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FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

School of Ocean and Earth Science

Bone-eating worms and wood-eating bivalves: characterising the ecology of deep-sea organic falls from multiple ocean basins

by

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Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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BONE-EATING WORMS AND WOOD-EATING BIVALVES:

CHARACTERISING THE ECOLOGY OF DEEP-SEA ORGANIC FALLS

FROM MULTIPLE OCEAN BASINS

Diva Joan Amon

Large organic inputs to the deep seafloor such as the remains of whales or pieces of wood are termed 'organic falls'. Despite over 30 years of research on these interesting deep-sea habitats, we still have only a basic understanding of their taxonomic composition and for some ocean basins, no natural or experimental studies have ever been conducted. The degree of connectivity between these isolated habitats, as well as how quickly organic matter is remineralised by specialist organic-fall fauna (e.g bone-eating *Osedax* worms and wood-eating *Xylophaga* molluscs) is poorly known.

In this thesis, I report the discovery of the first Antarctic whale fall and the diverse assemblage of fauna encrusting it (Chapter 2). The microdistribution of fauna on the whale bones provided evidence for the 'oil-gradient' hypothesis that more lipid-rich bones support a greater abundance of sulfophilic bacterial mats, which are also correlated with the abundance of grazing fauna. The abundance of *Osedax* species on bones however, showed a negative correlation with the bacterial-mat cover, and hence the greatest abundance was on bones predicted to have the lowest lipid content. The *Osedax* species discovered were investigated in detail (Chapter 3) and revealed two new species and a third previously-known species; *Osedax rogersi* sp. nov.*, Osedax crouchi* sp. nov. (described in this thesis and associated paper) and *Osedax antarcticus*. The new species, *O. crouchi* as well as another new species, *Osedax nordenskioeldi* sp. nov. (also described in this thesis and associated paper) and *Osedax antarcticus* were also found on implanted whale bones off Smith Island in the Bransfield Strait. These two localities are approximately 1800 km apart demonstrating the remarkable dispersal capability of species within this genus.

As well as the Antarctic study, I report on wood and bone-colonisation experiments on the Southwest Indian Ridge at two seamounts. A large number of species were found colonising the deployments; 53 species at Coral Seamount and 38 species at Atlantis Bank seamount with only 11 species in common and several putative new species present. Apart from Xylophaginae and *Idas* bivalves, few organic-fall specialists were present, possibly as there were major differences between the two seamounts suggesting that there were barriers to dispersal (Chapter 4). The wood deployments from each seamount were investigated in further detail using X-ray micro-computed tomography to examine the nature of intact *Xylophaga* borings, the comparative abundances and population size structures of the species, their rates of growth and their consumption rates of wood (Chapter 5). Two more sets of samples from the Mid-Cayman Spreading Centre and the Tongue of the Ocean, Bahamas were scanned also. The wood at each deployment site was colonized by a different species of *Xylophaga*. This novel analysis has shown that an individual *Xylophaga* can bore between 0.235 and 0.606 cm^3 of wood per year depending on the species, emphasising the importance of the genus *Xylophaga* with regard to wood remineralisation in the deep sea and its role as an ecosystem engineer.

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DECLARATION OF AUTHORSHIP

I, Diva J. Amon, declare that the thesis entitled ‘Bone-eating worms and wood-eating bivalves: characterising the ecology of deep-sea organic falls from multiple ocean basins' and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
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- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
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  All new taxon names mentioned in this thesis are disclaimed for nomenclatural purposes (ICZN Article 8.2).

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Date:…….

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1. Organic falls in the deep sea

The deep sea is an extremely energy-poor environment as photosynthetic primary production cannot occur owing to the lack of light (Rex and Etter, 2010). Thus, most of the life, excluding that at chemosynthetic environments, is supported by the downward flux of organic material from the euphotic zone (Angel, 2003; Gage, 2003). However, on average, less than 1% of surface production reaches the deep sea, mostly in the form of phytodetritus created by photosynthesis, larger particles such as crustacean moults and faecal pellets, and occasional large organic falls (Rowe and Staresinic, 1979; Gooday *et al.*, 1990; Tyler, 1995; Lampitt and Antia, 1997; Fischer *et al.*, 2000). Organic falls include the carcasses of marine mammals, squids, large fish, swarms of gelatinous animals such as salps, and a range of plant matter including seagrasses, macroalgae and terrestrial plant material (Rowe and Staresinic, 1979; Stockton and DeLaca, 1982; Smith and Baco, 2003; Billett *et al.*, 2006). Smith (1985) concluded that organic falls represent approximately 11% of benthic-community respiratory requirements.

Our understanding of organic falls has evolved from their being perceived as just large organic inputs to the seafloor to the more recent idea that they facilitate the creation of ephemeral unique ecosystems. They are not only extremely important in fulfilling the nutritional needs of many deep-sea species, which feed either directly on the substrates or prey on smaller fauna also attending the falls, but also provide shelter and substrates for animals to settle upon (Jones *et al.*, 1998; Herring, 2002). Large organic falls significantly influence species diversity and community structure by creating patches of organic enrichment and chemical or physical disturbance (Stockton and DeLaca, 1982; Smith, 1985; Grassle and Morse-Porteous, 1987; Gooday *et al.*, 1990; Hogler, 1994; Tyler, 1995; Jones *et al.*, 1998; Smith and Baco, 2003; Bernardino *et al.*, 2010). The exact complement of fauna present depends largely on the location and depth of the organic fall (Smith, 1985).

Whale falls and wood falls are the two main types of organic falls and will be focused on in this thesis. Trees and other terrestrial plant matter usually tend to decay naturally on the floor of a forest. However, on some occasions, trees end up in rivers being carried out into the ocean where they sink down to the depths and come to rest on the seafloor. These are termed wood falls and the paths of decay are completely different to terrestrial ones. Whale falls are the carcasses of cetaceans that have come to their final resting place on the ocean floor rather than on a beach or intertidal zone. Both of these processes can occur naturally, referred to as natural whale falls or wood falls. Alternatively, in the name of science, carcasses of beached whales and trees are towed out into deeper waters and sunk to allow experimentation and monitoring of the decompositional processes termed implanted whale falls and wood falls.

1.1 Whale falls

There have been seven natural whale falls reported thus far; in the waters off California (Smith *et al.*, 1989; Bennett *et al.*, 1994; Smith and Baco, 2003; Goffredi *et al.*, 2004), Japan (Fujioka *et al.*, 1993), Antarctica (Amon *et al.*, 2013), Vancouver (Lundsten *et al.*, 2010a) and Alaska (C. Smith, personal communication). There have also been several reports of individual cetacean bones being trawled or found (Turner, 1880; Tebble, 1966; Dell, 1987; Gibbs, 1987; Marshall, 1987; Waren, 1989; McLean, 1992; Marshall, 1994; Berrow *et al.*, 1995; Dell, 1995; Bolotin *et al.*, 2005; Bianucci *et al.*, 2007). A conservative estimate is that 50% of all dying whales sink to and remain at the seafloor (Smith and Baco, 2003). Certain areas, e.g. those underlying the migratory routes of whales and areas of intensive whaling, may receive these inputs more often than other ocean areas (Tyler, 1988; Smith *et al.*, 1989; Connolly and Kelly, 1996). The unpredictability of whale falls makes them very difficult to find and therefore study. Thus, most whale-fall studies are conducted on the experimentally-implanted carcasses of dead, beach-stranded whales (Jones *et al.*, 1998; Smith and Baco, 2003; Dahlgren *et al.*, 2006; Kemp *et al.*, 2006; Fujiwara *et al.*, 2007; Lundsten *et al.*, 2010b).

These concentrated food inputs are difficult to quantify but Smith *et al*. (1989) estimated that one adult from any of the nine largest species of whale provides more than 10000 kg wet weight of labile organic matter to the deep-sea floor. The contribution of whale falls to the overall vertical carbon flux in the deep sea has been estimated at two to three times less than the dominant source, phytodetritus (Jelmert and Oppen Berntsen, 1996). However, the significance of these events lies not with their total contribution to downward carbon flux, but with their creation of long-sustained point sources of energy and nutrients creating unique ecosystems (Jones *et al.*, 1998). It is thought that whale falls provide resources for varying periods, most a minimum of several months to a maximum of over a hundred years, depending on the depth, dissolved oxygen and the assemblage of decomposer organisms (Smith and Baco, 2003; Braby *et al.*, 2007).

1.1.1 The successional stages of whale falls

On death, a whale typically begins to sink through the water column to the seafloor. During this time, the carcass may rot and become filled with gases, causing the carcass to refloat, although this tends to depend on the water weight and depth of the carcass (Allison *et al.*, 1991). Eventually, the gases may build up to a level that can cause the whale to explode, releasing the gases and allowing the whale to sink to the seafloor (Reisdorf *et al.*, 2012). Once on the seafloor, scavengers play an important role in breaking up and distributing this resource to the benthos (Dayton and Hessler, 1972; Dahl, 1979; Sainte-Marie, 1992). It is thought that

scavenging animals recognise when a whale fall has arrived at the seafloor by distance chemoreception (Tamburri and Barry, 1991; Herring, 2002). The evidence for this is from numerous baited cameras, which show the rapid aggregation of animals such as grenadiers that arrive upcurrent, following the downstream odour plume from the bait (Sainte-Marie, 1992; Jones *et al.*, 1998; Herring, 2002). The odour plume may carry information on the distance, size, type, age and nature of the carcass (Sainte-Marie, 1992). It is also thought that scavengers may be alerted to whale falls by territorial collapse (Smith, 1985), mechanoreception of the bait impact on the bottom, or the sounds of aggregated feeders (Smith and Baldwin, 1982; Sainte-Marie, 1992). Sound travels quickly carrying information on the number and type of animals attending the whale fall (Smith and Baldwin, 1982).

Whale falls are dynamic communities, passing through three successional phases: 1) the mobile-scavenger stage, 2) the enrichment opportunist stage and 3) the sulfophilic stage (Smith *et al.*, 1989; Smith *et al.*, 2002; Smith and Baco, 2003). Smith *et al*. (1989) also proposed a fourth reef stage that occurs after the depletion of all organic material, during which the mineral remnants of the whale skeletons can be colonised primarily by suspension feeders exploiting flow enhancement by bone protrusion into the benthic boundary layer (Bennett *et al.*, 1994; Smith and Baco, 2003). Fujiwara *et al*. (2007) observed a reef stage after 2.5 years on twelve sperm whale carcasses between 219-254 m in which crinoids, basket stars, cnidarians and a benthic ctenophore were recorded. However some suspension feeding taxa such as serpulid and sabellid polychaetes found in the background community were observed on a Pacific whale skeleton exploiting enhanced flow conditions during the sulfophilic stage (Bennett *et al.*, 1994; Smith and Baco, 2003; Lundsten *et al.*, 2010b). This latter observation provides evidence for successional-stage overlap (third stage and fourth stage in this case), emphasising that stages may not be as clearly defined as previously perceived. Goffredi *et al*. (2004) also noted that the stages of succession overlap greatly at Monterey whale falls and Lundsten *et al*. (2010a) at a whale fall of Vancouver Island. The entire successional process may take as long as decades to hundreds of years (Smith *et al.*, 2002).

The first stage is characterised by the arrival of mobile scavengers at the carcass (Smith and Baco, 2003). The removal of whale soft tissue occurs by dense aggregations of large, active necrophages (Dayton and Hessler, 1972; Hessler *et al.*, 1978; Smith, 1985; Smith and Baco, 2003). Research has revealed that a wide range of megafauna attend whale falls including many species of demersal fishes from families such as Macrouridae, Ophidiidae, predatory sharks such as *Somniosus pacificus* Bigelow and Schroeder, 1944*,* the eel *Synaphobranchus kaupii* Johnson 1862*,* and hagfish such as *Eptatretus deani* Evermann and Goldsborough 1907 and *Myxine circifrons* Garman 1899 (Dayton and Hessler, 1972; Smith, 1985; Tamburri and Barry, 1991; Merrett and Haedrich, 1997; Jones *et al.*, 1998; Smith *et al.*, 2002; Smith and Baco, 2003;

Kemp *et al.*, 2006). These scavengers can respond to carrion falls over distances of 10 to 1000 m arriving within minutes to hours of it reaching the seafloor (Smith, 1985; Smith and Baco, 2003; Thistle, 2003).

Invertebrates such as isopods (Sekiguchi *et al.*, 1982), decapod shrimps (Thurston *et al.*, 1995), lithodid crabs such as *Paralomis multispina* Benedict 1895 (Smith, 1986), galatheid squat lobsters such as *Munidopsis* spp. (Jones *et al.*, 1998; Williams *et al.*, 2000; Smith and Baco, 2003; Kemp *et al.*, 2006), gastropods such as *Neptunea* spp. (Smith, 1985; Tamburri and Barry, 1991; Jones *et al.*, 1998), polychaetes such as *Hyalinoecia* spp. (Dayton and Hessler, 1972), octopods (Isaacs and Schwartzlose, 1975; Kemp *et al.*, 2006), holothurians (Pawson, 1976), asteroids, echinoids and ophiuroids such as *Ophiophthalmus normani* Lyman 1879 (Smith, 1985; Jones *et al.*, 1998; Thistle, 2003) also attend the carrion during this stage. Lysianassid amphipods such as *Orchomene obtuse* Sars 1891 and *Eurythenes gryllus* Lichtenstein in Mandt, 1822 represent a highly specialised response to whale falls in the deep sea (Hessler *et al.*, 1978; Thurston, 1979; Tamburri and Barry, 1991; Jones *et al.*, 1998; Gage, 2003). They can greatly reduce their metabolic rates between whale falls but still retain acute sensitivity to the arrival of carcasses on the seafloor (Smith and Baldwin, 1982; Thistle, 2003). When they detect the odour of a carcass, they rapidly increase their metabolic rate to allow for swift arrival at the organic fall. They have extensible guts to maximise consumption and shearing mandibles for voracious feeding (Dahl, 1979; Thurston, 1979). The ingested material is also quickly digested (95% in one to ten days) to create space for more food and to ease mobility (Hargrave *et al.*, 1995; Thistle, 2003). Most of the animals feed until satiation and leave the carcass but many may remain in the near vicinity to allow for easy return to the carcass after their guts are partially emptied (Smith, 1985), or to maximise digestive efficiency (Smith and Baldwin, 1982; Thistle, 2003).

Nutrients from the organic fall may be dispersed either as dissolved organic matter, small particles dislodged by scavengers and advected by currents, faeces, offspring, or as accrued body mass (Dayton and Hessler, 1972; Stockton and DeLaca, 1982; Smith and Baco, 2003). It is thought that this can provide up to 3% of the annual respiratory requirement for the deep-sea benthic community (Smith, 1985). At peak abundance around the carcass there may be tens of fishes, thousands of amphipods and many other invertebrates, but not necessarily all at the same time (Smith, 1985; Thistle, 2003). The concentrations of animals around carcasses are much greater than the surrounding areas but as the amount of flesh decreases, so too does the concentration of fauna present (Smith, 1985; Thistle, 2003). This successional stage appears to undergo its own temporal succession as the fragments of tissue diminish in size (Smith and Baco, 2003). Scavengers of much smaller body size take over from larger scavengers, ending with fauna as small as copepods (Smith and Baco, 2003). The efficiency and rapidity of the

response of the fauna during the mobile-scavenger stage can result in small carcasses being stripped from whale soft tissues to bones in a matter of days with tissue removal rates reaching as high as 40-60 kgd^{-1}, indicating that this community is tailored to processing organic falls (Smith, 1985; Jones *et al.*, 1998; Smith and Baco, 2003). Larger whales may take between four months and 24 months to be stripped of flesh after the arrival of the carrion on the seafloor, depending on the size of the animal (Smith and Baco, 2003).

