whale skeleton found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean. Red (non-colonized) and blue (colonized by Osedax) vertebrae were

sorted for infaunal assemblages analyses. Vertebrae and intervertebral disks shown in pale yellow were not used.

Table 2. Volume of different pieces of vertebra sorted for infaunal assemblage analyses.

Vertebra	Sample part	Dive	Sample ID	Volume (cm ³)
1	Superior part	1334	1334-103 1 Superior	40.0
1	Lateral part	1334	1334-103 1 Lateral	22.5
1	Inferior part	1334	1334-103 1 Inferior	40.0
1	Interior part	1334	1334-103 1 Interior	10.0
2	Superior part	1334	1334-103 2 Superior	55.0
2	Lateral part	1334	1334-103 2 Lateral	24.5
2	Inferior part	1334	1334-103 2 Inferior	70.0
2	Interior part	1334	1334-103 2 Interior	15.0
3	Superior part	1336	1336-103 3 Superior	37.5
3	Lateral part	1336	1336-103 3 Lateral	17.5
3	Inferior part	1336	1336-103 3 Inferior	60.0
3	Interior part	1336	1336-103 3 Interior	10.0
5	Superior part	1336	1336-310 5 Superior	60.0
5	Lateral part	1336	1336-310 5 Lateral	24.5
5	Inferior part	1336	1336-310 5 Inferior	67.5
5	Interior part	1336	1336-310 5 Interior	10.0
6	-	1336	1336-8	200.0
7	-	1336	1336-6	165.0
8	-	1336	1336-301	202.0

Statistical analyses for infaunal assemblage comparisons

Comparisons in species richness (i.e., number of species and rarefaction curves), faunal abundance and k-dominance curves were made within vertebrae only for vertebrae not colonized by *Osedax*, and between vertebrae (not colonized by *Osedax* Vs. colonized by *Osedax*). Rarefaction and k-dominance curves comparisons

in vertebrae not colonized by *Osedax* were made only for superior and inferior parts due to the low number of species and abundance in lateral and interior parts. Nematodes were not included in species richness analyses and rarefaction curves because they were not identified to species level and could also not be morphotyped with certainty. In any case, species abundance data was standardized by 100 cm³ to enable comparison between different sorted volumes, except for rarefaction curves, as they are conceived for comparison in samples of unequal size (Magurran, 1988). Vertebrae differences in number of species and abundance were tested by t-tests. In every case, normality and homogeneity of variances were previously tested using Shapiro-Wilk and Bartlett tests, respectively. In case samples were not normal or homoscedastic, data were \log_e+1 transformed to keep absences as zero. A comparison between assemblages of vertebrae not colonized by *Osedax* and colonized by *Osedax* was also made using Principal Component Analysis (PCA). Standardized data was transformed using \log_e+1 and a chi-square double standardization. Very rare and/or very low abundance species were excluded from PCA analyses. All statistical analyses were performed with the Vegan package (Oksanen et al., 2011) of software R (R Development Core Team, 2011).

3.3. Results

Epifaunal community assemblage and patterns along the skeleton

The nine Antarctic minke whale vertebrae presented two main contrasting patterns. Vertebrae 1-5 were covered by dense white bacterial mats on the upper parts and exhibited stained black areas closer to the sediment. Bacterial mats covered up to 35-40% of the surveyed area of bones and vertebra 5 presented the largest black area (ca. 17%; Figure 7). Interestingly, these vertebrae were still well preserved

compared to vertebrae 7-9, where the dense white bacterial mats and dark areas were either very small or not present. Instead, these bones presented holes of irregular shape and several centimeters of depth, some of them with lateral expansions below a thin bone cortical layer, exposing the bone trabecular system to the marine environment. The most degraded bones were vertebrae 7 and 9, with degraded bone surveyed areas of 19% and 45%, respectively (Figure 7). Vertebra 6 presented an intermediate pattern with black and degraded areas, but no bacterial mats (Figure 7). Similarly to what was found for bacterial mats and degraded areas, the epifaunal community assemblages were also distinct for vertebrae groups 1-5 and 6-9. *Munidopsis*, amphipods and polynoids were widespread among bones. However, *Osedax* and the small raphitomid gastropod only occurred in the second group of vertebrae, where they were abundant (Figure 7).

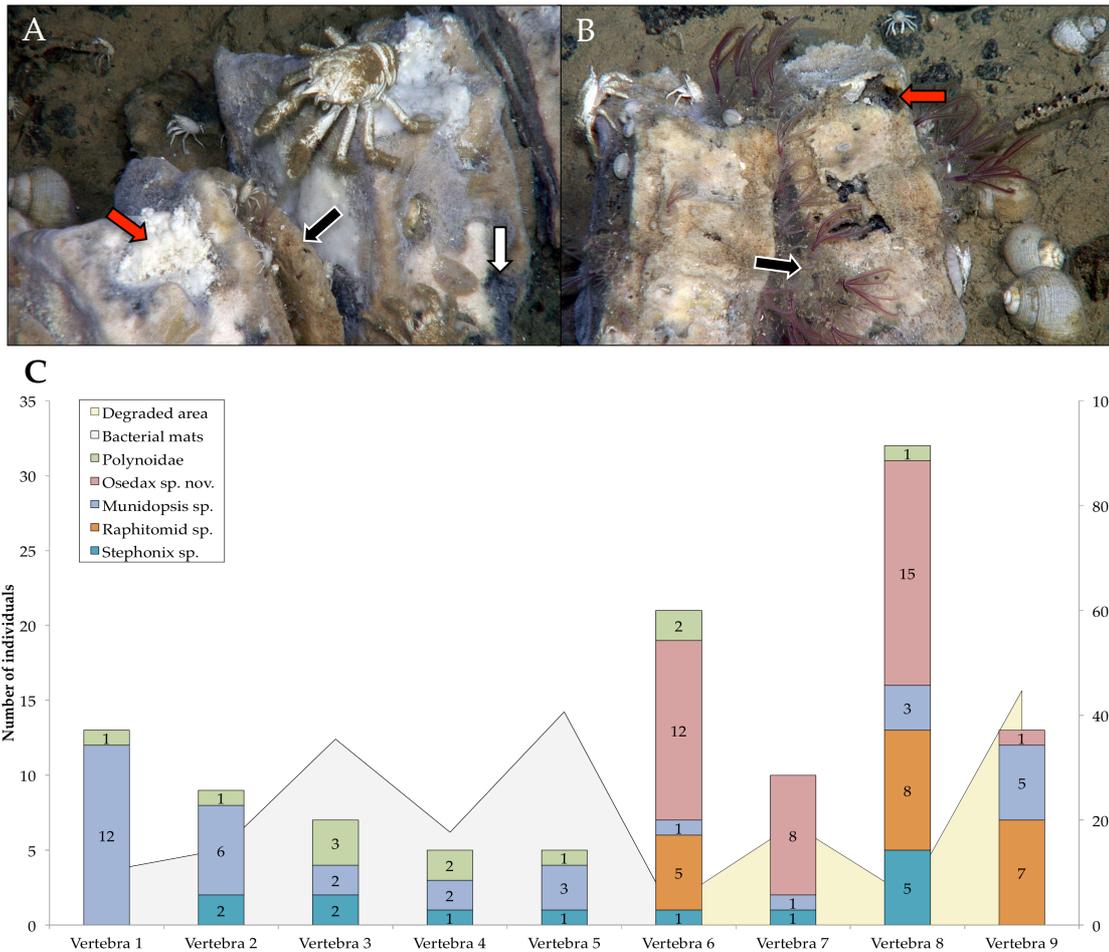


Figure 7. Epifaunal assemblages on vertebrae of the whale carcass found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean. **A.** Vertebrae colonized by dense white bacterial mats (red arrow) but not by *Osedax*. Note that that these vertebrae present dark areas (white arrow) and were still jointed by intervertebral discs (black arrow). **B.** Vertebrae heavily degraded (red arrow) and colonized by *Osedax* (black arrow). **C.** Fauna distribution along the bones (stacked bars) and the percentage of bone cover by dense white bacterial mats and bone degraded areas (shaded areas).

*Infaunal community structure in vertebrae not colonized by *Osedax**

Infaunal taxa were heterogeneously distributed within vertebrae not colonized by *Osedax* (vertebrae 1, 2, 3 and 5). Individuals were found only within the

first centimeter of the bone, which corresponded to the bone outer cortical layer and the first millimeters of the trabecular system depleted of lipids (Figure 8). This part of the bone was darker and was soft enough to easily sorted organisms using tweezers in contrast to the rest of the bone (deeper bone matrix) which was much more harder. All these bones (n=4) exhibited the same lipid distribution from the outside to the inside: (a) the first centimeter of bone (dense bone cortical layer and first millimeters of trabecular bone system) completely depleted of lipids, (b) a zone with high amounts of lipids filling the trabecular system, (c) a wide zone of trabecular system more or less depleted of lipids and (d) the innermost part of the bone completely filled with lipids (Figure 2). All fauna inhabited the trabecular system apparently without causing any damage to the bone structure (Figure 8).

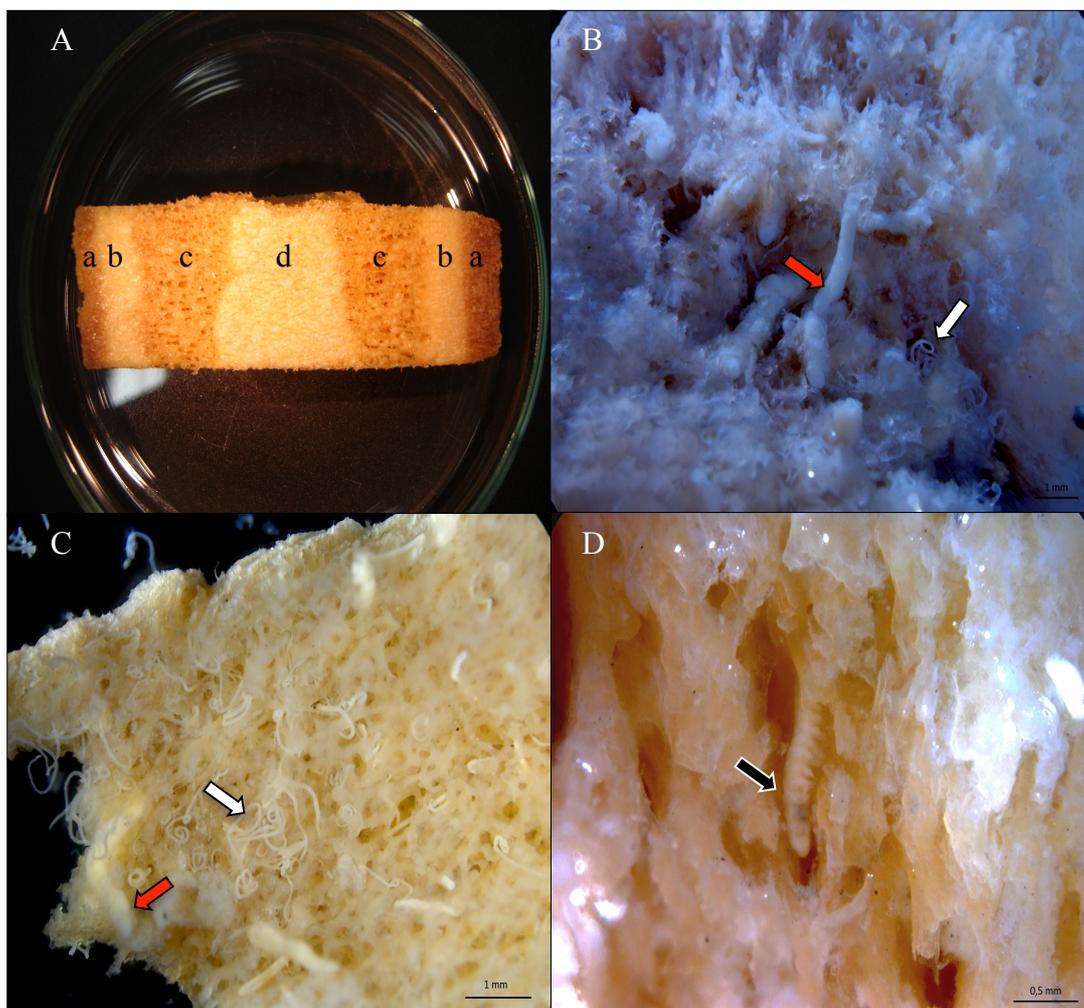
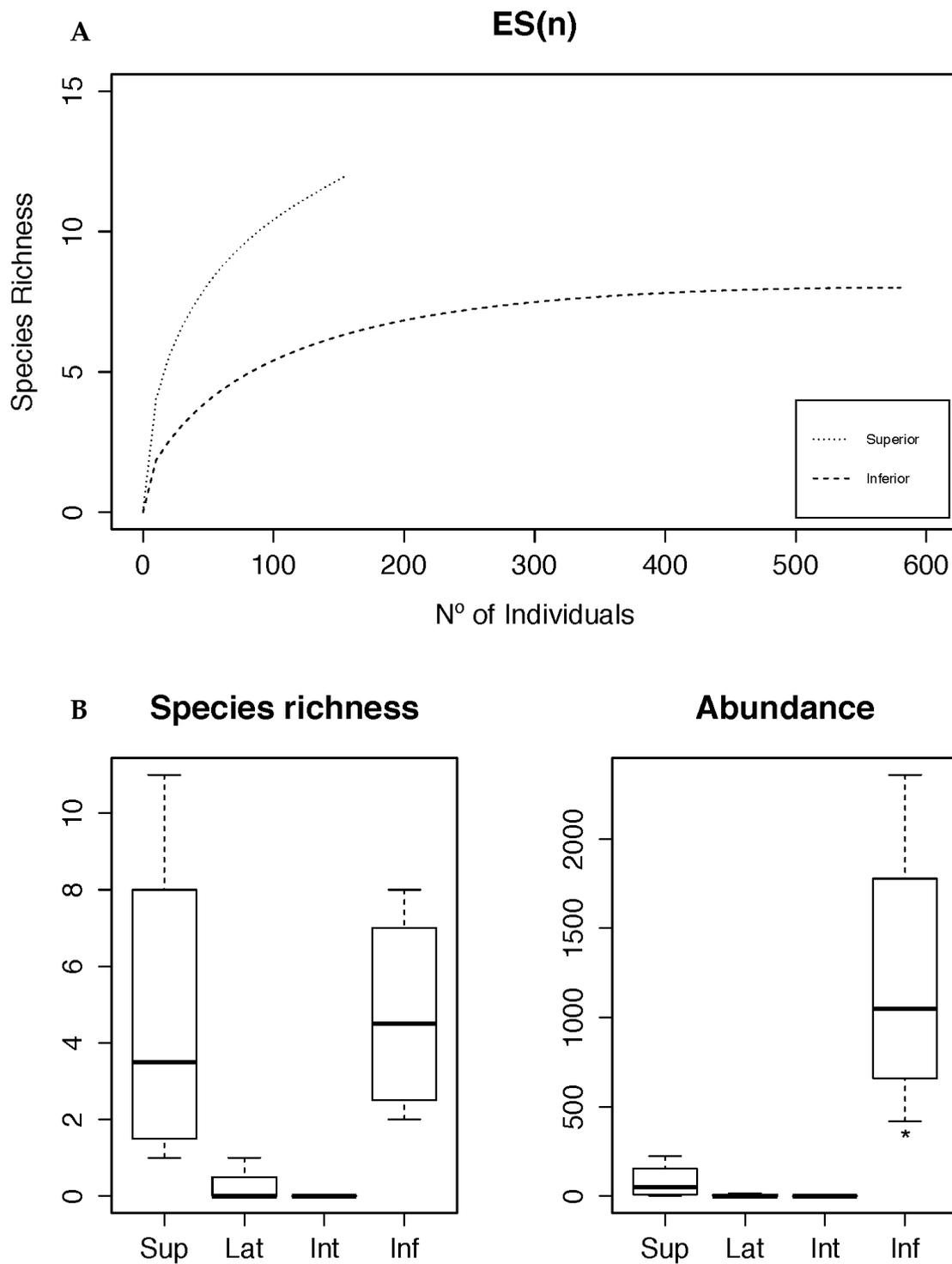