During the enrichment-opportunist stage or second stage, organically-enriched sediments and exposed bones are colonised by dense assemblages of heterotrophic macrofauna such as polychaetes and crustaceans (Levin *et al.*, 1994; Smith and Baco, 2003). Any remaining soft tissues and lipids within the bones are exploited during this stage (Smith and Baco, 2003). Tissues of primary consumers attending the carcass and detrital particles suspended in currents or deposited in sediments around the bones are also important nutritionally (Smith and Baco, 2003). The sediments in the immediate vicinity (one to three metres) of a whale fall experience an initial pulse in organic material that can be equivalent to roughly 2000 years of organic carbon flux (Smith and Baco, 2003). The enrichment-opportunist stage has a duration of months to less than two years depending on the size of the carcass (Smith and Baco, 2003).

Sediment macrofaunal densities can become as high as 20000 - 45000 m^{-2} during the enrichment-opportunist stage but species diversity is reduced within one metre of the skeletons (Smith *et al.*, 2002; Smith and Baco, 2003; Dahlgren *et al.*, 2004). A similar reaction has been typically noted at organically-enriched sediments in shallow waters (Pearson and Rosenberg, 1978; Bennett *et al.*, 1994; Levin *et al.*, 1994; Wiklund *et al.*, 2009b). At a Pacific whale fall, Smith and Baco (2003) observed extremely high densities of dorvilleid polychaetes such as *Ophryotrocha* spp. and the cumacean *Cumella* spp. Large numbers of gastropods and bivalves colonised sediments around whale skeletons and some of the bones were covered by the deep-sea whale-fall specialist polychaete *Vigtorniella flokati* Dahlgren *et al.,* 2004 (Smith and Baco, 2003; Dahlgren *et al.*, 2004). *V. flotaki* was seen clinging posteriorly to organic-rich bones and on sediments forming a writhing carpet at densities exceeding 8000 m^{-2}. Once the bones of the carcass begin to be exposed during this stage, the siboglinid genus *Osedax* may colonise them (more will be discussed on this genus later in this chapter).

Bacterial decomposition of the organic material, especially lipids within the whale bones, increases the sulfide availability and the community shifts to a sulfophilic stage (third stage) that usually persists for more than six years and up to decades (Bennett *et al.*, 1994; Naganuma *et al.*, 2001; Smith and Baco, 2003; Schuller *et al.*, 2004). Whale-skeleton compositions are thought to be 10% lipid and 25% protein on average but lipid content can be as high as 80% by weight (Allison *et al.*, 1991; Gage, 2003; Higgs *et al.*, 2011b). A 40-tonne whale carcass may

carry 2000-3000 kg of lipids in its skeleton (Lundsten *et al.*, 2010b). Early stages of lipid and organic-material decay involve aerobic heterotrophic bacteria depleting oxygen in the sediment or bones. Then anaerobic bacteria continue to decompose the organic material, using sulfate (rather than oxygen) as an electron acceptor, converting it to sulfide (Smith, 1992). It is also during this stage when free-living or endosymbiotic methanogens function (Allison *et al.*, 1991; Naganuma *et al.*, 1996; Smith and Baco, 2003; Treude *et al.*, 2009). These reducing, sulfide-rich conditions are then utilised by sulfide-oxidising symbionts as the substrate of chemosynthesis resulting in a large species-rich assemblage living on the skeleton as it emits sulfide, with the majority of fauna deriving nutrition from sulfide-based chemoautotrophy, via endosymbiosis or direct bacterial grazing (Smith, 1992; Bennett *et al.*, 1994; Kitazato and Shirayama, 1996; Deming *et al.*, 1997; Herring, 2002; Smith *et al.*, 2002; Smith and Baco, 2003; Tunnicliffe *et al.*, 2003). Over periods of several years, whale falls can create sulfidic conditions similar to those found at other chemosynthetic habitats such as cold seeps and hydrothermal vents (Treude *et al.*, 2009).

It has also been recently shown after analysing the composition of large whale skeletons from several species that the lipid content of whale skeletons are concentrated in the skulls and caudal vertebrae, and the cervical and thoracic vertebrae contain much less lipid (Higgs *et al.*, 2011b). Higgs *et al*. (2011b) also suggested that late-stage whale-fall communities in the deep sea would correspond mostly to these bones with high-energy availability. There is also a positive correlation between the size of carcass and the extent of the development of the sulfophilic community (Braby *et al.*, 2007). The macrofaunal communities on small skeletons, unlike on large skeletons, appear to be only sulfide-tolerant rather than chemoautotrophic (Smith and Baco, 2003). Juvenile skeletons appear to decompose and release the smaller reservoir of lipids quicker than those of adults (Smith and Baco, 2003). It appears that there is a minimum size/degree of calcification required for a whale skeleton to sustain chemoautotrophic communities for extended periods (years) (Smith and Baco, 2003). This stage emphasises the high degree to which organisms are adapted to feeding at organic falls as even the bones of the whale are utilised (Gage, 2003).

The first recognised chemoautotrophic assemblage on a whale skeleton was found in 1989 in the northeast Pacific (Smith *et al.*, 1989). Sulfide-oxidising bacteria such as species of *Beggiatoa* were observed growing in mats on the bones and also extended several centimetres into the sediment-water interface (Allison *et al.*, 1991; Bennett *et al.*, 1994; Deming *et al.*, 1997). *Beggiatoa* species typically live at an anaerobic-aerobic interface as they require sulfide as an energy source for carbon-dioxide fixation and oxygen for the oxidation of the sulfide (Deming *et al.*, 1997). Chemosynthesis in biofilms and bacterial-filamentous mats on animal and mineral surfaces provides food for large populations (hundreds of thousands) of grazing and

deposit-feeding animals (Kitazato and Shirayama, 1996; Naganuma *et al.*, 1996; Smith and Baco, 2003; Tunnicliffe *et al.*, 2003; Lundsten *et al.*, 2010a). Some of these bacterial mats can also be heterotrophic (Deming *et al.*, 1997).

Macrofaunal communities during the sulfophilic stage are large (exceeding 30000 individuals), species rich and trophically complex (Smith and Baco, 2003). The species diversity on large skeletons is higher than in any other deep-sea hard-substratum community including those of hydrothermal vents, cold seeps, manganese nodules and rocks, with an average 185 species per skeleton (Smith and Baco, 2003; Smith, 2006). This is thought to be explained by the unusually high trophic diversity on whale bones (Baco and Smith, 2003). Cumulative taxon richness at whale falls is primarily a product of time on the bottom, but other factors such as differences in oxygen levels may become more apparent with carcass age (Braby *et al.*, 2007; Lundsten *et al.*, 2010b). Bennett *et al*. (1994) observed that the sulfide-rich whale-skeleton faunal community during this stage was taxonomically distinct from the surrounding areas, with sometimes more than 97% of its individuals belonging to species that were very rare or absent from the background community. Fujiwara *et al*. (2007) also found similar results indicating that many of the animals attending whale falls are specialists. However, Lundsten *et al*. (2010) found the opposite; the majority of species found at whale falls are typical background deep-sea fauna opportunistically exploiting the organic enrichment that has resulted from the carcass. Whale skeletons and their associated chemoautotrophic assemblages may physically impact nearby sediment communities for years after the organic and sulfide enrichment effects of whale falls have dissipated by changing infaunal diversity and bioturbation (Smith *et al.*, 1998).

Whale-fall specialists appear to come from at least four different phyla and include whale-bone feeders (gastropods, siboglinids, munidopsids and sipunculids), bacterial grazers (limpets and dorvilleids), species utilising chemoautotrophic endosymbionts (bathymodiolins, vesicomyids and thyasirids), deposit feeders (ampharetids), facultative suspension feeders (bathymodiolins) and predators (polynoids) (Baco and Smith, 2003; Smith and Baco, 2003; Rouse *et al.*, 2004; Johnson *et al.*, 2010). Smith (2006) noted at least 32 species that appeared to be whale-fall specialists on Pacific whale falls. The siboglinid genus *Osedax* has been recorded from most whale falls studied so far and is one of the most prominent genera of whale-fall specialists. Sipunculids such as *Phascolosoma saprophagicum* Gibbs, 1987 and *Osedax* siboglinid worms feed directly on bone (Gibbs, 1987; Rouse *et al.*, 2004). *Rubyspira osteovora* Johnson *et al*., 2010 and *R. goffrediae* Johnson *et al*., 2010 of the Provannidae family also ingest bone particles but might also rely on thiotrophic bacteria to augment nutrition or as an alternative nutrition source once the bones are consumed (Braby *et al.*, 2007; Johnson *et al.*, 2010; Lundsten *et al.*, 2010a).

Molluscs and arthropods are the most speciose taxa at vents (Tunnicliffe *et al.*, 1998), molluscs at seeps whereas annelids account for 47-60% of all fauna at whale falls (Sibuet and Olu, 1998; Smith and Baco, 2003). Species that can colonise hard surfaces live directly on the bones, along with the filamentous bacterial mats (Smith *et al.*, 1989; Allison *et al.*, 1991; Deming *et al.*, 1997; Tunnicliffe *et al.*, 2003; Lundsten *et al.*, 2010a). A small percentage of the macrofauna found at the whale fall are found on bone surfaces lying below the sediment-water interface, while the majority occur on surfaces directly exposed to bottom water (Bennett *et al.*, 1994).

Whale falls at different depths are exposed to radically different environments caused by chemical, physical and biological factors (Dahlgren *et al.*, 2006). Taphonomic processes, such as the decomposition rate of bone, may also be strongly influenced by depth (Allison *et al.*, 1991; Dahlgren *et al.*, 2006). Shallow-water whale falls are surprisingly not well studied, with the first *in situ* experiment carried out in 2003 at 125 m off Sweden by Glover *et al*. (2005a) and Dahlgren *et al*. (2006), with several subsequent experiments at depths of approximately 30 m (Glover *et al.*, 2010). The carcass at 125 m was completely skeletonised after six months, slower than at deep-sea sites where this process can occur, for small cetacean carcasses, in as quickly as a few days (Jones *et al.*, 1998; Dahlgren *et al.*, 2006). Glover *et al*. (2010) suggested that the slow removal of the skin by scavengers and development of possibly toxic bacterial mats prevented the rapid consumption of flesh at the 30 m experiments. Fujiwara *et al.* (2007) found the opposite trend while observing 12 sperm whale carcasses at shallow depths. The succession was relatively rapid and the sulfophilic stage was shorter than that of deeper whale falls (Fujiwara *et al.*, 2007). This could possibly have been due to the biological decomposition being faster with the higher water temperatures at this site (12°C) versus 4°C at deep-sea sites, or the particular whale species used (Smith and Baco, 2003; Fujiwara *et al.*, 2007). Lundsten *et al*. (2010b) found that community differences at whale falls seem to be most strongly related to depth and water temperature, however environmental disturbances such as currents, turbidity flows, organic enrichment and sedimentation may also have a role (Braby *et al.*, 2007; Lundsten *et al.*, 2010b).

1.1.2 The impact of whaling on the faunal communities associated with whale falls

Whale populations are thought to be 10-50% of the totals prior to the beginning of industrial whaling in the 1800s (Clapham *et al.*, 1999; Smith and Baco, 2003) (Table 1.1). The impact of whaling was especially catastrophic for specific populations e.g. blue whales are still thought to be less than 2% of pre-whaling populations (Pershing *et al.*, 2010) (Table 1.1). Over one million whales were killed from 1920 to 1986, resulting in significant reductions in the population numbers of several species and presumably in the number of carcasses reaching the seabed

(Allen, 1980; Butman *et al.*, 1995; Gage, 2003). Pershing *et al*. (2010) calculated that the total carbon flux from eight baleen whale taxa is currently 2.8 x 10^4 tons C yr^{-1} and using estimates of pre-whaling abundance, the total flux would have been an order of magnitude greater or 1.9 x 10^6 tons C yr^{-1}. This is consistent with the calculations by Jelmert and Oppen-Bernsten (1996) showing that prior to whaling, there were 3.9 x 10^5 carcasses sinking per year, making whale falls six times more abundant than at present. It is thought that the decline in whale falls has had serious impacts on the ocean's ability to store and sequester carbon, and the availability of nutrients (Pershing *et al.*, 2010). This decline in cetacean numbers may also have had a significant impact on deep-sea benthic communities by redistributing and removing the source of organic-rich habitat islands and sulfide-rich dispersal 'stepping stones' (Butman *et al.*, 1995; Smith and Baco, 2003), although Jelmert and Oppen-Bernsten (1996) disagree with this theory instead stating that the pre-whaling contribution to biomass and spatial distribution was insignificant. Natural whale mortalities result in the gradual descent of carcasses to the deep-sea floor in regions where specific species of whales spend the most time, but whaling increased the number of carcasses being delivered to the seafloor in areas favourable to hunting, such as the Antarctic region (Butman *et al.*, 1995). This however significantly decreased after the early 1900s as new whale-processing technology allowed more efficient hunting and greater use of whale bone for oil, bone meal and fertiliser resulting in many fewer carcasses reaching the seafloor (Butman *et al.*, 1995). Current major impacts on whale populations are entanglement in fishing gear and ship strikes but to a lesser extent, aboriginal and scientific whaling, habitat degradation, pollution and disease (Clapham *et al.*, 1999).

The abundance of marine mammals pre-whaling lead to a specific set of fauna adapting entirely to surviving solely on the carrion (Tunnicliffe *et al.*, 2003) and now some of these whale-fall specialists may have been driven to extinction due to the spatial interruption, if not obliteration, of whale-fall habitats over the last 200 years (Butman *et al.*, 1995). It is thought that due to the varying degrees of whaling in differing ocean basins, the extent of whale-fall specialist extinctions may differ greatly with the North Atlantic being the greatest (30-50%), the Southern Ocean accelerating and the Northeast Pacific being the least severe (Smith, 2006). Fauna of other reducing environments such as hydrothermal vents, cold seeps and wood falls may also have had their dispersal capabilities reduced leading to their local extinction and ultimately leading to a reduction in deep-sea biodiversity (Butman *et al.*, 1995). Those species most dependent on whale falls are the most likely to become extinct, raising the possibility that whale falls only retain the most generalised subset of their original biota (Smith and Baco, 2003).

Considering today's rebounding whale populations since international intervention in the mid-1960s and the worldwide moratorium in 1986, if it is assumed that whale skeletons are able to support communities for up to five years and that only half of the whales that die each year sink

to the seafloor, Smith *et al*. (1989) calculated that there should be an average distance of nine kilometres between Gray-whale skeletons within their Pacific range depending on the successional stage. If a dispersal larval stage lasts 30 days, a direct line transit of 300 m per day would successfully take larvae to the next skeleton. Actual bottom currents are much faster (typically between 5-10 cm per second) (Smith *et al.*, 1989). In reality however, whale falls should be more closely spaced than calculated above, as whale mortalities are non-randomly distributed and are likely to be concentrated along whale migratory routes and in feeding grounds which occur near ocean margins (Tyler, 1988; Smith *et al.*, 1989; Butman *et al.*, 1995; Smith and Baco, 2003). However, studies into whale-fall ecology and biogeography with current populations may still fail to reveal the identity and characteristics of the species that may have already become extinct as a consequence of whaling (Butman *et al.*, 1995; Smith and Baco, 2003). This highlights the need to explore these poorly-known ecosystems prior to further anthropogenic alteration if we wish to preserve these unique deep-sea ecosystems (Smith and Baco, 2003).

Table 1.1. Historic (pre-whaling) and current abundances of nine species or species groups of whales.
As most available estimates refer to specific stocks, individual stock abundances were totaled for each species to yield global population numbers. 'Current Average' reflects the most recent estimates and may not reflect actual 2010 population numbers. (a) Average of estimates from Evans (1993) and Pershing *et al.* (2010). (b) Average of estimates from (Alter *et al.*, 2007) and (Stockton and DeLaca, 1982; Herring, 2002; Pershing *et al.*, 2010). (c) Average of estimates from (IWC, 2010) and (Pershing *et al.*, 2010). (d) Average of estimates from (Wolff, 1979; Harrold *et al.*, 1998; Rugh *et al.*, 2005) and (Pershing *et al.*, 2010). (e) Estimates from Whitehead and Planck (2002).

1.2 The bone-boring genus *Osedax*

1.2.1 Taxonomy

Osedax rubiplumus Rouse *et al*., 2004, *O. frankpressi* Rouse *et al*., 2004 and thus the genus, *Osedax*, were described in 2004 from a Gray-whale carcass off California at 2981 m but in fact specimens that morphologically correlate with the genus were perhaps first observed attached to submersible-recovered whale bones in the Oregon Subduction Zone in 1994 (E. Southward, notes and personal observation communicated via A. Glover)(Goffredi *et al.*, 2004; Rouse *et al.*,

Common name	Species	Historic Average[a]	Current Average[c]	% of Historic Estimate
Blue	*Balaenoptera musculus*	340280	4727	1.4
Bowhead	*Balaenoptera mysticetus*	89000	9450	10.6
Fin	*Balaenoptera physalis*	655200	71400	10.9
Gray	*Eschrichtius robustus*	60300[b]	18968[d]	31.5
Humpback	*Megaptera novaeangliae*	173350	52835	30.5
Minke	*Balaenoptera acutorostrata*	563500	738850	131.1
Right	*Balaena glacialis*	92050	9239	10.0
Sei	*Balaenoptera borealis*	324150	181490	56.0
Sperm	*Physeter macrocephalus*	1110000	452000[e]	40.7
Total		3407830	1538959	45.2
Total minus Minke whale		2844330	800109	28.1

2004; Hilario *et al.*, 2011) (Fig. 1.1). *Osedax* polychaete worms were observed protruding from whale bones in large numbers (Rouse *et al.*, 2004) (Fig. 1.2). The genus is now known to span the bathymetric range of 21 to 3000 m (Braby *et al.*, 2007; Glover *et al.*, 2013). *O. mucofloris* Glover *et al*., 2005 was the first species to be found in shelf-depth waters (125 m) and in the Atlantic Ocean (North Sea) (Glover *et al.*, 2005b; Schander *et al.*, 2010) (Figs. 1.1 and 1.2). This was also the first species of *Osedax* to be cultured on bones in a marine laboratory (Glover *et al.*, 2005b). The fact that *Osedax* species have now been found in the Atlantic, Pacific and Southern Ocean suggests a global distribution (Rouse *et al.*, 2004; Glover *et al.*, 2005b) (Fig. 1.1). There are currently 23 species of *Osedax* known worldwide not including those described in this thesis.

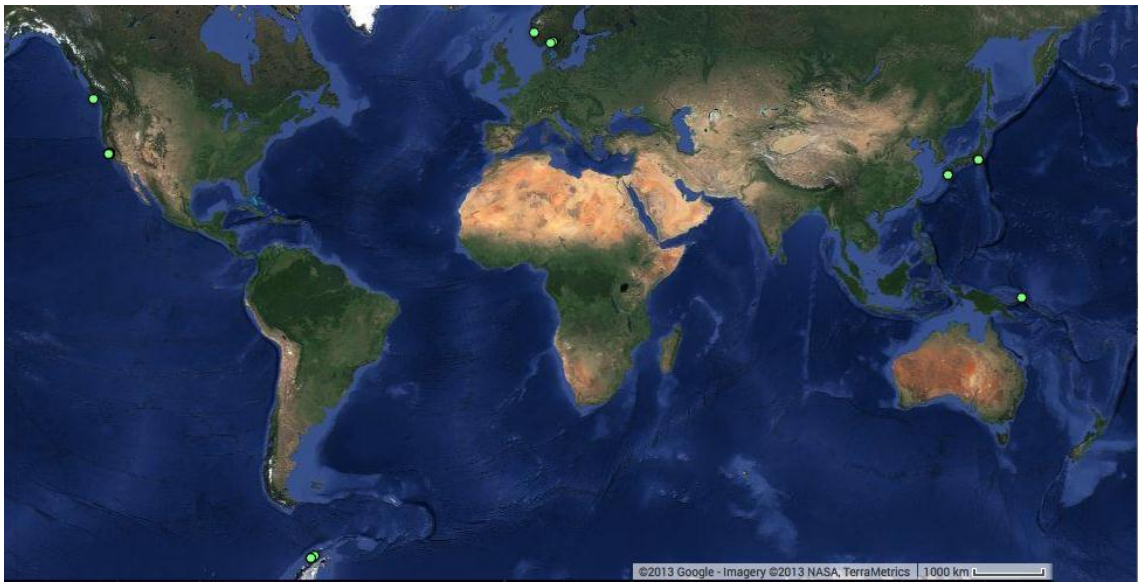

Figure 1.1 Known locations of the genus *Osedax*. Locations (green dots) have been taken from the literature and exclude any new records reported in this thesis. More than one species have been found at some locations.

Morphologically, *Osedax* has a crown which is composed of the oviduct and palps, and a contractile trunk which houses muscles, glands, a heart and blood vessels (Rouse *et al.*, 2004) (Fig. 1.2). The palps can vary in colour between the species but are usually some variation of red and are usually visible projecting above the bacterial mats and sediment on the bones (Rouse *et al.*, 2004; Glover *et al.*, 2005b; Fujikura *et al.*, 2006; Rouse *et al.*, 2008) (Fig. 1.2). *Osedax* are housed within a tube, which is usually gelatinous or made of mucous (*Osedax mucofloris*) and can retract completely into the tube in the bone upon disturbance by using a well-developed longitudinal muscle (Rouse *et al.*, 2004; Glover *et al.*, 2005b; Fujikura *et al.*, 2006; Rouse *et al.*, 2008; Huusgaard *et al.*, 2012). *O. frankpressi* and *O. rubiplumus* maintain populations between 10^5 and 10^6 adult females and can reach densities of 3-20 individuals cm^{-2} (Rouse *et al.*, 2004; Vrijenhoek *et al.*, 2008b). The abundance of *Osedax* species at the sites they have been observed at suggests that these worms may play an integral role in the cycling of

large organic inputs like vertebrate bones in the surrounding deep-sea environments (Rouse *et al.*, 2004).

Phylogenetic evidence places the entire *Osedax* clade as a highly-derived sister group to reducing-habitat siboglinids (Glover *et al.*, 2005b). This derived nature is in the significant morphological differences between *Osedax* species and other siboglinids; the loss of the opisthosome in adults, and the evolution of a branching root system (Glover *et al.*, 2005b). *Osedax* species do still have some similarities to siboglinids: the presence of palps, the loss of segmentation, the absence of a gut, and the nature of the chaetae on the opisthosome of the males (Glover *et al.*, 2005b). Genetic evidence suggests that dispersal events, rather than vicariance (as in obligate hydrothermal-vent taxa), have played a stronger role in shaping the distribution of known *Osedax* species with one possible dispersal route for shallow-water *Osedax* species being a trans-Arctic invasion between North Pacific and North Atlantic Ocean basins (Glover *et al.*, 2005b). Nucleotide-sequence analysis indicated that *O. frankpressi* and *O. rubiplumus* diverged from a common ancestor around 42 million years ago in the late Eocene (Rouse *et al.*, 2004). Molecular analyses of COI suggest that *O. mucofloris* is separated from *O. frankpressi* and *O. rubiplumus* by CO1 genetic distances of 18-23% and has high dispersal rates (Glover *et al.*, 2005b). This provides support for the idea of whale falls acting as 'stepping stones' for the dispersal of siboglinids (Glover *et al.*, 2005b). *O. frankpressi* and *O. rubiplumus* have mitochondrial diversities as great or greater than those found in eastern Pacific vent tubeworms (Vrijenhoek, 2010).

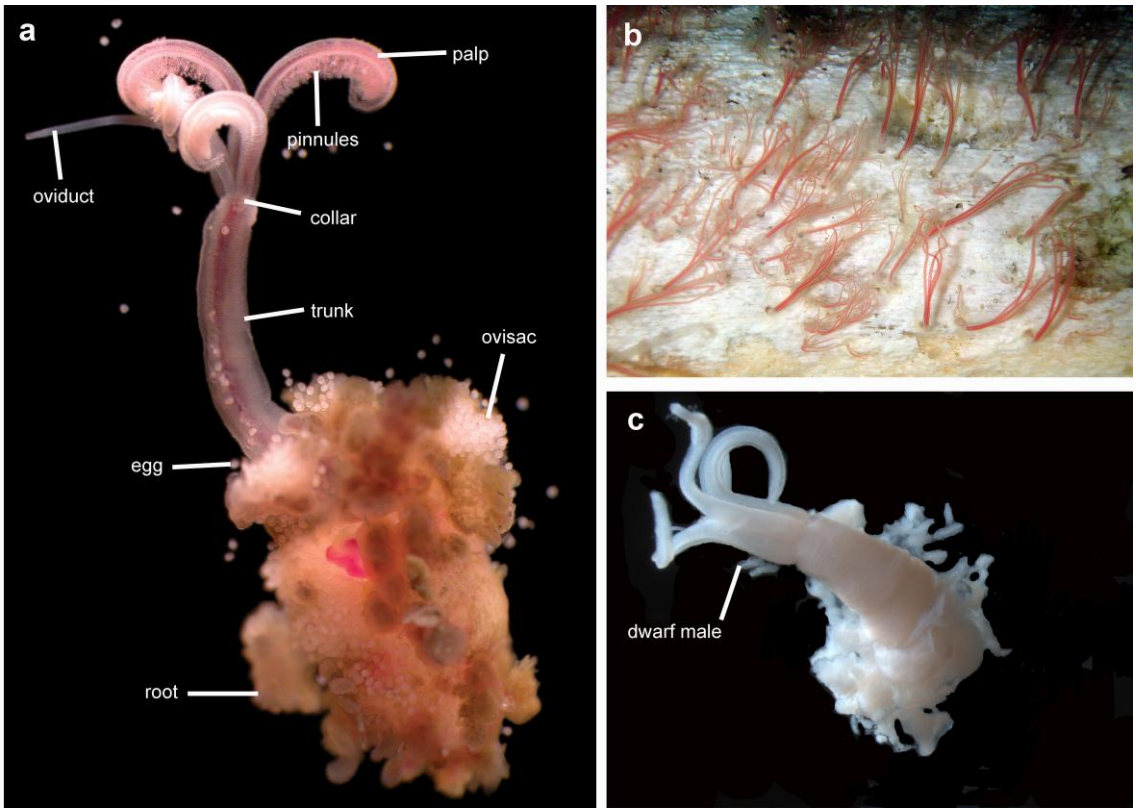

Figure 1.2 *Osedax.* (a) Photograph showing the general anatomical structures of the genus *Osedax* using a live dissected specimen of *O. mucofloris* as an example. Image courtesy Adrian Glover. (b) *Osedax antarcticus* in situ in bone. Image courtesy Thomas Dahlgren. (c) *Osedax antarcticus* with dwarf males present. Image courtesy Adrian Glover.

1.2.2 Bacterial symbiosis and nutrition in the genus *Osedax*

Members of the genus *Osedax* lack digestive systems like other siboglinids but also lack a trophosome, the organ that typically houses symbiotic bacteria in tube-dwelling annelids (Rouse *et al.*, 2004; Glover *et al.*, 2005b; Hilario *et al.*, 2011) (Fig. 1.2). Instead they contain a 'bulbous posterior ovisac covered by a sheath of coloured tissue that branches into a vascularised root system (branching or lobulate) that invades the bone marrow' creating a shallow depression (Rouse *et al.*, 2004; Glover *et al.*, 2005b) (Fig. 1.2). Katz *et al*. (2011) however, has called for a review of the above scientific view of the absence of a trophosome in *Osedax*, stating that the roots region, the ovisac region and the trunk form one continuous compartment with the trophosome situated at the posterior end of this compartment. The term 'trophosome' should be applied to the posterior symbiont-housing tissue in *Osedax* because of the organisational and functional similarities of tissue that contains endosymbionts, regardless of the origin of the tissue (Katz *et al.*, 2011).

Analyses by Rouse *et al*. (2004) of the sheaths of *Osedax frankpressi* and *O. rubiplumus* revealed numerous bacteriocytes containing large rod-shaped bacteria of the order

Oceanospirillales, known for the heterotrophic aerobic degradation of complex organic molecules and therefore responsible for the nutrition of this genus. These symbiotic microbes provide a nutritional bridge between high-energy compounds and the exploitation by *Osedax* species (Goffredi *et al.*, 2005; Goffredi *et al.*, 2007; Hilario *et al.*, 2011). The heterotrophic bacteria produce proteolytic enzymes that are capable of hydrolysing diverse carbon sources that are dominated by collagen and cholesterol from within mammal bones (Goffredi *et al.*, 2005; Goffredi *et al.*, 2007; Jones *et al.*, 2008). Data suggest that both host and endosymbionts rely on animal bones for nutrition, and fatty acids synthesised by the endosymbionts are transferred to the host (Goffredi *et al.*, 2005; Goffredi *et al.*, 2007; Katz *et al.*, 2010). Recently it has been speculated that nutrition must be provided to the symbionts by the host and not the other way around (Katz *et al.*, 2011). The symbionts are housed in an internal tissue, isolated from the presumed nutrient source by several host cell layers and cannot directly access organic bone material. Bone needs to be broken down and taken up by the host before symbionts can use it.

The diversity and concentration of *Oceanospirillales* found in *Osedax* worms varied among host species and across time, with *Osedax mucofloris* showing the highest among those surveyed (Glover *et al.*, 2005b; Goffredi *et al.*, 2007; Verna *et al.*, 2010). Goffredi *et al*. (2007) found that juvenile *O. frankpressi* had only a single endosymbiont phylotype but adults had several endosymbiont phylotypes. Analyses of spawned oocytes of *O. rubiplumus* and *Osedax* sp. 'orange collar' by Rouse *et al*. (2009), showed no evidence of bacterial endosymbionts required by female worms for nutrition, indicating that the bacteria must be acquired later from the environment as seen in other siboglinids. Verna *et al*. (2010) and Goffredi *et al*. (2007) concluded from observed diversity and distribution patterns that the endosymbionts are transmitted horizontally from the environment with repeated infection events occurring as the host root tissues grow into the bones. Factors that can influence endosymbiont diversity and distribution include host specificity, endosymbiont competition and the genetic variability of the free-living endosymbiont population due to environmental factors (Goffredi *et al.*, 2007; Verna *et al.*, 2010).

This finding of heterotrophic symbiosis in *Osedax* species differs markedly from the chemolithoautotrophic symbioses in other deep-sea animals that rely on sulfide- or methane-oxidising endosymbionts and hence suggests that the evolutionary history of bacterial symbioses among the Siboglinidae is very varied (Van Dover, 2000; Rouse *et al.*, 2004; Goffredi *et al.*, 2005). The reliance on the bones of marine mammals, hydrocarbon degradation and the morphology of the symbiont-bearing ovisac and root system of *Osedax* spp. make this particular form of bacterial endosymbiosis unique (Rouse *et al.*, 2004). The interiors of bones are sulfide-rich (Deming *et al.*, 1997) and *Osedax* spp. have been observed growing in regions

of blackened bone (Glover *et al.*, 2005b). The black colour is thought to be as a result of the reduction of iron by sulfur in an anaerobic environment (Kitazato and Shirayama, 1996). Even though *Osedax* species are known to exhibit heterotrophic nutrition, there is no evidence as yet to suggest that these animals are not also using sulfide-based chemoautotrophy; further investigation is required (Glover *et al.*, 2005b). *Osedax mucofloris* also had epibiotic bacteria present over the surfaces of the trunk, palps and pinnules (Verna *et al.*, 2010). The role of these epibionts is currently unknown.