Figure 8. Bone matrices and fauna of vertebrae not colonized by *Osedax* of the whale carcass found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean. A. Cross-section of a vertebra showing the lipid distribution patterns: a) Dense bone cortical layer and first millimeters of trabecular system, where all fauna was found; b) Trabecular system filled with high amounts of lipids (whitish color); c) Wide zone of the trabecular system with small amounts of lipids; d) Lipid-rich innermost part of the bone. Diameter of Petri dish = 8.5 cm. **B and C.** Detail of the first millimeters of trabeculae from the inferior parts of vertebra 2 and vertebra 4, respectively. This area was heavily colonized by nematodes (white arrow) and *Capitella iatapiuna* (red arrow). **D.** *Dorvilleid* polychaete (black arrow) inhabiting the open spaces of the trabecular system in the upper part of Vertebrae 2. Dense white bacterial mats heavily colonized this area.

A total of 3239 individuals belonging to 14 species/morphotypes were found in these vertebrae (see Appendix). Nematodes represented the 77.4% of the total abundance (Figure 11), but were not identified to species level or morphotyped. Polychaetes were the most species-rich group with 11 species/morphotypes and the dominance of *Capitella iatapiuna* (18.8%). In addition, *Ophryotrocha* spp. (~3%) were the most species-rich genus with eight morphotypes. Other less abundant organisms included two hesionid species, *Hesiocaeca* sp. nov. and *Pleijelius* sp. nov. 1, the chrysopetalid *Vigtorniella* sp., the spionid polychaete *Lindaspio* sp. nov., harpacticoid copepods and a bivalve, which all together account for only ~0.8% of the total abundance.

Although the number of species were not significantly different between the superior (mean = 4.75 ± 4.5 species) and inferior (mean = 4.75 ± 2.75 species) bone parts ($t = 0$, $df = 4.971$, $p = 1$), the expected number of species was higher in the superior (ES(150) = 12 species) than in inferior parts (ES(150) = 6 species) (Figure 9). However,

the abundance of organisms was significantly higher ($t = -3.5061$, $df = 3.902$, $p = 0.02578$) in inferior parts (mean = 1218.5 ± 825.0 ind. 100cm^{-3}) than in superior parts (mean = 81.4 ± 101.1 ind. 100cm^{-3}) (Figure 9). As previously mentioned, no organism was found in the inner parts of bones and only 4 individuals of *Capitella iatapiuna* were found in the lateral part of vertebrae 2 (Appendix 1 and 2). Species assemblage structure also differed between the superior and inferior parts. While inferior parts were highly dominated by nematodes (81.6%) and *Capitella iatapiuna* (15.9%), other species did occur in very low abundances (Figure 4). On the other hand, *Capitella iatapiuna* (51.1%) and *Ophryotrocha* spp. (31.3%) dominated superior parts, with nematodes accounting for only 5.5% of the total abundance (Figure 10).



*Figure 9. Species richness and abundance of organisms in different parts of vertebrae not colonized by *Osedax* of the whale carcass found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean. A. Rarefaction curves for superior ($n=4$) and inferior bone parts ($n=4$). Nematodes were not included in rarefaction analyses because they were not*

identified to species level. **B.** Boxplots of species richness (number of species) and abundance of individuals per 100 cm³ for superior (n=4), lateral (n=4), interior (n=4) and inferior (n=4) parts. Rectangles show the interquartile range, from the first quartile (the 25th percentile) to the third quartile (the 75th percentile). The whiskers go from the minimum value to the maximum value. Black horizontal lines inside rectangles represent the mean. Nematodes were not included in species richness analyses because they were neither identified to species level nor morphotyped. Statistically significant differences were only tested between inferior and superior parts due to the low abundance and absence of organisms in the lateral and interior parts, respectively. (*) Statistically significant at $p < 0.05$.

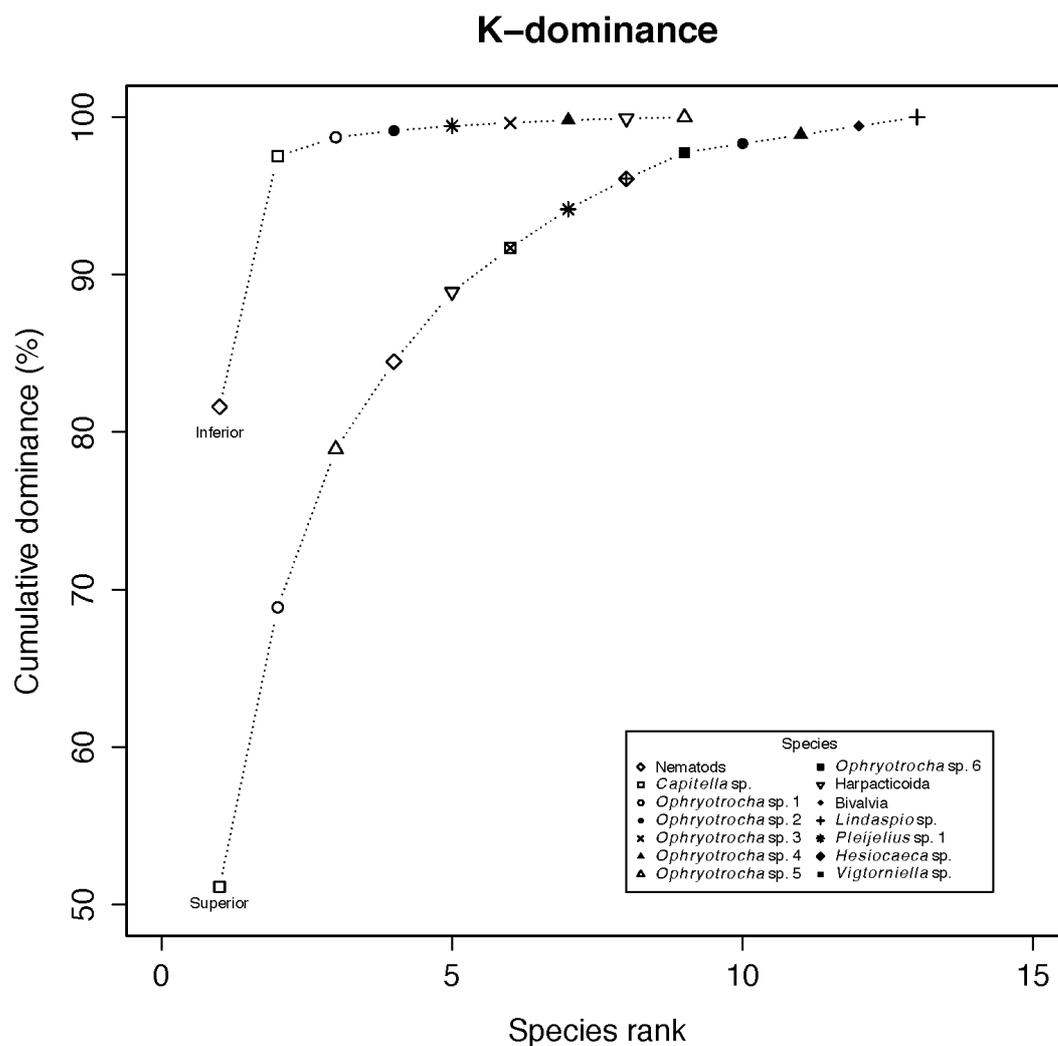


Figure 10. Assemblage K-dominance curves of inferior (n=4) and superior (n=4) parts of vertebrae not colonized by Osedax of the whale carcass found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean.

Comparison between infaunal community structures of vertebrae colonized and not colonized by Osedax

A total of 10080 individuals were found in vertebrae colonized by *Osedax* belonging to 23 different species/morphotypes (Appendix). Organisms were found deeper than the first few millimeters of the trabecular system and even below the first dense lipid-rich layer of the bone (Figure 8A). Although in very different proportions, dominant species were the same as in vertebrae not colonized by *Osedax* (Figure 11). Nematodes were still dominant with 52.2% of the total abundance of species. Polychaetes were also the more species-rich phylum (15 species) with the dominance of *Capitella iatapiuna* (38.9% of the total abundance of species). *Ophryotrocha* spp. (4.9%) was also the most species-rich genus with 6 different species (Figure 11 and Appendix 1 and 2). In general, rarer species were more common (15 species) and represent ca. 4.0% of the total abundance (Figure 11 and Appendix 1 and 2).

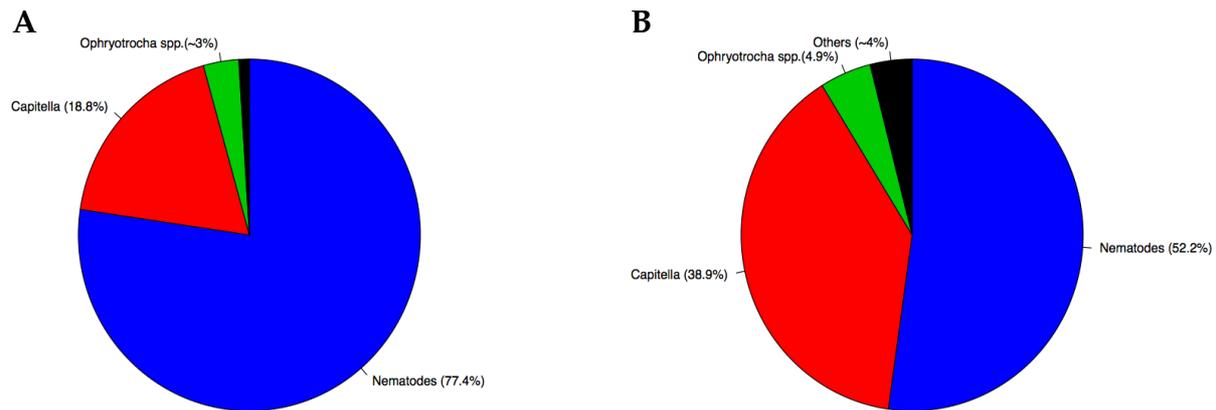


Figure 11. Relative abundance of different species/morphotypes in vertebrae not colonized by *Osedax* (A) and colonized by *Osedax* (B) of the whale carcass found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean. The eight morphotypes of *Ophryotrocha* genus and rare species found among different vertebrae are pooled together under “*Ophryotrocha* spp.” and “Others”, respectively (Appendix 1 and 2).

Vertebrae colonized by *Osedax* had significantly more species (mean = 16.0 ± 2.6 species) than vertebrae not colonized by *Osedax* (mean = 7.5 ± 3.7 species) ($t = -3.5447$, $df = 4.999$, $p = 0.01648$) (Figure 12). Even taking into account differences in sample size the expected number of species was higher in vertebrae colonized by *Osedax* ($ES(700) = 15.2$ species) than in not colonized ($ES(700) = 13.0$ species). Furthermore, these vertebrae were significantly more colonized (mean = 1807.3 ± 599.2 ind. 100 cm^{-3}) than vertebrae not colonized by *Osedax* (mean = 531.9 ± 381.6 ind. 100 cm^{-3}) ($t = 3.2283$, $df = 3.204$, $p = 0.04398$) (Figure 12). Faunal assemblages were distinct between both vertebrae types (Figure 13), mainly because the numerous rare species that inhabited the vertebrae colonized by *Osedax*.

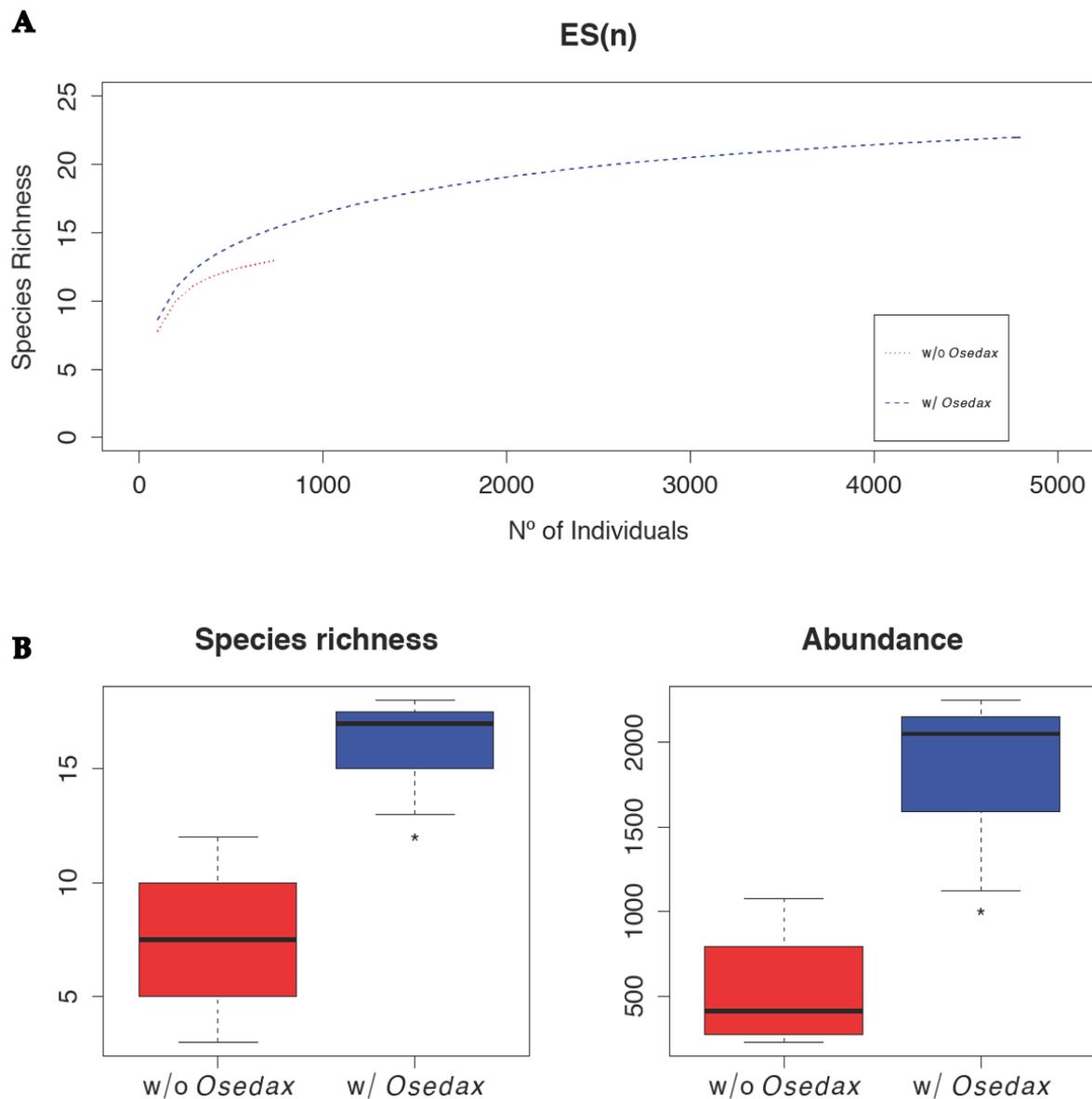


Figure 12. Species richness and abundance of organisms in vertebrae not colonized and colonized by *Osedax* of the whale carcass found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean. **A.** Rarefaction curves for vertebrae not colonized by *Osedax* ($n=4$) and colonized by *Osedax* ($n=3$). Nematodes were not included in rarefaction analysis because they were neither identified to species level nor morphotyped. **B.** Boxplots of species richness (number of species) and abundance of individuals per 100 cm^3 for vertebrae not colonized by *Osedax* ($n=4$) and vertebrae colonized by *Osedax* ($n=3$). Rectangles show the interquartile range, from the first quartile (the 25th percentile) to the third quartile (the 75th

percentile). The whiskers go from the minimum value to the maximum value. Black horizontal lines inside rectangles represent the mean. Nematodes were also not included in species richness analyses for the same reason stated above. (*) Statistically significant at $p < 0.05$.