Katz *et al*. (2010) and Higgs *et al*. (2011a) proposed that *Osedax* species bore into the bone using chemical corrosion, as *Osedax* do not have any means by which they can bore mechanically. The surfaces of the roots are highly convoluted and the epidermal cells have an extensive microvillous border which is well equipped for the uptake of dissolved compounds and the secretion of digestive enzymes (Katz *et al.*, 2010). Also the epidermal cells of the root region are extremely rich in rough endoplasmic reticulum and possess a large nucleolus, both characteristics for intense protein synthesis highlighting the adaption of the epidermis of *Osedax* (Katz *et al.*, 2010). Chemical corrosion of the bone was confirmed recently when it was found that epithelial cells of the roots and ovisac are able to secrete acids, which is proposed to dissolve the carbonate matrix of the bone, then allowing the absorption of bone-derived nutrients (Tresguerres *et al.*, 2013). *Osedax mucofloris* features a highly-adapted respiratory system which is optimised to this unique nutrition adaptation with roots embedded in anoxic bones and elevated oxygen demand due to aerobic heterotrophic endosymbionts (Huusgaard *et al.*, 2012).

Over an eight-month study by Higgs *et al.* (2011a), 6% of Minke whale bone was degraded suggesting that the presence of *Osedax* can lead to the rapid degradation of whale bones. *Osedax* are therefore a critical factor in regulating the longevity of whale bones and thereby affect the succession of associated megafauna, making this a foundation species for whale-fall communities (Braby *et al.*, 2007). The borings of *Osedax mucofloris* were wide, shallow sub-surface cavities restricted to the densest layer of bone, had a maximum penetration of 2.63 mm and were at least one order of magnitude bigger than microbial borings found in bone (Higgs *et al.*, 2010). *Osedax* species display several root morphotypes which would presumably correspond to differing boring morphologies (Higgs *et al.*, 2010; Kiel *et al.*, 2013).

The successful colonisation of bones by *Osedax* is likely to be dependent on a range of factors such as the size and food content of the bone, bone calcification, disturbance or removal by scavenging organisms, competition with bacterial mats and the degree of sedimentation (Goffredi *et al.*, 2007; Glover *et al.*, 2008; Amon *et al.*, 2013). Different species of *Osedax* have been observed to show preference to different types of bones in whales. *Osedax rubiplumus*

tends to favour cortical bone and *O. frankpressi* prefers vertebrae (Goffredi *et al.*, 2007). *O. japonicus* Fujikura *et al.,* 2006 was found growing upon cephalic tissues, blubber and bones of a sperm whale carcass off Japan at approximately 200 m (Fujikura *et al.*, 2006). It was unclear whether this was due to the whale species, the *Osedax* species or both (Fujikura *et al.*, 2006). Physiological differences such as methane, sulfide and oxygen levels, temperature, pressure, and depth may play a role in the ecological distribution of *Osedax* species (Goffredi *et al.*, 2007). These environmental boundaries may be influenced by the specific nature of their integration with local microbes (Goffredi *et al.*, 2007; Amon *et al.*, 2013). This genus is predated upon by tanner crabs and brachyuran crabs (Rouse *et al.*, 2008; Vrijenhoek *et al.*, 2008a).

Osedax spp. were previously thought to be whale-fall specialists but it is now known that they are not limited to whale bones but can also successfully live on the bones of other vertebrates such as mammalian quadrupeds, pinnipeds, birds and teleosts (Jones *et al.*, 2008; Vrijenhoek *et al.*, 2008a; Schander *et al.*, 2010; Kiel *et al.*, 2011; Rouse *et al.*, 2011). Vertebrate bones (excluding whales) with lower lipid concentrations may provide a very rare but still possible food resource for *Osedax* species (Jones *et al.*, 2008; Vrijenhoek *et al.*, 2008a). A study by Rouse *et al*. (2011) using shark cartilage showed no successful colonisation by *Osedax.* This was thought to be because the experiments were too short in duration to allow for settlement and also because small bones and cartilage are not as likely to be used by *Osedax* as they usually do not contain enough collagen and may also be easily buried in sediments (Glover *et al.*, 2008; Vrijenhoek *et al.*, 2008a). Even if many fish bones are lost to scavengers or burial, *Osedax* should be able to exploit at least a portion of this immense resource which constantly falls to the seafloor globally (Rouse *et al.*, 2011). The colonisation of vertebrate bones indicates that *Osedax* do not require particular substrates found only in cetacean carcasses but can exploit a variety of habitats not limited to areas where whales are plentiful, and this possibly helps these worms to flourish in a world with fewer cetaceans (Jones *et al.*, 2008; Vrijenhoek *et al.*, 2008a).

1.2.3 The reproductive biology and life history of the genus *Osedax*

In contrast to other siboglinids, most species of *Osedax* shows marked sexual dimorphism in size. *Osedax* have dwarf males ranging in size from 0.1-1.1 mm with the abundance depending on the species, whereas females typically have trunks 3-4 mm long and palps 1-2 cm long (Rouse *et al.*, 2004; Rouse *et al.*, 2008; Vrijenhoek *et al.*, 2008b; Worsaae and Rouse, 2010) (Fig. 1.2). Tubes of the females of *Osedax frankpressi* and *O. rubiplumus* contained numerous dwarf males (up to 607 each) filled with spermatids and sperm that clustered around the oviducts of females creating a skewed male-to-female sex ratio of 17:1, assuring sperm availability (Rouse *et al.*, 2004; Whiteman, 2008; Worsaae and Rouse, 2010; Hilario *et al.*,

2011). It appears that females of *Osedax* accrue males over time or attract more when larger (Rouse *et al.*, 2004; Rouse *et al.*, 2008; Vrijenhoek *et al.*, 2008b; Whiteman, 2008). Not all females of *Osedax* have harems of dwarf males however e.g. immature females (Rouse *et al.*, 2004; Vrijenhoek *et al.*, 2008b; Rouse *et al.*, 2009). Recruitment of these males was induced by rearing larvae with adult females in *O. japonicus* (Miyamoto *et al.*, 2013).

Rouse *et al*. (2004) observed that the paedomorphic males retained morphological traits similar to that of siboglinid trochophore larvae, including an anterior ciliary band and posterior opisthosomal chaetae used to attach to the females tubes (Vrijenhoek *et al.*, 2008b; Worsaae and Rouse, 2010; Miyamoto *et al.*, 2013). Males feed off yolk droplets which decrease with growth and are typically full of spermatozoa that increase with growth (Whiteman, 2008). There was no evidence from light and confocal microscopy of a mouth or gut in males (Rouse *et al.*, 2009; Worsaae and Rouse, 2010). Occasional 'ghosts' of males lacking sperm and yolk and presumably dead, have also been observed (Vrijenhoek *et al.*, 2008b). Male longevity is not understood as yet, so it is currently thought that males simply deplete their yolk reserves to manufacture sperm, then die, while new males are continuously recruited from the larval pool (Vrijenhoek *et al.*, 2008b).

Sex may be environmentally determined in *Osedax*, with the larvae that settle on exposed bones maturing as females and those that then land on females becoming males as is seen in the echiuran annelid *Bonellia viridis* Rolando, 1821 (Rouse *et al.*, 2004; Glover *et al.*, 2005b; Rouse *et al.*, 2008; Vrijenhoek *et al.*, 2008b). When sexually undifferentiated *Bonellia* larvae land on the extended feeding proboscis of a female, they are transformed into dwarf males by the action of a masculinising hormone, bonellin (Berec *et al.*, 2005; Rouse *et al.*, 2008). Whiteman (2008) hypothesised that the ephemeral nature of bones as a food resource, coupled with their unpredictable locations, and the competition and succession among *Osedax* species has driven the extreme sexual size dimorphism and evolution of environmental sex determination in these taxa.

Osedax mucofloris is believed to reproduce continuously like *O. rubiplumus* and *Osedax* sp. 'orange collar' females, which also have high fecundities (Dahlgren *et al.*, 2006; Rouse *et al.*, 2009). *Osedax* are thought to be more fecund that most other siboglinids in part due to the large proportion of the female bodies of *Osedax* worms dedicated to the ovaries (Rouse *et al.*, 2004; Glover *et al.*, 2005b; Fujikura *et al.*, 2006; Rouse *et al.*, 2009; Hilario *et al.*, 2011). These traits permit the exploitation of widely-scattered and ephemeral vertebrate-bone habitats by allowing rapid colonisation, rapid growth rates and asynchronous reproduction as seen in other siboglinids (Lutz *et al.*, 1994; Tyler and Young, 1999; Glover *et al.*, 2005b; Jones *et al.*, 2008; Rouse *et al.*, 2008; Vrijenhoek *et al.*, 2008a; Rouse *et al.*, 2009). Allozyme analysis and

mitochondrial diversity of the male harems and host females indicated random sexual mating with no inbreeding and that as many as 10^6 females contributed to a common larval pool and males were not supplied by a limited number of neighbouring females (Vrijenhoek *et al.*, 2008b).

Fertilisation is presumed to be internal as the males are found within the lumen of the female's tube (Rouse *et al.*, 2008). Mature sperm gather anteriorly in the body of the male and a ciliated sperm duct runs into the head where sperm exits the male, but the mechanism of sperm delivery and the site of fertilisation are still unknown (Rouse *et al.*, 2008; Worsaae and Rouse, 2010). Fertilisation rates of the spawned oocytes of *Osedax* sp. 'orange collar' varied between 0-100 % but most females showed nearly 100 % fertilisation rates (Rouse *et al.*, 2009). Oocytes are produced in females with minimum trunk widths of 0.3 mm and are held in the ovisac which is connected to the oviduct within the trunk region (Rouse *et al.*, 2004) (Fig. 1.2). *Osedax japonicus* was observed with several hundred eggs of around 100 µm in the oviduct with transparent mucus surrounding the bodies (Fujikura *et al.*, 2006) and *O. mucofloris* released more than 100 eggs from the ovisac upon disturbance (Glover *et al.*, 2005b). *O. rubiplumus* females were observed at 1820 m freely spawning hundreds of large oocytes (Rouse *et al.*, 2009). Prior to emission, white oocytes filling the oviduct were visible and each spawning event lasted a few minutes and consisted of a series of emissions of loosely connected streams which rapidly dissipated in the current (about 60 seconds long) interrupted by pauses of 20-30 seconds (Rouse *et al.*, 2009). Females of *Osedax* sp. 'orange collar' were observed in aquaria spawning an average of 335 (±130) eggs in continuous streams with occasional pauses per day (Rouse *et al.*, 2009). This number varied greatly however, indicating that not all females spawned every day (Rouse *et al.*, 2009).

The fertilised oocytes became free-swimming ciliated trocophore larvae after 24 hours, which swam for nine to sixteen days before settling (Rouse *et al.*, 2009; Miyamoto *et al.*, 2013). The ciliation observed on larvae may point to locomotory abilities which aid dispersal (Rouse *et al.*, 2009). They showed marked similarity to the *Osedax* dwarf males removed from the female tubes but lacked the distinguishing presence of sperm and spermatids in their bodies (Rouse *et al.*, 2009). This larval life span appears to be shorter than in closely related siboglinids (Rouse *et al.*, 2009). *Osedax* are capable of colonisation and growing to sexual maturity in as little as one month to three months (Glover *et al.*, 2005b; Rouse *et al.*, 2008; Vrijenhoek *et al.*, 2008b; Miyamoto *et al.*, 2013).

The post-embryonic development of *Osedax japonicus* has recently been deduced (Miyamoto *et al.*, 2013). The larvae were observed to settle on the bones, then elongate their bodies and crawl around on the bones instead of swimming (Miyamoto *et al.*, 2013). Larvae still had a prototroch,

gut, yolk and two pairs of chaetae at this point. Mucus was secreted to create a tube one day after settlement and two ventral palps began to develop from the dorsal side of the prostomium (Miyamoto *et al.*, 2013). A heart began to beat two days after settlement and the root began to dig into the bone. The trunk and ventral palps elongated four days after settlement. Infection of the root by symbiotic bacteria occurred shortly after this stage. Pinnules projected from the ventral palps seven days after settlement. Juvenile worms were complete morphologically ten days after settlement but only extended their bodies outside of the bone two weeks after settlement (Miyamoto *et al.*, 2013).

Recruitment in *Osedax* may be impacted by environmental disturbances and these in turn are much higher in shallow water according to Braby *et al*. (2007). *Osedax* spp. populations at a whale fall at 385 m were much slower recruiting (approximately nine months) than at depths of 1018 m and 1820 m (approximately two months) implying that shallower depths may make recruitment rarer (Braby *et al.*, 2007). *Osedax frankpressi* and *O. rubiplumus* females with varying trunk widths (0.2-0.5 mm) have been observed on the same carcass suggesting ongoing recruitment (Rouse *et al.*, 2004). *O. rubiplumus* is by far the largest species in the genus found thus far and is thought to be an early and short-term coloniser (Braby *et al.*, 2007; Rouse *et al.*, 2009). *O. frankpressi* was observed on a whale fall at 2893 m off California, replacing *O. rubiplumus* on the bones (Braby *et al.*, 2007; Lundsten *et al.*, 2010b). *Osedax* sp. 'spiral' was found by Braby *et al*. (2007) on this whale fall also and appeared to be a late successional species that attacked sediment-covered bone fragments with its filamentous roots. Lundsten *et al*. (2010b) reported the initial colonisation by *Osedax* sp. 'orange collar' at another whale fall, succeeded five months later by *O. roseus* Rouse *et al.,* 2008. *Osedax* sp. 'yellow patch' were observed also and may be a late successional species (Lundsten *et al.*, 2010b). These two examples may be evidence for the temporal succession of *Osedax* species, however the mechanisms responsible (dispersal, metabolic requirements and others) are still unclear (Braby *et al.*, 2007). Temporal succession may suggest that females of each species have a relatively short period of time in which to reproduce (Whiteman, 2008). Species may be segregated with depth and time; *O. japonicus* was found south of Japan at 220-250 m, but not at 925 m in Sagami Bay (Pradillon *et al.*, 2009). Instead 8 other species of *Osedax* arrived successively on the bones. Several of those species also occur off California indicating a wide geographic distribution. Similar segregations with depth have also been found off California.

1.2.4 The fossil evidence of the genus *Osedax*

Attempts to determine the evolutionary age of the genus *Osedax* lead to two scenarios: 1) *Osedax* split from its siboglinid relatives about 45 million years ago when archeocete cetaceans first appeared and then diversified during the late Oligocene and early Miocene when toothed

and baleen whales occurred or 2) *Osedax* split from its siboglinid relatives during the Cretaceous (130 million years ago) and began to diversify during the Paleocene, at least 20 million years before the origin of large marine mammals (Vrijenhoek *et al.*, 2009; Kiel *et al.*, 2010). Higgs *et al*. (2010) used micro computed-tomography (CT) to ascertain the morphology of borings produced by *Osedax mucofloris* on a Minke whale bone exposed on the seafloor for eight months. These findings were used to allow for the identification of *Osedax* activity at a fossil whale fall from the Pliocene of Italy and hence aided the deciphering of the evolutionary history of the genus *Osedax* (Higgs *et al.*, 2010; Higgs *et al.*, 2011c). The fossil borings were distinguished from those of other known borers by several diagnostic features (Higgs *et al.*, 2011c). This study also provided the first evidence of *Osedax* in the Mediterranean.

Kiel *et al*. (2010) found fossil whale bones from early Oligocene sediments in Washington that showed traces similar to those made by members of the genus *Osedax* today. The geologic age of these fossils coincided with the radiation of whales, which strengthens the idea of an evolutionary link between *Osedax* and its main modern substrate (Kiel *et al.*, 2010; Hilario *et al.*, 2011). Major portions of the skull were corroded away, likely due in part to the activities of *Osedax* (Kiel *et al.*, 2010). The traces attributed to *Osedax* started as boreholes with diameters of 0.10-0.45 mm on the surface and lead to cavities inside the bone (Kiel *et al.*, 2010). This size was consistent with the sizes of small species of *Osedax*. The boring density was high with distances between boreholes of 0.7-4.4 mm (Kiel *et al.*, 2010). Muniz *et al.* (2010) also found tubular borings in a fragment of the neurocranium of a fossil baleen whale in Spain, thought to be from the osteophagous behaviour of annelids or sipunculids. The maximum observed length was 40 mm, the diameters varied between 0.9 and 1.9 mm and boring density was high with individual tubes only 1.0-3.0 mm apart (Muniz *et al.*, 2010).

However, Cretaceous origins are supported by the discovery of plesiosaur skeletons associated with communities of small molluscs, showing that these reptile skeletons supported invertebrate communities resembling those colonising deep-sea whale skeletons today (Kaim *et al.*, 2008; Kiel, 2010). Also Kiel *et al*. (2011) have provided the first evidence of *Osedax* living on fossil Oligocene marine flightless diving bird bones (family Plotopteridae). Boreholes were observed in significantly higher densities on the bird femur than previously seen on mammal bones with up to 40 individuals cm^{-2} (Kiel *et al.*, 2010; Kiel *et al.*, 2011). Kiel (2013) also found evidence of *Osedax* in Oligocene whale teeth and fish bones. These findings have implications for the evolutionary age of the genus *Osedax* as marine birds and large fish have existed continuously since the Cretaceous, enabling this genus to survive during the Paleocene, after the disappearance of large marine reptiles at the end of the Cretaceous and before the rise of whales in the Eocene (Kiel *et al.*, 2011; Rouse *et al.*, 2011). These two recent discoveries have removed

a significant obstacle to the Cretaceous-*Osedax* hypothesis (Kiel *et al.*, 2011; Rouse *et al.*, 2011).

1.3 Wood Falls

1.3.1 The ecology of wood falls

Sunken parcels of plant remains including wood, leaves, bark, twigs, husks of coconuts, fruits, seagrass and *Sargassum* provide important oases of organic enrichment in the deep sea (Wolff, 1979; Bernardino *et al.*, 2010). The precise spatial and temporal locations of wood and other plant falls are unknown but most are found in tropical regions, especially associated with monsoon, hurricane and typhoon seasons when large amounts of plant debris can be carried down rivers ending up in the deep sea (Wolff, 1979; Scheltema, 1994). Terrestrial plant inputs to the deep sea are also noticeable in temperate areas during the spring thaw of rivers. The amounts of terrestrial plant material entering the deep sea may be increasing due to the intensifying of the monsoon and storms as result of climate change but this may still be inconsequential when the last few millennia are considered. Globally the significance of wood falls to the overall energy budget of the deep-sea environment and carbon mineralisation on the seafloor is still obscure (Gage, 2003). The fossil record for wood falls with associated chemosynthetic fauna is sparse with only about 30 sites from the late Eocene to early Miocene known and two Jurassic commmunities (Kiel and Goedert, 2006; Kaim *et al.*, 2008).

When deposited in the deep sea, sunken wood will undergo biodegradation resulting in oxygen being depleted within the wood and the attraction of sulphate-reducing bacteria (Palacios *et al.*, 2006). Oxygen is also taken up due to the respiratory activity of fauna (Bienhold *et al.*, 2013). Anaerobic degradation of the organic matter results in the production of volatile fatty acids and reduced compounds such as hydrogen sulfide, hydrogen and methane (Palacios *et al.*, 2006). Fungi and heterotrophic bacteria are the primary decomposers of wood in the deep sea but only a few species have the extracellular cellulases needed to digest cellulose and lignin, resulting in the hydrolysis of hemicellulose, cellulose and lignin possibly being the main limiting steps in wood degradation (Wolff, 1979; Palacios *et al.*, 2006; Fagervold *et al.*, 2012; Bienhold *et al.*, 2013).

Duperron *et al*. (2008) provided the first molecular evidence for the presence of possible thiotrophic symbioses in sunken-wood ecosystems. As a result, it is now known that the anaerobic degradation of sunken wood supports chemosynthetic communities in the same way as the bacterial decomposition of lipids from whale bones in the deep sea; hydrogen sulfide is a major by-product (Leschine, 1995; Duperron *et al.*, 2008; Laurent *et al.*, 2009; Fagervold *et al.*,

2012; Bienhold *et al.*, 2013). Laurent *et al*. (2013) found that sulfide-tolerant species are some of the first to colonise shallow-water wood falls and then dominate over several weeks (Laurent *et al.*, 2013). Once sulfide levels decrease, the sulfide-tolerant species are replaced by less-tolerant opportunistic species (Laurent *et al.*, 2013). Available data indicate that the decomposition of plant remains in the deep sea occurs in a similar way as in shallow waters but just at a reduced rate (Wolff, 1979).

The animals associated with sunken wood use the hard substrate as a shelter and substratum, and also as a direct or indirect organic source (Turner, 1977; Wolff, 1979; Scheltema, 1994; Nishimoto *et al.*, 2009) (Fig. 1.3). These environments have allowed for the development of opportunistic species, serve as areas of larval dispersal and contribute to changes in diversity, niche specialisation and enrichment (Turner, 1973; Bernardino *et al.*, 2010; Bienhold *et al.*, 2013). Species diversity has been noted to decrease significantly over time in sediments adjacent to the wood falls, most likely due to stress resulting from intense organic loading of sediments (up to 20-30% organic carbon) (Bernardino *et al.*, 2010). The composition of a sunken-wood community is thought to be dependent on the species of wood, succession stage, water depth and area of the ocean (Pailleret *et al.*, 2007a; Nishimoto *et al.*, 2009).

Bienhold *et al*. (2013) observed that successional stages of the decomposition of plant remains in the deep sea occur in a similar way to whale falls (Bernardino *et al.*, 2010; Bienhold *et al.*, 2013). The wood was initially colonised by specialist fauna like *Xylophaga* and other wood-boring bivalves, bacteria and opportunistic fauna (amphipods and crustaceans) (Fig. 1.3). This lead to the production and dispersal of wood chips and faecal matter by wood boring bivalves increasing organic carbon in the sediment (Turner, 1977; Wolff, 1979; Pailleret *et al.*, 2007a; Voight, 2007; Bernardino *et al.*, 2010; Bienhold *et al.*, 2013). This in turn attracted detritus-feeding organisms (polychaetes) and also predatory fauna, which enhanced respiration rates and lead to the development of sulfidic niches attracting chemosynthetic fauna. Bernardino *et al.* (2010), in a study of bathyal wood falls off Southern California, found sulfide-tolerant species such as the dorvilleid genus *Ophryotrocha* only became abundant more than 1.5 years after implantation, in accordance with the slow build up of porewater sulfides at wood parcels. The first chemoautotrophic species, *Idas* was only found three years after implantation. Bernardino *et al*. (2010) found that macrofaunal enhancement was still high after 5.5 years of wood implantation (Bernardino *et al.*, 2010). However, these successional stages may however differ depending on factors such as geographic location, season and the type and size of the wood (Bienhold *et al.*, 2013). Organically-enriched sediments around wood falls may also provide important habitat islands for the persistence and evolution of opportunistic and more specialised species dependent on organic- and sulfide-rich conditions at the deep-sea floor (Smith and Baco, 2003; Bernardino *et al.*, 2010).

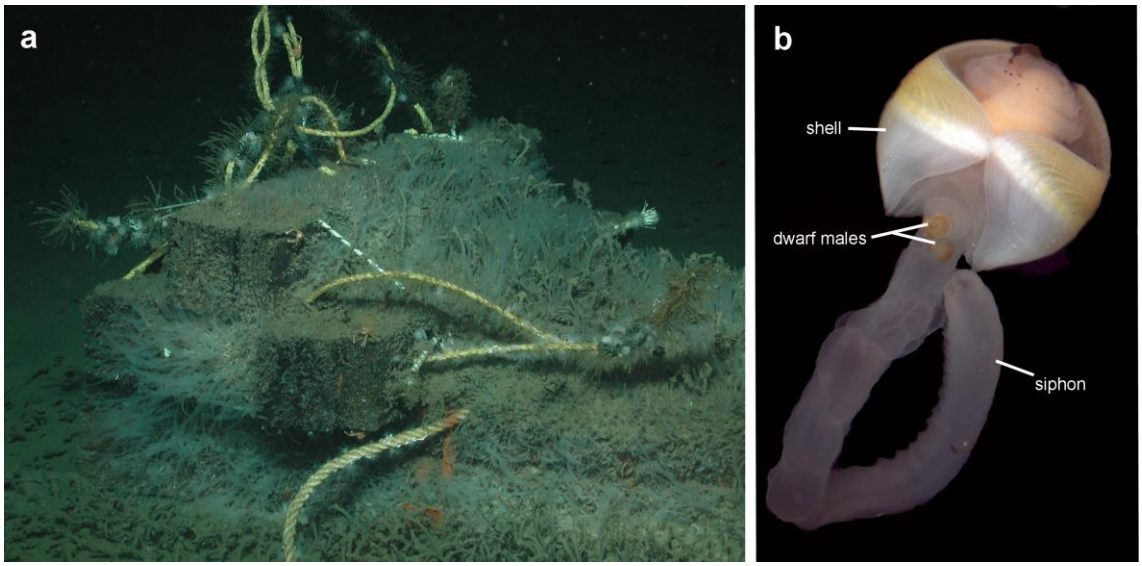

Figure 1.3 *Xylophaga.* (a) Photograph showing an implanted wood fall in situ off California at 1670 m in 2002. The numerous tubes seen projecting from the wood are the siphons of *Xylophaga washingtonia*. Image courtesy Craig Smith. (b) The general anatomical structures of the genus *Xylophaga* using a live dissected specimen of an unknown species. Presumed dwarf males are also present. Image courtesy Adrian Glover.

At wood falls, bivalves such as *Idas* and the wood-boring *Xylophaga* are some of the most common genera present. Old *Xylophaga* cavities in wood are occupied by several other types of animals with polychaetes (Glyceridae, Dorvilleidae, Ampharetidae and Amphinomidae) usually dominating but also copepods, isopods, cumaceans, sipunculids (*Phascolosoma* sp.) and ostracods (Turner, 1977; Wolff, 1979; Maddocks and Steineck, 1987; Pailleret *et al.*, 2007a; Johnson *et al.*, 2008; Bernardino *et al.*, 2010; Samadi *et al.*, 2010; Boggemann *et al.*, 2012; Borda *et al.*, 2012; Bienhold *et al.*, 2013). Chitons, limpets (Acmaeidae, Pseudococculinidae, Cocculinidae and Lepetodrilidae), amphipods, echinoids, ophiuroids, asteroids, holothurians and echiurans have also been found feeding on plant remains (Turner, 1977; Wolff, 1979; Paterson and Baker, 1988; Pailleret *et al.*, 2007a; Becker *et al.*, 2009; Samadi *et al.*, 2010; Borda *et al.*, 2012; Kobayashi *et al.*, 2012; Bienhold *et al.*, 2013). Galatheids are also very common at wood falls (Turner, 1977).

Some animals are wood-fall specialists such as the wood-boring genus *Xylophaga,* discussed further in Section 1.4. The galatheid *Munidopsis andamanica* MacGilchrist, 1905 uses wood and the biofilm found on wood as its two main food sources (Hoyoux *et al.*, 2009). It is thought that *M. andamanica* has developed lasting associations with fungal and bacterial resident gut microorganisms that probably aid in the digestion of plant-based material (Hoyoux *et al.*, 2009). Other species of *Munidopsis* such as *M. nitida* Milne Edwards, 1880, *M. bispinoculata* Baba 1988 and *M. pilosa* Henderson 1885 are thought to be primary consumers rather than scavengers feeding on fragments of hard organic substrates (wood, bone and shell) degraded by microorganisms (Hoyoux *et al.*, 2012). It has been suggested that the amphipod *Hirondellea*

gigas Birstein and Vinogradov, 1955 residing in the oligotrophic hadal depths of Challenger Deep has evolved digestive enzymes capable of digesting sunken wood debris (Kobayashi *et al.*, 2012). *Asterechinus elegans* Mortensen, 1942 is a wood-feeding echinoid found in the West-Pacific region exclusively on sunken wood (Becker *et al.*, 2009; Bienhold *et al.*, 2013). Ophiuroids, *Ophiambix aculeatus* Lyman 1880, *O. epicopus* Paterson and Baker, 1988 and *O. meteoris* Bartsch, 1983, have been found inside wood borings created by molluscs, with wood fragments in the guts (Paterson and Baker, 1988). Although cocculiniform limpets are usually not considered as borers, specimens of *Pectinodonta* sp. may seriously contribute to wood degradation as they were found in notable grooves they likely dug (Pailleret *et al.*, 2007a). Other animals such as dorvilleids, ampharetids and cumaceans are not found in background communities or even more than one metre away from the wood fall, suggesting they are enrichment opportunists that come to exploit wood falls (Bernardino *et al.*, 2010).

1.4 The wood-boring genus *Xylophaga*

1.4.1 Taxonomy

The wood-boring bivalve-mollusc genus *Xylophaga* belongs to the subfamily Xylophaginae which is also comprised of *Xyloredo* and *Xylopholas*. The Xylophaginae fall within the superfamily Pholadoidea and the family Pholadidae. The genus *Xylophaga* derives its name from *xylo* = wood, *phaga* = eating and is comprised of over 50 species thus far, of which a third have been described since 1996 (Knudsen, 1961; Turner, 2002; Voight, 2008; Voight and Segonzac, 2012) (Figs. 1.3 and 1.4). The Xylophaginae are often confused with the family, Teredinidae despite there being significant morphological differences (Turner, 2002). Teredinidae are also the principal degraders of wood in shallow temperate and tropical marine waters (0-150 m), whereas many *Xylophaga* species tend to be found in deeper water (Distel *et al.*, 2011).

Xylophaga rapidly respond to and colonise terrestrial plant remains that wash offshore, and *Thalassia* seagrass rhizomes, which sink into the deep sea (Knudsen, 1961; Wolff, 1979; Gage, 2003). *Xylophaga* species are quite diverse with four to six species occurring in relatively small oceanic areas (Turner, 2002; Voight, 2008). The large number of species of *Xylophaga* occurring in the deep sea may be due to the patchy distribution of plant debris resulting in this genus being subjected to a high degree of isolation bringing about speciation (Knudsen, 1961; Turner, 2002; Vrijenhoek *et al.*, 2009). Turner (1973) concluded ‘the predictability of the arrival of plant remains on the seafloor allowed for the evolution of the Xylophaginae, but the unpredictability of the timing of the arrival to the seafloor, led to their opportunism’. There is

particular interest in this genus due to the economic losses caused by the degradation of wooden structures placed in the sea (Tyler *et al.*, 2007). As human activities such as fishing, mining and research push into deeper areas of the ocean, Xylophaginae are becoming increasingly considered as pests (Turner, 2002).