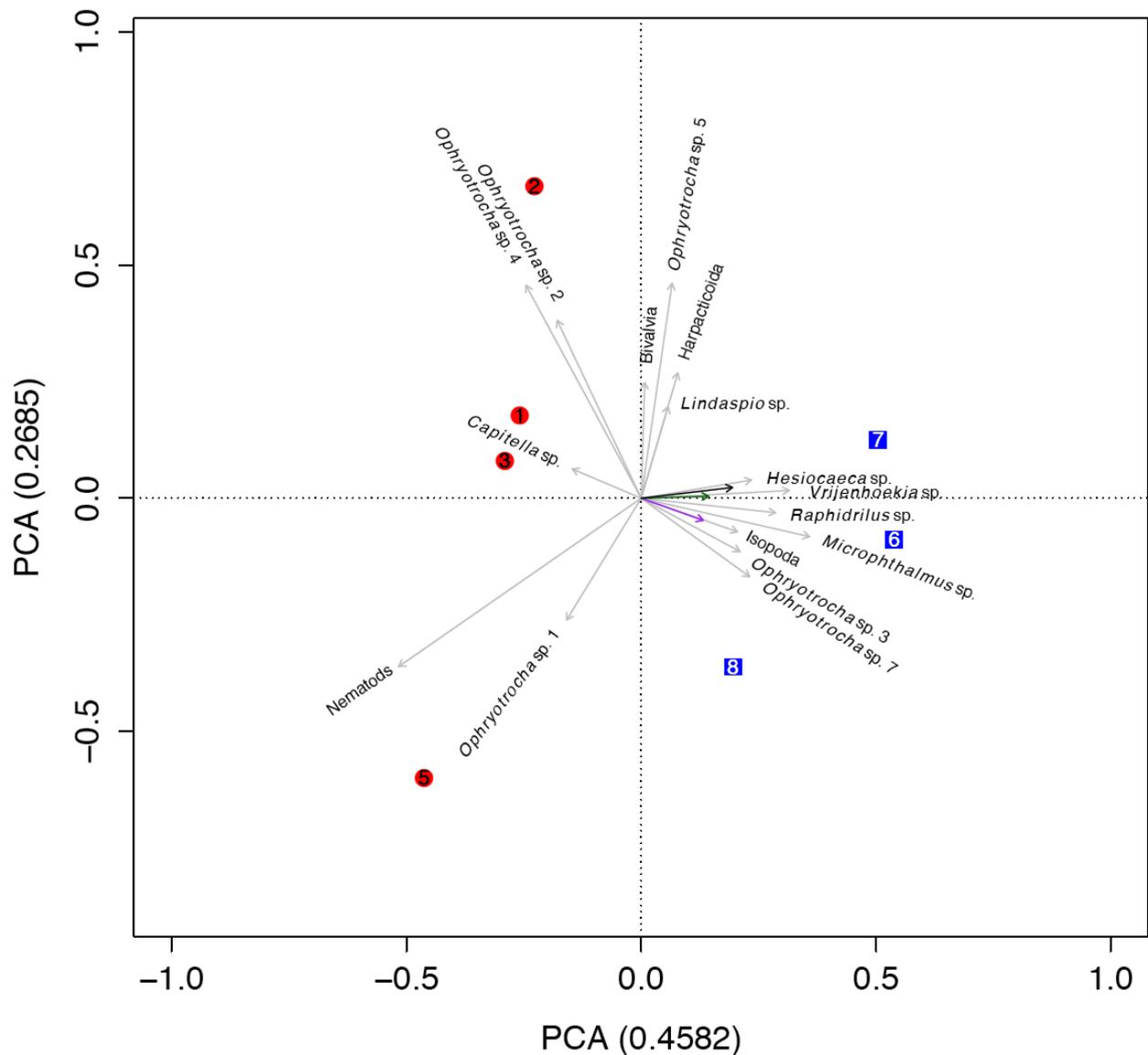


Figure 13. Principal component analyses of assemblages in vertebrae not colonized (red circles) and colonized by *Osedax* (blue squares) of the whale carcass found in São Paulo Ridge at 4204 m depth, SW Atlantic Ocean. Black, green and purple arrows

correspond to *Cyclopoida*, *Pleijelius sp. 1* and *Raphitomid sp.*, respectively, for a better visualization of the data.

3.4. Discussion

Whale skeletons have enormous quantities of oil trapped inside bones, but these lipid reserves are heterogeneously distributed among different types of bones (Higgs et al., 2011b). Oil-rich bones such as jaws and lumbar and caudal vertebrae are expected to harbor higher abundances of sulfophilic organisms in latter ecological successional stages, as they also emit higher and steadier reduced compound fluxes (Higgs et al., 2011b). These lipid-rich bones are also thought to be more resistant to bioerosion because they are highly hydrophobic (Higgs et al., 2011b). Ontogenetic differences in the degree of calcification and lipid content of bones (Smith and Baco, 2003) may explain why *Osedax* is able to completely degrade juvenile whale-falls in subdecadal time scales while adult skeletons could last for ~80 years (Smith and Baco, 2003; Smith, 2006; Lundsten et al., 2010b; Higgs et al., 2011b; Smith et al., 2015). Despite all vertebrae analyzed in the present study were from the caudal section (Sumida et al., submitted) and presumably had no variation in lipid content, we could still detect significant changes in community structure among bones.

Epifaunal community structure

Dense white bacterial mats heavily covered the first five vertebrae, which were also in a well-preserved state (Figure 7). Similar bacterial mats have been found in other whale-fall communities and have been identified as filamentous-

chemoautotroph species, such as sulfide-oxidizing *Beggiatoa*-like species (Smith et al., 1989; Allison et al., 1991; Deming et al., 1997; Smith and Baco, 2003). These bacteria live in the oxic-anoxic bone interface oxidizing free sulfide produced during the degradation process of inner-bone lipids (Deming et al., 1997; Treude et al., 2009). Higgs et al. (2011b) state that such bacterial mats are a good proxy to identify more “sulfophilic” bones, i.e., bones that emit more sulfide. Vertebrae 1-5 also presented many dark areas, probably due to precipitation of metallic sulfide compounds (Allison et al., 1991). This also corroborates the presence of high sulfide effluxes (Figure 7). In addition, one of the polynoid polychaetes found was *Bathykurilla* sp. nov., which belong to a genus known for relying on chemosynthetic bacterial mats (Glover et al., 2005a). Thus, we suggest that these 5 vertebrae were in a sulfophilic stage harboring mainly chemosynthetic bacterial assemblages.

The remaining four vertebrae colonized by *Osedax* (vertebrae 6-9) were different in faunal assemblage composition and aspect, with evident degraded areas measuring several centimeters in depth (Figure 7). The holes observed in these vertebrae clearly exhibited many similarities with burrows described for different *Osedax* species (Higgs et al., 2014). It is also important to highlight that this particular *Osedax* species forms clusters of several individuals, all of them surrounded by a dense mucous layer (Figure 7). The collapse of several burrows of the same cluster may explain the large size of some of the observed holes. Therefore, we suggest that extinct clusters of *Osedax* were the main cause of the observed bone degradation.

Heterotrophic anaerobic sulfur-reducing bacteria living inside bones produce the reduced compound fluxes exploited by chemosynthetic species, such as the *Beggiatoa*-like bacteria. Evidence suggests that their activity may be limited by the diffusion of dissolved seawater sulfate through the bone cortical layer and the hydrophobic lipid-rich bone matrices (Allison et al., 1991; Deming et al., 1997;

Treude et al., 2009). Furthermore, *Osedax* burrows are thought to be a point where sulfate-reducing bacteria may penetrate deeper into the bone matrix and may also serve as conduits for sulfide emission (Higgs et al., 2011b; Smith et al., 2015). However, degraded areas where *Osedax* is no longer present expose the trabecular system to seawater (Figure 1) allowing the diffusion of oxygen and other solutes through the bone matrix. This would not only enhance sulfur availability but could also change inner-bone bacterial community structure and in consequence, reduced-compound fluxes. This may be the reason why chemoautotroph bacterial mats were absent in the surface of vertebrae 6-9. This suggests that contrary to vertebrae 1-5, these vertebrae probably did not emit enough sulfide fluxes to support chemosynthetic-based microbial assemblages. These findings are similar to those observed in other juvenile skeletons in the deep-sea, where *Osedax* colonization accelerated skeleton degradation and shortened the sulfophilic stage (Braby et al., 2007; Lundsten et al., 2010b).

Infaunal community structure and the colonization process of inner bone matrices

Although vertebrae not colonized by *Osedax* were in a good state of conservation, rich assemblages colonized the cortical layers and the first few millimeters of trabeculae. However, infaunal assemblages differed in composition, species richness and abundance among the different bone areas. Lateral and inferior parts were either very low colonized or fully inhabited, respectively (Figure 9). Superior and inferior parts were densely colonized although harboring different invertebrate assemblages (Figure 10), which were more diverse in superior parts but significantly more abundant in lower parts (Figure 9). These differences are probably

related to the different bone physical-chemical microenvironments found in whale skeletons.

The inner environment of bones is thought to be completely anoxic as previous studies measure no oxygen in parts below the cortical layer outer face (Huusgaard et al., 2012). Upper parts bearing sulfide-oxidizing bacterial mats suggest the presence of an oxic-anoxic interface due to the permanent exposition to the water column but also a sulfide-rich anoxic environment inside bones as a result of sulfate-reducing metabolism. Sulfide oxidation cause a reduction in pH acidifying bone environment, which in turn may affect the physical structure of bones by dissolving the dense cortical layers (Allison et al., 1991; Smith et al., 2015). On the other hand, the blackish inferior parts highlight the capacity of sediment iron to bind sulfide diminishing sulfide fluxes. In fact, despite the high rates of sulfate reduction metabolism found in black sediments around whale-falls, only low concentrations of free sulfide were measured probably due to reactions with sediment iron (Goffredi et al., 2008; Treude et al., 2009; Goffredi and Orphan, 2010; Smith et al., 2014, 2015). Sediment could fill in trabeculae penetrating deeper into bones and, thus modify the environment inside bones with the precipitation of iron sulfides (Allison et al., 1991). Lateral parts did not show any of the previously discussed patterns and interior bone parts were completely filled by lipids without any evidence of degradation or exposure to the marine environment.

Some of the organisms found along different bone areas also suggest the idea that whalebones present several microenvironments harboring different assemblages. For instance, *Ophryotrocha* spp., which are known for feed on chemosynthetic bacteria (Wiklund et al., 2009a), were much more abundant in upper bone matrices (31.3%), where dense white microbial mats were widespread, than in inferior parts (2.1%) (Figure 10). Similarly, *Capitella iatapiuna* (Silva et al., *submitted*)

dominated superior parts with 51.1% of the total abundance, whereas represented only 15.9% in lower bone areas (Figure 10). *Capitella* is also known to inhabit highly reducing anoxic habitats, where it feeds on chemosynthetic bacteria (Tsutsumi et al., 1990; Gamenick et al., 1998).

Overall abundance patterns show similarities with bacterial colonization patterns in bones. Evidence suggests that reducing bacteria colonize bones from the outside and slowly penetrate bone inner parts degrading lipids at very low rates (Deming et al., 1997; Treude et al., 2009). For instance, in a 7-year old whale fall, sulfate-reducing bacteria only penetrated few millimeters of a vertebra. Outstandingly, the periphery of a vertebra of a 50-year old whale fall were densely colonized by bacteria whereas its inner parts were still intact (Deming et al., 1997; Smith et al., 2015; Treude et al., 2009). In our vertebrae, all organisms were found within the bone cortical layer and the first millimeters of the trabecular system, which were completely devoid of lipids and darker in color suggesting bacterial activity (Figure 8). The bone cortical layer is the densest and hardest structure of bones, but fauna was curiously easier to sort by tweezers in this area than the trabecular system. Bacterial penetration through the bone matrix and the consequent degradation of the bone cortical layer lipids seems to also allow macrofauna to access and colonize whalebones matrices. However, either for microbes and macrofauna, the trabecular system lipids may be a difficult barrier to go through. This would explain why only the cortex and the first few millimeters of the trabecular system were colonized while the rest of the matrix was completely intact. Macrofauna may certainly affect bacterial degradation by promoting seawater diffusion through bone matrices, sediment penetration and facilitating, competing or predated on microbes, especially the relatively larger species such as *Capitella iatapiuna* (Silva et al., *in press*).

The colonization of bacteria in areas in contact with sediments is generally more efficient than that occurring in areas exposed to the water column (Deming et al., 1997; Smith et al., 2015). I found the same pattern for macrofauna, with inferior parts significantly more colonized mainly due to the large abundance of nematodes (81.6% of the total abundance). In upper parts, nematodes represented only 5.5% (Figure 10). It is likely that the richer invertebrate assemblages present in upper areas play a main role controlling nematode abundance. Similar patterns were found in whale-fall sediments where nematode abundance was inversely correlated with macrofauna due to competition or predation (Debenham et al., 2004).

Infaunal assemblages of vertebrae colonized by Osedax: shedding light on the ecological role of Osedax

Faunal assemblages of vertebrae colonized by *Osedax* were significantly more diverse and abundant (Figure 12). Vertebrae colonized by *Osedax* also revealed distinct infaunal assemblages (Figure 13). The colonization of these vertebrae by *Osedax* may be the cause of the observed dramatic differences between assemblages.

Bone degradation by extinct clusters of *Osedax* heavily eroded vertebrae breaking out the cortical layer and penetrating deeper into the trabecular system (Figure 7). The degradation of the bone's hardest structure may have allowed fauna to pass through this barrier and easily access the bone inner resources. On the other hand, degradation of the bone cortical layer may have greatly affected the inner bone microbial assemblages similarly to what was observed for epifaunal assemblages (Figure 7). This may create new microenvironments inside bones and restrict the innermost zones to heterotrophic anaerobic bacteria, such as sulfur-reducing bacteria. Fauna less tolerant to anoxic and sulfidic conditions may then be

able to colonize bone matrices. In fact, *Osedax* may not only facilitate inner bone colonization but can also create conditions for the presence of other whale-fall assemblages. For instance, Johnson et al. (2010) suggest that other species that feed on bones, such as *Paralomis* crabs and the bone-eating specialist *Rubyspira* gastropods, need *Osedax* degradation to exploit bones.

The present study is based on a juvenile whale skeleton and although *Osedax* may not substantially affect the community ecological succession stages in adult whale carcass, it seems likely that *Osedax* cause similar effects on bone macrofaunal assemblages. Adult whale carcasses may last several decades on the seafloor and the most lipid-rich bones are difficult to exploit by *Osedax* (Smith et al., 2015). *Osedax* high respiration rates in such anoxic sulfide-rich environments as well as the lower collagen content could explain the low abundances of *Osedax* in such bones (Higgs et al., 2011b; Huusgaard et al., 2012). This may also explain the negative correlation between chemoautotrophic bacterial mats and *Osedax* abundance observed in other studies of adult whale skeletons (Amon et al., 2013), which is not the case here as all vertebrae were from the caudal part of a juvenile skeleton (see above). Nevertheless, *Osedax* is also able to colonize adult lipid-rich bones, causing evident erosion, and even to completely degrade the less lipid-rich bone, such as ribs, (Smith et al., 2015), which may also facilitate the entrance of fauna.