Xylophaga were thought to be primarily deep-sea fauna but have been found at shallow warm depths (Turner, 2002; Voight, 2008). Species of *Xylophaga* are found between 0 (*Xylophaga dorsalis* Turton 1819 and *X. globosa* Sowerby I, 1985*)* and 7250 m (*X. grevei* Knudsen, 1961*)* and range from the equator to near the poles (Knudsen, 1961; Kudinova-Pasternak, 1975; Schiotte, 2005; Voight, 2008) (Fig. 1.4). 13 species are known from greater than 3000 m (Knudsen, 1961; Harvey, 1996; Turner, 2002; Voight, 2007, 2008, 2009; Voight and Segonzac, 2012). It was thought that some deep-sea species make shallow-water incursions at high latitudes where the water column is isothermic (Voight, 2008); *X. nidarosiensis* Santhakumaran, 1980 and *X. noradi* Santhakumaran, 1980 have been found at 10 m off Norway (Santhakumaran, 1980), but *X. multichela* Voight, 2008 was found at 106 m off the Pacific coast of Guatemala indicating that these incursions do not only occur in cold areas (Voight, 2008) (Fig. 1.4). Shallow waters appear to have less competition than found at depth, where up to five species have been found on a single piece of wood (Hoagland and Turner, 1981; Voight, 2008). Most of the distribution of the genus *Xylophaga* thus far appears to be in close proximity to coastlines however *Xylophaga* have been found to thrive on abyssal plains despite those locations being well away from any source of terrestrial vegetation (Voight and Segonzac, 2012) (Fig. 1.4).

Xylophaga species can be loosely grouped based on characteristics such as the morphology of the mesoplax, siphons and muscle scars, and the method of reproduction (Turner, 2002; Voight, 2007). The genus *Xylophaga* is characterised by siphons that are often capable of retracting but are usually extended out of the wood into the water column (Turner, 2002) (Fig. 1.3). Siphonal characteristics to note include the relative length of the two siphons, the presence or absence of cirri at the apertures and the type of siphonal folds, which may or may not have lappets or fringes (Turner, 2002). The mesoplax is a one- or two-part transverse plate, usually wider than long that straddles the valves at the umbos and partially or completely covers the posterior end of the anterior adductor muscle (Turner, 2002). The presence or absence of a ventral portion and tubes on the mesoplax are other characteristics that are very important for species identification (Turner, 2002).

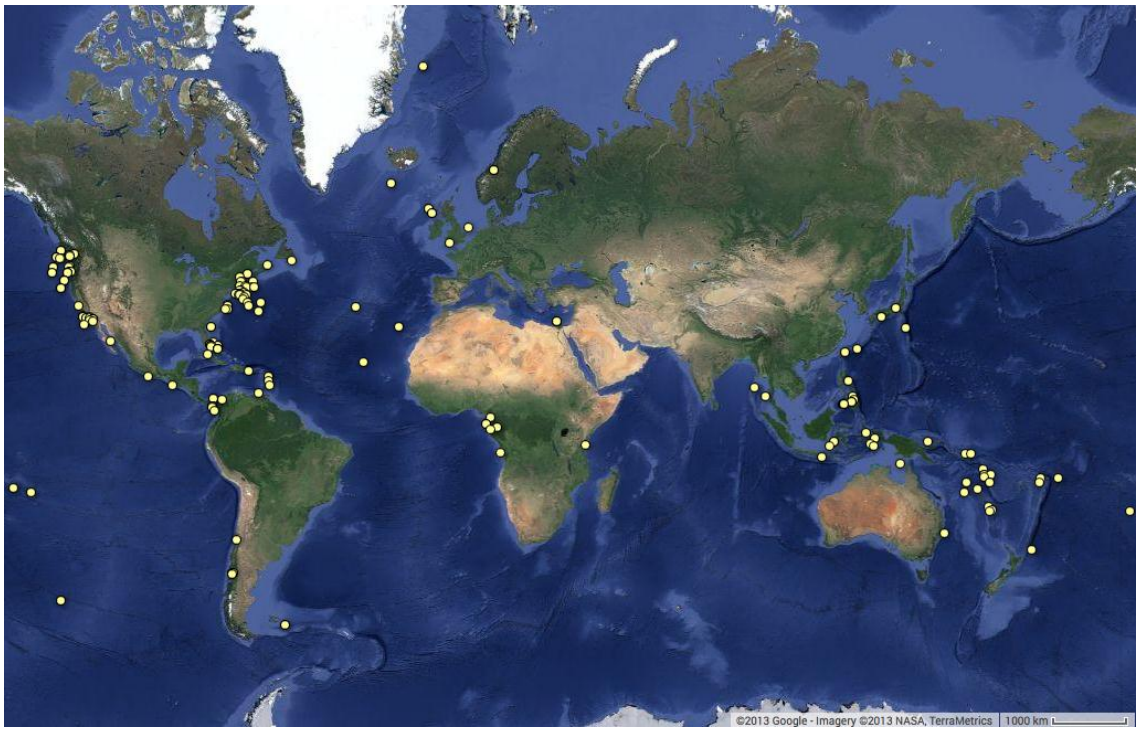

Figure 1.4 Known locations of the genus *Xylophaga*. Locations (yellow dots) have been taken from the literature and exclude any new records reported in this thesis. More than one species has been found at some locations.

Wood-boring families Pholadidae and Teredinidae appear to have been worldwide by the Jurassic (Hoagland and Turner, 1981). Purchon (1941) proposed that Xylophaginae and Teredinidae both arose from a common ancestral wood-boring pholad by adopting wood as a nutritive source in addition to its previous use for protection. Distel *et al.* (2011) have confirmed that xylotrophy has occurred once in a lineage of Bivalvia that then diversified into shallow-water and deep-water branches. Their data also suggested that the appearance of xylotrophy coincided with the acquisition of bacterial endosymbionts (Distel *et al.*, 2011). Fossil evidence indicates that the oldest *Xylophaga* come from the lower Cretaceous and it is thought that *Xylophaga* branched off at an early stage of development of Pholadidae (Knudsen, 1961). Faecal chimneys in boreholes of about eight millimetres diameter in wood fragments were also observed and were presumed to have been built by xylophagid bivalves (Kiel, 2008; Kiel *et al.*, 2009). Other fossilised *Xylophaga* from the Oligocene-early Miocene have been found in Washington State, and were imaged to look at the digestive systems using computed tomography (Kiel *et al.*, 2012). Faecal pellets of woody detritus have also been found to be abundant in the concretionary matrix surrounding wood (Turner, 1973; Kiel, 2008).

Knudsen (1961) put forward the theory that the genus *Xylophaga* originated in shallow water in the tropic regions where abundant plant debris was available. Analyses by Distel *et al*. (2011) suggested that the last common ancestor of Teredinidae and Xylophaginae burrowed in and fed

on wood, and had a caecum for wood storage and digestion. It possessed unpaired gill demibranchs that contained xylotrophic symbionts in bacteriocytes housed within the interlamellar tissue. It was not wormlike but instead had a typical bivalve body plan with the intestines traversing the heart and the visceral organs located between the anterior and posterior adductor muscles and largely enclosed by the valves (Distel *et al.*, 2011). Like modern teredinids, this common ancestor may have possessed apophyses and formed lined burrows that were sealed by paired pallets which have been lost in *Xylophaga* but functioned together to prevent dehydration when the animals were exposed to the atmosphere before the invasion of deep water by a shallow-water ancestor (Distel *et al.*, 2011). This hypothesis requires each of these unusual shared traits to have emerged just once in Bivalvia, rather than twice as is demanded by the currently accepted taxonomy (Distel *et al.*, 2011).

1.4.2 Bacterial symbiosis and nutrition in the genus *Xylophaga*

Food chains based on wood and presence of the genus *Xylophaga* exist in the deep sea (Turner, 2002) (Fig. 1.3). *Xylophaga* use their ridged shells as rasps to bore into wood found on the seafloor (Voight, 2008). Teredinids tend to bore with the grain of the wood but *Xylophaga* bivalves bore at right angles to the grain of the wood, possibly acting to reduce competition by physically partitioning the wood between xylophagids and teredinids as both are obligatory wood-borers (Knudsen, 1961; Voight, 2008). *Xylophaga*, like teredinids, ingest and digest terrestrial plant material aided by cellulolytic endosymbionts (such as *Teredinibacter turnerae*) held within the gills that are capable of fixing nitrogen (Waterbury *et al.*, 1983; Distel *et al.*, 1991; Distel and Roberts, 1997; Turner, 2002; Yang *et al.*, 2009; Taylor and Glover, 2010). Distel and Roberts (1997) confirmed the presence of endosymbionts in *Xylophaga atlantica* Richards, 1942 and *X. washingtonia* Bartsch, 1921 that resembled those found in teredinids morphologically and in the location in the bacteriocytes in the abfrontal region of the gills. The symbionts are distributed in clusters throughout the bacteriocytes, with mitochondria interspersed in the cytoplasm between clusters (Distel and Roberts, 1997). They are straight to gently-curved rods ranging in width from 0.4-0.7 µm and up to 5.0 µm in length contained within vesicles ranging from 10-20 µm in diameter (Distel and Roberts, 1997). The bacterial symbionts are therefore separated from the external environment by the plasma membrane, cytoplasm, and vesicular membrane of the host (Distel and Roberts, 1997).

Xylophaga also have a large wood-storing caecum formed by an outpocketing of the stomach (Purchon, 1941; Turner, 1973; Distel and Roberts, 1997; Turner, 2002). *Xylophaga* are the most important fauna involved in decomposing and converting terrestrial cellulosic plant material and wood into a usable form, such as animal biomass, in the deep sea (Turner, 1973; Distel and Roberts, 1997; Bienhold *et al.*, 2013). As a result, they are considered keystone species in this

environment (Turner, 1977; Bienhold *et al.*, 2013). By degrading the wood, they facilitate the development of anoxic zones and anaerobic microbial processes (Bienhold *et al.*, 2013).

Plant material was always thought to be the major source of nutrition for xylophagids, but recently Bernardino *et al*. (2010) found that adult xylophagids had an increased bacterial carbon contribution probably reflecting microbial symbiosis in *Xylophaga* sp., but as a juvenile, wood material was the major contributing factor to nutrition (Distel and Roberts, 1997). Burrowing seldom reaches more than five times the length of the valves and there is often a chimney of fecal pellets lining the posterior end of the burrow (Turner, 2002). Faecal pellets are not expelled with sufficient force to carry them out of the burrow and so they accumulate as a compact mass consolidated with mucus preventing any extensive movements of the animal (Purchon, 1941). There is thought to be high predation pressure on *Xylophaga* by organisms such as flatworms of the family Euryleptidae, crabs, the mussel *Idas argenteus* Jeffreys, 1876 and the echinoderm *Xyloplax janetae* Mah, 2006 (Turner, 2002; Voight, 2007, 2008; Ockelmann and Dinesen, 2011). *I. argenteus* were observed predating on the spat of *Xyloredo ingolfia* Turner, 1972 (Ockelmann and Dinesen, 2011). Predation can cause the substrate to last longer as an energy source for these wood-boring bivalves (Ockelmann and Dinesen, 2011).

1.4.3 The reproductive biology and life history of the genus *Xylophaga*

A hypothesis by Turner (1973) states that the breeding season of the Xylophaginae, and possibly other invertebrates dependent on plant remains for food and substratum, may be linked with the rainy season in the tropics and the spring runoff in temperate zones as large amounts of wood are flushed into the sea then. However, Tyler *et al*. (2007) found that regardless of the time of year that wood was deployed to the deep sea, xylophagid molluscs, like *Xylophaga depalmai* Turner, 2002 invaded it and grew quickly to sexual maturity. Gametogenesis was said to be continuous in *X. depalmai* once initiated and there was shown to be no correlation between mean oocyte size and adult weight for differing wood substrata (Tyler *et al.*, 2007). *X. washingtonia* is also thought to breed throughout the year (Turner, 2002). Turner (1973) found that oocytes were very small (45 μm) and plentiful (up to 30000 in some species) and reproduction was continuous. This reproductive life history is probably as a result of the fact that *Xylophaga* must reproduce rapidly before they consume their own habitat (Turner, 1973). The geographical distribution of *Xylophaga* are dependent on the presence of suitable plant material and the dispersal capability of the free-swimming larvae (Turner, 1973; Wolff, 1979). Once the larvae have settled and metamorphosed into the adult form, they are incapable of moving to another piece of wood and if the wood disintegrates, they lose protection and food and subsequently die (Knudsen, 1961; Turner, 1973, 2002).

Three mechanisms of fertilisation have been noted in the family Teredinidae and may be analogous to the Xylophaginae: 1) sex products are released into the water where fertilisation occurs 2) sperm are released by the male into the water column which the female then draws in using her siphon and fertilisation occurs internally and 3) sperm from the male is inserted into the female siphon using his siphon so fertilisation occurs internally (Eckelbarger and Reish, 1972). Self-fertilisation and hermaphroditism are also known to occur in teredinids (Eckelbarger and Reish, 1972). Tyler *et al*. (2007) found some individuals of the species *Xylophaga depalmai* did exhibit simultaneous hermaphroditism.

Knudsen (1961) documented that nine of seventeen species with large eggs appeared to brood their young on the outside of their shell or siphon and since then more species have been noted including *Xylophaga multichela, X. tubulata* Knudsen, 1961*, X. grevei, X. abyssorum* Dall, 1886*, X. africana* Turton, 1822*, X. whoi* Turner, 2002*, X. clenchi* Turner and Culliney, 1971*, X. obtusata* Knudsen, 1961*, X. supplicata* Taki and Habe, 1950 and *X. gagei* Harvey, 1996 (Knudsen, 1961; Harvey, 1996; Turner, 2002; Voight, 2008). *X. profunda* Turner, 2002 has also been found with as many as 75 young of about 0.30 mm in length attached to the umbonal area and *X. panamensis* Knudsen, 1961 with 50 embryos about 0.38 mm in length attached to the ventral edge of the shell (Knudsen, 1961; Turner, 2002). It has only been assumed and has not actually been proven that the juveniles clinging to the shells and mantles of these adult *Xylophaga* species are actually the young of the specimen to which they are attached (Turner, 2002). Juveniles of *X. depalmai* were found unattached suggesting multiple methods of reproduction and dispersal in the genus *Xylophaga* (Tyler *et al.*, 2007; Voight, 2008). *X. atlantica* is oviparous and does not brood its young (Turner, 2002). The larvae of *X. washingtona* are free-swimming, thought to spend all of their life in the sea and probably do not rise more than 3-8 metres above the seafloor; young specimens have never been found attached to the shells of adults (Turner, 2002).

The long-held view of brooding within Xylophaginae (Turner, 1955; Knudsen, 1961; Wolff, 1979) has been recently challenged based on new evidence that the brooding larvae are in fact dwarf males (Ockelmann and Dinesen, 2011; Haga and Kase, 2013). Recent studies by Ockelmann (2011) showed *Xyloredo ingolfia* brood one to six spat, usually behind the umbones, which appear to act as functional dwarf males. Recently developed spat had developing sperm found in two bags which were probably modified testes (Ockelmann and Dinesen, 2011). These bags were thought to be transferred to the mantle cavity of the female for fertilisation to occur, implying that chemical communication between females and dwarf males must exist (Ockelmann and Dinesen, 2011). Ockelmann and Dinesen (2011) hypothesised that once the spat had delivered their sperm, development could continue. It is thought that considerable time elapses between sperm transfer and commencement of wood boring (Ockelmann and Dinesen,

2011). *Xylophaga atlantica* probably have dwarf males also (Ockelmann and Dinesen, 2011). *X. supplicata* was also shown to have progenetic dwarf males rather than brooding juveniles (Haga and Kase, 2013). Haga and Kase (2013) also showed that specimens exhibit a protandric transition from male to female via a simultaneous temporary hermaphroditic stage. As a result of their study, Haga and Kase (2013) have proposed that all species of *Xylophaga* that 'brood juveniles' are actually carrying dwarf males instead (Fig. 1.3). If this is the case, 42.4% of the species below 1000 m have dwarf males and thus it may be an adaptation to the sporadic distribution of wood substrate (Haga and Kase, 2013). This is supported by the observation of functional dwarf males in the teredinid, *Zachsia zenkewitschi* Bulatoff and Rjabtschikoff, 1933 (Yakovlev and Malakhov, 1985). Haga and Kase (2013) suggested that the morphology of the siphon of the host individual, together with the positioning of dwarf males plays an important role in fertilisation.