CHAPTER 4

CONCLUSIONS

Deep-sea whale-fall communities were first described in 1989 and soon became the focus of many researches due to the faunal overlap with other chemosynthetic communities and their role as stepping-stones for dispersion (Smith et al., 1989). After 26 years of research on deep-sea whale falls our understanding of these communities has increased dramatically (Smith et al., 2015). However, the scarce data available beyond the Northeast Pacific Ocean makes the study of whale-fall communities in other ocean basins necessary to fully understand the evolution, ecology and especially the poorly known biogeography of these deep-sea communities. In the first chapter of this thesis I aimed to characterize the biological community of the first deep-sea whale fall of the whole Atlantic Ocean in order to integrate and compare my results with the current knowledge and to explore faunal overlaps with whale falls and other cognate communities of around the world.

Many new species with novel adaptations have been described in deep-sea whale falls since their discovery. Probably the most striking example is the bone-eating worm genus *Osedax* (Rouse et al., 2004). Although many of the faunal patterns in whale-fall communities have already been described, there is still a lack of knowledge of the basic ecology, such as the effects of these iconic specialists on the otherwise poorly known bone assemblages. Thus, in the second chapter of this thesis I explored in more detail the bone epifaunal and infaunal assemblage patterns and the ecological role of the whale-fall specialist *Osedax*.

Here, I integrate the results of both chapters in order to discuss whale fall biogeography and the stepping stone hypothesis as well as the ecological effects of *Osedax* in whale skeleton assemblages.

Insights of whale-fall biogeography and the stepping-stone hypothesis:

The SW Atlantic whale fall fauna found in this study presented a close affinity between that of the NE Pacific, especially with genera found in the Monterey Canyon and off southern California (Smith and Baco, 2003; Goffredi et al., 2004; Smith, 2006; Braby et al., 2007; Lundsten et al., 2010b). I also found a large generic overlap with other chemosynthetic ecosystems. These findings have deep implications for the almost unknown biogeography of whale-fall communities and contrast/conform with patterns proposed for other chemosynthetic communities, such as vents and seeps.

Despite the importance of evolutionary and ecological relationships among biological communities at different deep-sea chemosynthetic habitats, the biodiversity and biogeography of hydrothermal vents and cold seeps has been by far much more studied than whale-fall communities. Vent fields can be ephemeral and separated by huge distances, occurring mainly along active mid-oceanic ridges and back arc spreading centers (reviewed in Van Dover et al., 2002). Vents show great endemism and different biogeographic provinces fit well with different basins and their history of geological events (reviewed in Van Dover et al., 2002; Moalic et al., 2012). On the other hand, seeps may be longer lasting and widespread along all continental margins (e.g., Levin, 2005). These environments, however, do not present such endemism and other factors such as depth rather than geography may better explain their faunal distributions (e.g., Olu et al., 2010; Teixeira et al., 2013).

Here I find for the first time an inter-basin distribution for many whale-fall specialists and other genera previously only known to occur in other chemosynthetic-based ecosystems (i.e., vents, seeps and wood parcels). Strikingly, some of the fauna found in the present study appears to be related to that of the NE Pacific. Five genera or 12% of all genera reported in this study were previously found exclusively in the NE Pacific (see Table 3). For instance, specialists such as the gastropod *Rubyspira* sp. nov., the polynoids *Bathyfauvelia* sp. and *Bathykurila* sp., the ampharetid *Grassleia* sp. and the hesionid *Vrijenhoekia* sp. nov. comprise genera with distribution hitherto restricted to the Pacific (Glover et al., 2005a; Johnson et al., 2010; Pleijel et al., 2008; Reuscher et al., 2012). In fact, for *Rubyspira* and *Vrijenhoekia* this is the first record anywhere outside the Monterey Canyon and expands their bathymetric ranges by more than 1300 m depth (see Johnson et al., 2010; Pleijel et al., 2008).

There was also a substantial overlapping with vent and seep fauna, such as *Grassleia*, a NE Pacific vent and seep inhabitant (Reuscher et al., 2012), and *Bathykurila*, that occurs in NE Pacific vents and whale falls (Glover et al., 2005a). The polynoid polychaete genus *Bathyfauvelia* is also registered for the first time on a chemosynthetic-related habitat. Other genera present were also found in cognate communities of the Atlantic Ocean, such as the new hesionid polychaetes *Hesiocaeca* sp. nov. and *Pleijelius* sp. nov. 1 and 2, and the spionid *Lindaspio* sp. nov., previously registered in NW Atlantic methane hydrates (Desbruyères & Toulmond, 1998), NW Atlantic wood-fall experiments (Salazar-Vallejo and Orensanz, 2006) and SE Atlantic oil fields (Bellan et al., 2003), respectively (Table 3).

Table 3. Genera reported in the present study and their previous geographic records. Most genera are shared between the NE Pacific Ocean whale falls and hydrothermal vents and cold seeps.

Genus	Previously known from	Habitat
<i>cf. Grassleia</i>	NE Pacific	Hydrothermal vents and cold seeps
<i>Vrijenhoekia</i>	NE Pacific	Whale falls
<i>Bathykurila</i>	NE Pacific	Hydrothermal vents and whale falls
<i>Bathyfauvelia</i>	NE Pacific	Abyssal plain
<i>Rubyspira</i>	NE Pacific	Whale falls
<i>Hesiocaeca</i>	NE Pacific/NE Atlantic	Cold seeps (NE Atlantic) and whale falls (NE Pacific)
<i>Pleijelius</i>	NE Atlantic	Wood falls
<i>Vigtorniella</i>	N Pacific/NE Atlantic	Whale falls
<i>Lindaspio</i>	NE Pacific/SW Atlantic	Hydrothermal vents (NE Pacific) and oil seeps (SW Atlantic)
<i>Osedax</i>	All Pacific/NE Atlantic/Southern Ocean	Whale falls

These findings support the stepping-stone hypothesis of Smith et al. (1989) and suggest that dispersal rather than vicariance is a major driver for diversification in whale fall ecosystems (see Glover et al., 2005b; Smith et al., 2015). In fact, whale falls are likely to occur worldwide although heterogeneously distributed (Smith and Baco, 2003). Some large baleen whales, such as humpbacks, migrate from high-latitude high-productivity feeding areas to low-latitude low-productivity breeding areas along continental margins in all oceans and to some specific oceanic islands (e.g., Hawaii) (reviewed in Roman et al., 2014). In addition, other species such as sperm whales, exhibit cosmopolitan distribution and can potentially sink everywhere in the ocean, especially supplying the deep ocean at equatorial latitudes

(Smith et al., 2015). For some areas, carcasses may be relatively close to each other, e.g., Smith et al. (2003) estimated average nearest neighbor distances for whale falls from gray whales to occur every 5-16 km in the NE Pacific Ocean.

In fact, some genera of the whale fall specialists appear to have a worldwide distribution, such as the bone-eating genus *Osedax*. *Osedax rubiplumus* illustrates well this idea having been reported in both sides of the Pacific Ocean and even in the Southern Ocean, which suggests a large inter-basin connectivity (Smith et al., 2015). Furthermore, the present *Osedax* phylogeny does not seem to fit any specific geographical or bathymetrical pattern suggesting that dispersion is an important factor for the evolution of *Osedax* species (Glover et al., 2005b, 2013). The new *Osedax* species found in the present work is similar to *O. frankpressi*. It is the deepest *Osedax* species found to date, extending the genus depth range by more than 1300 m (Goffredi et al., 2004; Rouse et al., 2004) and it is the first found in the deep Atlantic Ocean. Similarly, mtCOI studies cluster *Rubyspira* sp. nov. with the two previously described species (*R. osteovora* and *R. goffrediae*; Johnson et al., 2010), both of them from the Monterey Canyon (NE Pacific). The occurrence of other Pacific genera in our study, such as *Vrijenhoekia* and *Bathykurila*, also supports the idea that many whale-fall specialist lineages may be distributed worldwide (Table 3).