Turner (1973) proposed three possible methods of spawning and colonisation for the Xylophaginae: 1) the adults are able to detect when new wood is in the near vicinity and thus are triggered to spawn (unlikely); 2) the larvae have the ability to detect new wood in the vicinity and swim towards it; or 3) the larvae are produced in great abundance, have the ability to delay metamorphosis and are dispersed via the currents, settling when a chance encounter brings them into contact with wood. However, if the larvae remain in the plankton for long periods of time, one would expect to find them in plankton tows but Turner (1973) found none. Since the larvae have no velum, Knudsen (1961) thought that larvae can either disperse by crawling along the bottom or crawling and swimming short distances but do not have a real pelagic stage. *Xyloredo ingolfia* also have very modified sperm, as seen in *X. dorsalis* which have long thin heads, lack an acrosome, are vermiform and exhibit little motility (Ockelmann and Dinesen, 2011). The highly modified sperm of some species of Xylophaginae indicates an absence of free spawning (Turner, 1955; Ockelmann and Dinesen, 2011).

The colonisation process of the genus *Xylophaga* is so efficient however, it indicates that there must be a pool of larvae always ready to invade wood as it becomes available (Young, 2003). Bernardino *et al*. (2010) found that post colonisation of wood by *Xylophaga*, the abundance of juveniles increased to around 21000 individuals m^{-2} in the sediment, presumably resulting from a recruitment response to the available wood substrate. Brooding species of *Xylophaga* inhabiting the wood parcels may have been the source of juveniles to surrounding sediment (Voight, 2007; Bernardino *et al.*, 2010). The sediment-dwelling *Xylophaga* species did not attain adult size, even though adults were present on the wood parcels and thus the sediment population may be a sink population due to crowding on the wood (Bernardino *et al.*, 2010). Ockelmann and Dinesen (2011) hypothesised that recruitment by pheromone-induced settling of larvae would enable wood-associated species to locate and colonise their ephemeral and patchy

environment in the deep sea. However, an experiment by Voight and Segonzac (2012) demonstrated that *Xylophaga alexisi* Voight and Segonzac, 2012 can settle despite no source population being located at the site suggesting that *Xylophaga* can disperse over long distances and perhaps survive long periods in the plankton (Voight and Segonzac, 2012).

All juveniles examined tended to resemble the adults in their soft parts and the size of the juveniles varied between species (Knudsen, 1961). It is not known how juveniles begin to attack the woody material but Knudsen (1961) reported that the young were never seen boring from their mothers' burrows so apparently boring always begins from the exterior. These molluscs live in the highest densities in the first 20 cm above the sea-sediment interface of the substratum with numbers decreasing vertically thereafter up into the water column (Turner, 1973).

1.4.4 The growth rates of the genus *Xylophaga*

Variations in growth rates may be due to a variety of factors, most importantly: genetics, length of the growing season, temperature, substrate qualities and inter and intraspecific interactions like crowding (Romey *et al.*, 1994). The genus *Xylophaga* provided some of the earliest data concerning growth in the deep sea (Turner, 1977). *Xylophaga* are classic r-selected species in the deep sea; they grow at rapid speeds when compared with many other deep-sea fauna, reaching adult size within months of settlement (Turner, 1973; Dean, 1993; Romey *et al.*, 1994; Young, 2003). *Xylophaga depalmai* grew at a mean rate of around 0.03 mm per day whereas *X. alexisi* was estimated to grow at 0.33 mm per month but may have been stunted by crowding during experimentation (Tyler *et al.*, 2007; Voight and Segonzac, 2012). Wood-boring bivalves typically show an S-shaped growth curve with initial rapid growth allowing the primary colonisers to establish themselves prior to the arrival of other organisms, being followed by an asymptote when individuals reach adulthood and divert their energy from growth to gamete production (Hoagland and Turner, 1981; Romey *et al.*, 1994).

Time-series studies by Romey *et al.* (1994) at a site off Cape Cod at a depth of 100 m revealed that the first set of individuals of the species *Xylophaga atlantica* to settle on wood panels grew much faster than individuals that settled later in the year (0.085 mm per day vs. 0.031 mm per day respectively). The modal growth rate was much greater at the experimental site at 200 m at 0.246mm per day thought to be due to the warmer average temperatures there (Romey *et al.*, 1994). Differences in growth rates due to season, substrate and previous density were also apparent (Romey *et al.*, 1994). Physical crowding may account for some of the decline in growth rate (Turner, 1973; Romey *et al.*, 1994). Many of the largest individuals also ran out of wood and had fallen out of the panels (Romey *et al.*, 1994).

All *Xylophaga* species known to date show considerable variation in size depending on the type of wood into which they are boring (Turner, 2002). The softer the wood, the deeper *Xylophaga* are able to penetrate and the greater the development of faecal chimneys (Turner, 2002). The burrows of some specimens can be up to 20 mm in length and there can be around 150-170 burrow openings cm^{-2} or 100 to >500 individuals present per dm^3 (Turner, 1973; Voight and Segonzac, 2012; Bienhold *et al.*, 2013). The borings of *Xylophaga* are oblong pear shaped and a single piece of wood may be subjected to multiple attacks. This was observed by Knudsen (1961) in a twig, which contained adults in the interior and recently-settled juveniles closer to the exterior. Turner (1973) concluded that the high fecundity, rapid sexual maturation, rapid colonisation, rapid growth rate, high population densities, high reproductive rates, ease of dispersal and apparent ease of use of transient habitats makes these wood borers classic examples of opportunistic species living in an ephemeral habitat.

1.5 Similarities between the genera *Osedax* and *Xylophaga*

Despite the fact that *Osedax* and *Xylophaga* are from two different phyla and live on two different types of organic falls, there are many ecological and functional similarities between these two specialist genera. Some of these have been mentioned in Glover *et al*. (2013). The following characteristics may allow these fauna to exploit the ephemeral ecosystems they inhabit.

1) Both genera occur at organic falls: ephemeral hard substrates that project from the sediment, are rich in organic carbon and are capable of fuelling chemosynthetic ecosystems. *Xylophaga* occur on wood and *Osedax* on whale bone. Turner (1973) hypothesised that the geographical distribution of *Xylophaga* are dependent on the presence of their wood substrate and their dispersal in the larval stage. Perhaps the same can be said for *Osedax* and its bone substrate.

2) Both genera bore into their substrate for nutrition and have bacterial symbionts, which aid heterotrophic feeding. *Osedax* bore into the calcified matrix to extract collagen and cholesterol for nutrition via bacterial symbionts, using acids secreted from their root system, whereas *Xylophaga* use their shells to bore into wood consuming it via bacterial symbionts in the process.

3) Both genera have large bathymetric ranges. *Osedax* have been found from 21 to 3000 m and *Xylophaga* from 0 to 7250 m, but both have been predominantly found in the deep sea thus far.

4) Both genera are sessile as adults and disperse via the larval stage. Once *Osedax* and *Xylophaga* have settled and bored into the substrate while attaining the adult form, no more movement is possible.

5) Sexual dimorphism is exhibited in both genera. The females appear to be large in size whereas the males are dwarfed and are seen to settle in the tubes of female *Osedax* and on a variety of places on the female *Xylophaga.*

6) A large effect is had on the substrate due to the life history of both genera. *Osedax* and *Xylophaga* breakdown the substrate for nutrition and in doing so, if left uninhibited, will end up destroying the substrate eventually, resulting in their death. Both of these genera are keystone species in the decomposition of organic falls and conversion of the substrates into more useable forms.

1.6 The overlap of fauna between organic falls and other chemosynthetic habitats

Hydrothermal vents, cold seeps and large organic falls all provide alternative energy sources to the downward flux of marine phytodetritus in the deep sea. Both wood and whale bones during the enrichment and sulfophilic stages can be anaerobically degraded producing hydrogen sulfur, which can support chemosynthetic communities (Duperron *et al.*, 2008; Duperron *et al.*, 2009; Laurent *et al.*, 2009; Gaudron *et al.*, 2010). As a result, organic falls in the deep sea are colonised by invertebrate communities with phylogenetic relationships to the chemotrophic fauna on hydrothermal vents, cold seeps and other reducing environments (Tunnicliffe *et al.*, 2003; Kiel *et al.*, 2009; Gaudron *et al.*, 2010). The overlap of some fauna at higher taxonomic levels between organic falls and other chemosynthetic habitats implies that organic falls may possibly act as evolutionary 'stepping stones' along the seafloor for the dispersal of a subset of fauna between reducing habitats or vice versa (Hecker, 1985; Smith *et al.*, 1989; Distel and Roberts, 1997; Smith and Baco, 2003). Even though few species are shared when compared with the overall numbers present, the similarities of these taxonomic groups (families and genera) indicate a strong evolutionary connection through common ancestors even if there is not extensive gene flow today (Tunnicliffe *et al.*, 1996; Sibuet and Olu, 1998; Tunnicliffe *et al.*, 2003). Whilst vent and seep fauna are restricted to certain geologic settings, organic falls are cosmopolitan, occurring throughout the worlds oceans (Glover *et al.*, 2005b). Whale bones and wood falls may thus provide the only refuges for sulfide-based chemoautotrophic communities over vast reaches of deep-sea floor (Bennett *et al.*, 1994). The immediate sediments surrounding organic falls create a sedimentary reducing habitat similar to deep-sea seeps in terms of sulfide production (low intensity) but limited spatially and temporally (Treude *et al.*, 2009; Bernardino *et al.*, 2010; Bienhold *et al.*, 2013). Bones and wood on the other hand probably provide sustained low-emissions of sulfide probably more similar to hydrothermal vents (Treude *et al.*, 2009). At organic falls, especially during the sulfophilic stage, there are several different trophic

levels/sources of nutrition allowing for high species richness. This can be paralleled to hydrothermal vent communities and may also help to explain the overlap of fauna (Smith and Baco, 2003).

Not many of the polychaetes at whale falls have been identified to species level and as a result it is not clear which may be specialised on the organic-fall habitat, or whether they also occur in the background fauna and use the organic fall as an additional food source rendering them opportunistic (Wiklund *et al.*, 2009a). Dorvilleids such as *Ophryotrocha* and chrysopetalids such as *Vigtorniella* have been reported from most reducing habitats - hydrothermal vents, cold seeps, anoxic sediments below fish farms, wood falls and whale falls (Wiklund *et al.*, 2009a; Bernardino *et al.*, 2010). Bernardino *et al*. (2010) found that dorvilleids were particularly plentiful at wood falls representing at least three genera found at seeps, vents and whale falls suggesting that wood islands may create a complex of niches for this group and that overlap does occur (Baco and Smith, 2003). The polynoid *Bathykurila guaymasensis* Pettibone, 1989 has been found at whale falls and hydrothermal vents, the hesionid species, *Hesiocaeca methanicola* Desbruyeres & Toulmond, 1998 was found inhabiting seep habitats and whale falls, and the ampharetid *Amelinna* sp. has been found at wood falls and cold seeps (Bennett *et al.,* 1994; Tunnicliffe *et al.,* 1998; Glover *et al.,* 2005)(Wiklund *et al.*, 2009a; Bernardino *et al.*, 2010). These species specialise on bacterial mats, which are plentiful at all of these habitats, allowing easy overlap between reducing habitats (Bennett *et al.*, 1994; Tunnicliffe *et al.*, 1998; Glover *et al.*, 2005a; Wiklund *et al.*, 2009a).

There have been few records of siboglinids other than *Osedax* found at organic falls and this is a major objection to the stepping-stone hypothesis (Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Deming *et al.*, 1997; Feldman *et al.*, 1998; Baco *et al.*, 1999; Van Dover, 2000; Smith and Baco, 2003; Rouse *et al.*, 2004; Glover *et al.*, 2005a; Pleijel *et al.*, 2008; Wiklund *et al.*, 2009a; Lundsten *et al.*, 2010a). *Escarpia spicata* Jones, 1985, is known from vent areas in the Guaymas Basin, cold seeps and from the sulfide-rich sediments surrounding a whale carcass off California (Feldman *et al.*, 1998). *Lamellibrachia barhami* Webb, 1969 is known from cold seeps, wood falls and a whale fall (Lundsten *et al.*, 2010a). The presence of *E. spicata* and *L. barhami* in so many environments suggests that they may be opportunistic species with a wide tolerance for varying habitats (Feldman *et al.*, 1998).

Chemosynthetic bivalve molluscs from the families Vesicomyidae, Solemyidae (*Acharax*), Thyasiridae, Lucinidae and Mytilidae are some of the most prominent fauna at whale falls, hydrothermal vents, wood falls and cold seeps (Smith *et al.*, 1989; Bennett *et al.*, 1994; Berrow *et al.*, 1995; Naganuma *et al.*, 1996; Smith and Baco, 2003; Bolotin *et al.*, 2005; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Fujiwara *et al.*, 2009; Bernardino *et al.*, 2010). The sulfides

produced from decomposing organic falls are used by bivalves through chemosynthetic symbionts similar to those of vent and seep bivalves (Gros and Gaill, 2007; Gros *et al.*, 2007; Duperron *et al.*, 2009; Lorion *et al.*, 2010). Symbioses in many of these families are obligate but nutrition is via filter feeding of organic material and chemosynthesis (Smith *et al.*, 1989; Tunnicliffe *et al.*, 2003; Raulfs *et al.*, 2004; Taylor and Glover, 2010). Many of these organic-fall bivalves are conspecific with vent and seep species, and they occur in reproductively-viable populations at organic falls adding support for the dispersal 'wooden steps to deep-sea vents' hypothesis (Smith and Baco, 2003; Samadi *et al.*, 2007). This states that deep-sea mussels inhabiting cold seeps and hydrothermal vents are believed to have evolved from mytilids living on either whale carcasses or sunken wood which then used these two habitats as evolutionary 'stepping stones' down the continental slope (Smith and Baco, 2003). This view is based on molecular and fossil evidence (Smith *et al.*, 1989; Distel *et al.*, 2000; Smith and Baco, 2003; Samadi *et al.*, 2007; Lorion *et al.*, 2010; Ockelmann and Dinesen, 2011). Distel *et al.* (2000) proposed that bathymodiolin mussels first adapted to low sulfide habitats in the deep sea (wood and whale falls) and then more recently moved on to sulfide-rich environments (seeps and vents).

Numerous gastropod species have also been found overlapping between reducing environments: *Pyropelta* species at whale falls and vents (Bennett *et al.*, 1994), Cocculinidae including *Cocculina craigsmithi* McLean, 1992 found aggregating on blackened portions of bone at whale falls and the Juan de Fuca vents, *Mitrella permodesta* found at Monterey Canyon seeps and exposed bone surfaces in high densities (Smith *et al.*, 1989; Bennett *et al.*, 1994), *Protolira thorvaldssoni* Waren, 1996 known from vents, seeps and whale bones (Sasaki *et al.*, 2010), and several *Lepetodrilus* species found at vents, wood falls and whale falls (Johnson *et al.*, 2008; Rogers *et al.*, 2012b). *Xylodiscula* is a whale-fall gastropod genus that overlaps with sunken wood and seagrass (Marshall, 1994; Smith and Baco, 2003). Kiel *et al*. (2009) documented the oldest wood falls with the presence of provannid, skeneiform and limpet gastropods, and thyasirid and nuculanid bivalves from the late Cretaceous found in Japan. The species observed were the same as those found on plesiosaur bones and at hydrocarbons seeps in the same sediments supporting the hypothesis that there has been great faunal overlap between reducing habitats (Kiel *et al.*, 2009).