Thus, it is feasible to imagine a “worldwide whale-fall corridor” along continental margins, somewhat similar to the distribution of cold-seeps, but also along equatorial areas. Both “corridors” would allow fauna dispersion by a stepping-stone dispersal mechanism. This mechanism may be important for some chemosynthetic-generalist invertebrates with worldwide distributions, such as vesicomid clams (Smith et al., 1989; Tunnicliffe and Juniper, 1990), and also for whale-fall specialists.

Bone assemblages and the ecological role of Osedax

So far the ecological role of *Osedax* has been thought to be just restricted to the degradation of whale skeletons and their effects on the main ecological succession patterns. Several experiments demonstrate that large populations of *Osedax* spp. completely degrade juvenile carcasses in sub-decadal time scales (Goffredi et al., 2004; Braby et al., 2007; Lundsten et al., 2010b). These findings led some authors to state that *Osedax* is a foundation genus that largely determine the longevity of whale-fall communities in the deep seafloor (Braby et al., 2007). Some ecological succession stages are deeply affected and eventually truncated due to the degradation produced by *Osedax*, specially in the case of the sulfophilic stage (Braby et al., 2007; Lundsten et al., 2010b). However, this do not hold true for adult whale carcasses. Adult whale skeletons are more calcified and present higher lipid contents both factors preventing a significantly fast *Osedax* degradation (Smith et al., 2015), specially in lipid-rich bones (Higgs et al., 2011b). These bones, such as lumbar and caudal vertebrae, can last in the deep-sea for ~80 years even though they may be colonized and partially eroded by *Osedax* (Smith, 2006; Smith et al., 2015).

To the best of my knowledge here I present the first study that fully characterize both the deep-sea whalebone epi- and infaunal assemblages. This allowed me to deeper explore the ecological role of *Osedax* in whale-fall biodiversity. I found that even well preserved vertebrae, i.e., vertebrae not colonized and degraded by *Osedax*, may harbor rich and abundant infaunal assemblages. These bones exhibited different microenvironments due to the interaction of biotic and abiotic factors, which in turn support different infaunal assemblages. Faunal colonization of bones is similar to those of bacterial communities and I suggest that interactions between fauna and microorganisms may play a main role not only in the

degradation of inner lipids but also of the cortical layer. The degradation of the hardest bone structure may facilitate the process of colonization of bones.

Osedax colonization causes greater impacts in bone cortical layer than those of other fauna and bacterial communities. This significantly enhances species richness and abundance of infaunal assemblages by modifying the bone physical structure and the chemical environment of the bone inner matrices. These impacts deeply affect epifaunal assemblages as well as the ecological succession stages. Therefore, I conclude that *Osedax* is an important ecosystem engineer that not only play a main role in the degradation of the largest particles that reach the deep-sea but also in shaping biodiversity patterns during this process.

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Appendix 1

Species morphotypes found in vertebrae. Abundances are given per 100 cm³

Species/morphotype	V 1	V 2	V 3	V 5	V 6	V 7	V 8
Ampharetidae	0	0	0	0	0.5	0	0
<i>Ophryotrocha</i> sp. 8	0	0	0	0	0	0.6	0
Nermertea	0	0	0	0	0	1.2	0
<i>Vigtorniella</i> sp.	0	1.8	0	0	0	0	0
<i>Pleijelius</i> sp. 2	0	0	0	0	2.0	0	0
<i>Stephonyx</i> sp.	0	0	0	0	0	0	2.5
<i>Ophryotrocha</i> sp. 6	0	3.0	0	0	0	0	0
Bivalvia	0	0.6	0	0	0	0.6	0
Raphitomid sp.	0	0	0	0	0	1.2	0.5
Cyclopoida	0	0	0	0	1.0	2.4	0
<i>Ophryotrocha</i> sp. 7	0	0	0	0	8.0	0	2.0
<i>Vrijenhoekia</i> sp.	0	0	0	0	10.0	12.1	0
<i>Lindaspio</i>	0	0.6	0	0	0.5	0.6	0
<i>Ophryotrocha</i> sp. 4	0.9	2.4	0.8	0	0	0	0
Isopoda	0	0	0	0	1.0	2.4	1.0
<i>Raphidrilus</i> sp.	0	0	0	0	6.5	6.1	0.5
<i>Microphthalmus</i> sp.	0	0	0	0	27.0	14.5	2.0
<i>Ophryotrocha</i> sp. 2	4.4	2.4	1.6	0	2.0	0.6	0
<i>Ophryotrocha</i> sp. 5	0.9	11.5	0	0	2.5	14.5	0.5
<i>Hesiocaeca</i> sp.	0	1.2	0.8	0	24.5	7.9	3.0
<i>Pleijelius</i> sp. 1	5.3	0	2.4	0	22.0	23.0	5.4
Harpacticoida	1.8	3.0	1.6	0	5.5	26.1	1.0
<i>Ophryotrocha</i> sp. 3	1.8	1.2	0	0.6	57.0	22.4	9.9
<i>Ophryotrocha</i> sp. 1	20.4	2.4	11.2	5.5	54.5	43.6	46.0
<i>Capitella iatapiuna</i>	15.1	298.2	68.0	9.2	908.0	1020.0	183.2
Nematoda	270.2	749.1	139.2	488.0	918.0	1046.7	867.3

Appendix 2. *Species/morphotypes found in different sorted parts of vertebrae. Abundances are given per 100 cm³*

Species/ morphotype	V 1 Sup	V 1 Lat	V 1 Int	V 1 Inf	V 2 Sup	V 2 Lat	V 2 Int	V 2 Inf	V 3 Sup	V 3 Lat	V 3 Int	V 3 Inf	V 5 Sup	V 5 Lat	V 5 Int	V 5 Inf	V 6	V 7	V 8
Nematoda	0.0	0.0	0.0	760.0	12.7	0.0	0.0	1755.7	5.3	0.0	0.0	286.7	0.0	0.0	0.0	1174.8	918	1046.7	867.3
<i>Capitella iatapiuna</i>	2.5	0.0	0.0	40.0	138.2	16.0	0.0	588.6	24.0	0.0	0.0	126.7	1.7	0.0	0.0	20.7	908	1020.0	183.2
<i>Ophryotrocha</i> sp. 1	0.0	0.0	0.0	57.5	5.5	0.0	0.0	1.4	37.3	0.0	0.0	0.0	15.0	0.0	0.0	0.0	54.5	43.6	46.0
<i>Ophryotrocha</i> sp. 2	0.0	0.0	0.0	12.5	1.8	0.0	0.0	4.3	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	2.0	0.6	0.0
<i>Ophryotrocha</i> sp. 3	0.0	0.0	0.0	5.0	0.0	0.0	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	57.0	22.4	9.9
<i>Ophryotrocha</i> sp. 4	0.0	0.0	0.0	2.5	1.8	0.0	0.0	4.3	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ophryotrocha</i> sp. 5	0.0	0.0	0.0	2.5	32.7	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	14.5	0.5
<i>Ophryotrocha</i> sp. 6	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ophryotrocha</i> sp. 7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	0.0	2.0
<i>Ophryotrocha</i> sp. 8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0
Harpacticoida	0.0	0.0	0.0	5.0	9.1	0.0	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	26.1	1.0
Cyclopoida	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	2.4	0.0
Isopoda	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	2.4	1.0
Amphipoda	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5
Bivalvia	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0
<i>Lindaspio</i> sp.	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.6	0.0
<i>Pleijelius</i> sp. 1	0.0	0.0	0.0	15.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.0	23.0	5.4
<i>Pleijelius</i> sp. 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0
<i>Hesiocaeca</i> sp.	0.0	0.0	0.0	0.0	3.6	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.5	7.9	3.0
<i>Vigtormiella</i> sp.	0.0	0.0	0.0	0.0	5.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Vrijenhoekia</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	12.1	0.0
<i>Microphthalmus</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.0	14.5	2.0
Ampharetidae sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
<i>Raphidrilus</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5	6.1	0.5
Raphitomid sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.5
Nemertea sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0