The giant colonial marine ciliate *Zoothamnium niveum* Ehrenberg, 1838 is a symbiont-harbouring protozoan that has been found on sunken plant material as well as on a deployed whale bones off Japan at five metres (Kawato *et al.*, 2010). This indicates that this ciliate is cosmopolitan and typically resides in reducing environments in both shallow and deep water (Kawato *et al.*, 2010). There have also been records of pandalid shrimp (*Pandalopsis ampla* Spence Bate, 1888), isopods, amphipods, cnidarians such as actinarians (*Stomphia* sp.),

pennatulaceans (*Halipteris californica* Moroff, 1902) and echinoderms found at more than one chemosynthetic habitat (Marshall, 1987; Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Deming *et al.*, 1997; Baco *et al.*, 1999; Van Dover, 2000; Smith and Baco, 2003; Rouse *et al.*, 2004; Lundsten *et al.*, 2010a).

As organic-fall environments are similar to those of cold seeps and hydrothermal vents in the deep sea, it is hypothesised that the larval ecology of many organic-fall specialists will be similar, allowing broad dispersal and effective local recruitment at new sites (Turner, 1973; Bennett *et al.*, 1994; Tyler and Young, 1999; Van Dover, 2000; Marsh *et al.*, 2001). Future studies of organic-fall communities and their relationships to fauna at vents and seeps are required (Van Dover, 2000; Smith and Baco, 2003; Bernardino *et al.*, 2010).

1.7 Thesis aims and objectives

Despite over 30 years of research on organic falls, there are still many gaps in our knowledge. We still have only a basic understanding of the taxonomic composition of organic falls, and for some ocean basins (such as the Indian Ocean), no natural studies or experiments have been conducted. The degree of connectivity between isolated habitats for specialist organic-fall fauna (e.g. *Osedax* and *Xylophaga*) is poorly known, again owing primarily to a lack of quality identifications and taxonomy. Key to understanding evolutionary histories is the degree of overlap in faunal assemblages between organic-falls and other chemosynthetic habitats such as vents and seeps. Finally, we are also extremely ignorant of the functional ecology of these systems, in particular how quickly the organic matter is remineralised by these remarkable specialist organisms. This is particularly important for our general understanding of deep-sea ecosystem function as humans start to influence this habitat for the first time. With this in mind, I will focus on the following aims, which form the data chapters of this thesis:

Chapter 2 - Analyse the ecology of the first natural whale fall found in a polar deep-water environment in terms of the taxonomic composition of the faunal assemblage, determine the successional stage and likely age and species of the carcass, analyse the whale-fall community structure, and test the 'oil-gradient' hypothesis.

Chapter 3 - Describe three new species of *Osedax* from two locations in the polar Southern Ocean morphologically and ecologically. These new species will be characterised phylogenetically also and their relationships with other species of *Osedax* compared.

Chapter 4 - Analyse the ecology of implanted wood and bone organic falls from two seamounts in the Southwest Indian Ocean Ridge in terms of the taxonomic composition and functional ecology of the colonising faunal assemblage.

Chapter 5 - Investigate the ecosystem functional role of *Xylophaga* at wood falls using micro-computed tomography. Not only were these wood falls different in terms of the region of study (Mid-Cayman Spreading Centre, Southwest Indian Ocean Ridge and Bahamas) but also depth, deployment length, colonising *Xylophaga* species and type of wood used. The population structure of colonising *Xylophaga* will also be evaluated, as well as, the impact of *Xylophaga* on the wood substrate.

Chapter 6 - The final chapter aims to synthesise our knowledge of organic-fall ecology with a view to creating new hypotheses for future research.

A summary of the locations of study can be seen in Figure 1.5 and are as follows:

1) A natural whale fall in the Southern Ocean at 1444-1446 m.
2) Whale bones implanted in the Bransfield Strait at 550-650 m.
3) Whale bones implanted on two seamounts in the Southwest Indian Ocean Ridge at 732-750 m.
4) Wood implanted on two seamounts in the Southwest Indian Ocean Ridge at 732-750 m.
5) Wood implanted in the Mid-Cayman Spreading Centre at 4773-4970 m.
6) Wood implanted in the Tongue of the Ocean, Bahamas at 480-520 m.

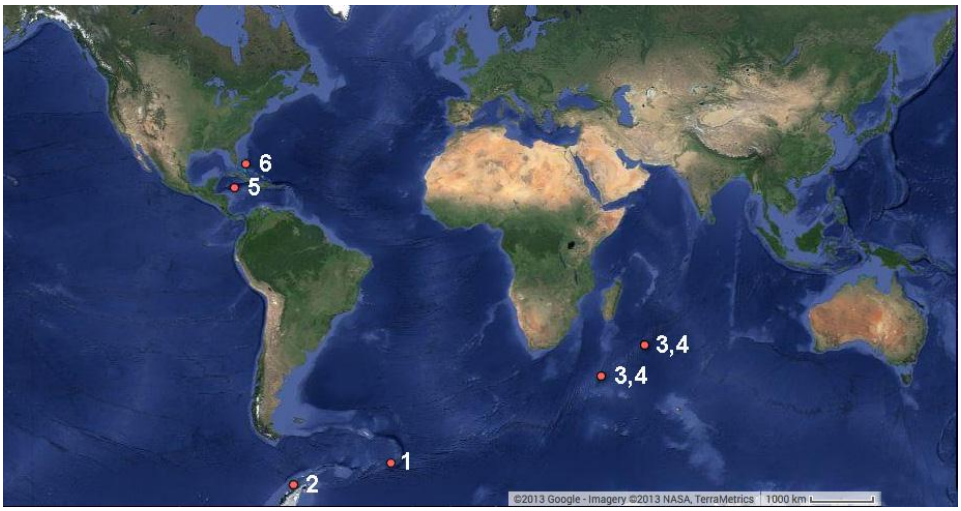

Figure 1.5 The sites of study during this thesis. The locations are represented by red dots. The numbers assigned to each red dot correspond with those in the list above Figure 1.5.

2. The discovery of a natural whale fall in the Antarctic deep sea

A modified version of this chapter was published as:

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Large cetacean carcasses at the deep-sea floor, known as 'whale falls', provide a resource for generalist-scavenging species, chemosynthetic fauna related to those from hydrothermal vents and cold seeps, and remarkable bone-specialist species such as *Osedax* worms. Here we report the serendipitous discovery of a late-stage natural whale fall at a depth of 1444 m in the South Sandwich Arc. This discovery represents the first natural whale fall to be encountered in the Southern Ocean, where cetaceans are abundant. The skeleton was situated within a seafloor caldera, in close proximity (<250 m) to active hydrothermal vents. We used a DNA barcoding approach to identify the skeleton as that of an Antarctic Minke whale (*Balaenoptera bonaerensis*). The carcass was in an advanced state of decomposition, and its exposed bones were occupied by a diverse assemblage of fauna including nine undescribed species. These bone fauna included an undescribed species of *Lepetodrilus* limpet that was also present at the nearby hydrothermal vents, suggesting the use of whale-fall habitats as stepping stones between chemosynthetic ecosystems. Using Remotely Operated Vehicle (ROV) videography, we have quantified the composition and abundance of fauna on the whale bones, and tested a hypothesis that varying concentrations of lipids in the bones of whales may influence the microdistribution of sulfophilic whale-fall fauna. Our data supported the hypothesis that more lipid-rich bones support a greater abundance of sulfophilic bacterial mats, which are also correlated with the abundance of grazing limpets (*Pyropelta* sp.). The abundance of *Osedax* sp. on bones however, showed a negative correlation with the bacterial-mat percentage cover, and hence greatest abundance on bones predicted to have lowest lipid content.

2.1 Introduction

Recent studies of both natural and experimentally-implanted whale skeletons have provided a remarkable insight into the fate of the planet's largest creatures after their death. Whale remains on the seabed, termed 'whale falls', provide a large amount of organic enrichment, shelter and substrate to the deep-sea floor and thus produce a habitat that is distinct from that of the surrounding benthic community (Smith *et al.*, 1989; Jones *et al.*, 1998; Smith *et al.*, 2002; Smith and Baco, 2003; Smith, 2006). Such carcasses can be consumed by a diverse community of both generalist-scavenging species such as fish and crustacea, and highly specialised feeders such as 'bone-boring' *Osedax* worms (Baco and Smith, 2003; Smith and Baco, 2003; Rouse *et al.*, 2004).

Faunal assemblages occupying whale falls vary in species diversity and community structure, which may reflect stages of decomposition on the seafloor (Bennett *et al.*, 1994; Smith and Baco, 2003; Smith, 2006), although such stages may be difficult to distinguish for some carcasses (Lundsten *et al.*, 2010b). The first recognised stage, known as the mobile-scavenger stage, is characterised by the removal of soft tissue from the carcass by organisms such as hagfish, sharks, and crustaceans, and is thought to last from four to at least 24 months (Smith *et al.*, 1989; Smith and Baco, 2003). The second stage is the enrichment-opportunist stage, during which organically-enriched sediments and exposed bones are colonised by dense assemblages of heterotrophic fauna exploiting the remaining soft tissue and bones for months to years depending on the size of the carcass (Levin *et al.*, 1994; Smith and Baco, 2003). During the third stage, sulfide derived from the anaerobic breakdown of bone lipids by microbes fuels a species-rich assemblage on and around the bones; this stage may last for decades (Smith, 1992; Bennett *et al.*, 1994; Deming *et al.*, 1997; Smith and Baco, 2003; Smith, 2006). A significant component of the fauna found during this stage derives nutrition from this sulfide-based chemoautotrophy, via microbial endosymbiosis or direct bacterial grazing (Bennett *et al.*, 1994; Smith *et al.*, 2002; Smith and Baco, 2003; Smith, 2006). This third stage may also facilitate the dispersal of some faunal species between other chemosynthetic environments by providing 'stepping stones' between ephemeral, sulfide-rich habitats (Smith *et al.*, 1989; Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Smith and Baco, 2003; Lorion *et al.*, 2009; Lundsten *et al.*, 2010a). The final stage is thought to be a 'reef stage', where the remains of bones form a hard-substrate habitat for suspension feeders such as anemones, although to date, it has not been recorded in the scientific literature (C. Smith, personal communication). The enrichment-opportunist, sulfophilic and reef stages are thought to overlap to some extent (Smith *et al.*, 2002; Goffredi *et al.*, 2004; Braby *et al.*, 2007). Estimates of the duration of each stage and whale falls in their entirety are still poor as so few carcasses have been studied in any detail, but

it is estimated that the entire decomposition process can take anywhere from years to >100 years depending on the size of the carcass, the fauna present and the ecological setting (Smith *et al.*, 2002; Smith and Baco, 2003; Schuller *et al.*, 2004; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Lundsten *et al.*, 2010b).

The discovery of natural whale falls on the seafloor is extremely rare. To date, only six natural whale falls have been discovered through chance encounters and studied with ROVs or submersibles in depths ranging from 150 to 4037 m (Smith *et al.*, 1989; Fujioka *et al.*, 1993; Smith and Baco, 2003; Goffredi *et al.*, 2004; Lundsten *et al.*, 2010a), although considerably more have been observed in photosurveys but remain unsampled (C. Smith, personal communication). In addition to these, there are a number of records of whale bones unexpectedly trawled up from depth with attached fauna (Tebble, 1966; Dell, 1987; Marshall, 1987; Waren, 1989; McLean, 1992; Marshall, 1994; Dell, 1995; Bolotin *et al.*, 2005). Discovering natural whale falls is important to deep-sea science because they provide a unique view into the natural processes of the taphonomy of large cetaceans on the seafloor: i.e. their settlement, decomposition and ultimately fossilization (Allison *et al.*, 1991). Natural whale falls are also important as the majority of whale falls studied have been implanted within the last twenty years and as a result, are relatively young. With natural whale falls, it is possible that carcasses have been decomposing on the seafloor for longer periods (Schuller *et al.*, 2004).

One important aspect of whale-fall ecology that has hitherto not received attention is the variation in community structure along a whale skeleton in the sulfophilic stage. A summary of data has recently emerged on the specific composition of whale bones possibly influencing faunal communities (Higgs *et al.*, 2011b). Whale bones are thought to be composed of 10% lipid and 25% protein on average, but lipid content can be as high as 80% by weight (Allison *et al.*, 1991; Gage, 2003; Higgs *et al.*, 2011b). A 40-ton whale carcass may carry 2000-3000 kg of lipids in its skeleton (Lundsten *et al.*, 2010b). Organisms living on the whale skeleton are likely to be dependent on the lipid-protein matrix of the bone, either directly or indirectly via chemoautotrophic bacteria (Bennett *et al.*, 1994; Deming *et al.*, 1997; Smith *et al.*, 2002; Smith and Baco, 2003). The 'oil-gradient hypothesis' states that the abundance of sulfophilic organisms in late-stage whale falls will correspond to changes in oil throughout the whale skeleton over time (Higgs *et al.*, 2011b). In older whale-fall communities, the majority of fauna is expected to be found on the humerus and lower vertebrae (lumbar and caudal), as these are the most oil-rich and therefore, sulfidic of bones. One of the most prevalent types of sulfophilic fauna at whale falls are sulfur-oxidising bacterial mats which cover the bones (Smith and Baco, 2003). Higgs *et al*. (2011b) hypothesised that these bacterial mats may act as a useful proxy for the sulfide output and therefore lipid content of the bones, as these are the basis of the grazing food chain during this stage of whale falls (Treude *et al.*, 2009).

During both the enrichment-opportunist and the sulfophilic stage of whale falls, a unique genus of siboglinid polychaetes, *Osedax*, consume the bones of the skeleton heterotrophically (Rouse *et al.*, 2004; Glover *et al.*, 2005b). It was initially speculated that *Osedax* relied for nutrition on hydrocarbon degradation (Rouse *et al.*, 2004), then collagen and cholesterol (Goffredi *et al.*, 2005), and finally primarily on collagen alone (Goffredi *et al.*, 2007; Higgs *et al.*, 2011a). These worms are thought to use acid-secreting enzymes on the surface of a root-like structure to bore into the bones (Higgs *et al.*, 2011a). It has only been realized recently how this genus affects the taphonomy of whale skeletons, contributing to their rapid degradation by boring into the bones at high densities (Braby *et al.*, 2007; Higgs *et al.*, 2011c). There are limited data however, on whether the genus *Osedax* has a preference for certain bone types or positions on whale skeletons, or even the role of *Osedax* in different successional stages (Goffredi *et al.*, 2005; Fujikura *et al.*, 2006; Braby *et al.*, 2007; Fujiwara *et al.*, 2007). Questions still to be addressed include whether *Osedax* colonise early and persist through to the sulfophilic stage, and whether degradation by *Osedax* inhibits the progression of whale skeletons into the reef stage.

The aims of this paper were to investigate the ecology of a late-stage natural whale fall, determine the successional stage and likely age of the carcass, analyse the whale-fall community structure, and test the 'oil-gradient' hypothesis. DNA barcoding of the bones was used to determine the whale species. All organisms found growing on the bones and in the immediate vicinity of the skeleton were identified, including a range of species undescribed to science. Using a high-definition refinement of ROV videography, we also examined the ‘intra’ whale-fall variations in community composition allowing us to test the 'oil-gradient hypothesis' for the first time. This included the examination of the distribution of bacterial mats along the bones of differing lipid content. The hypothesis was also extended to species that depend on the bacterial mats and the bone-specialist *Osedax*.

2.2 Methods

2.2.1 Observation and sample collection at the whale fall

The skeleton was found in the South Sandwich Arc, which is part of a complex tectonic system located south of the Polar Front in the East Scotia Sea at 59°41.671’S, 28°21.089’W, at a depth of 1444-1447 m (Fig. 2.1). The vicinity of the whale fall contained several chemosynthetic environments such as white-smoker vent fields and areas of diffuse flow, currently being investigated as part of the UK-funded ChEsSO (Chemosynthetic Ecosystems of the Southern Ocean) project. The whale fall was encountered serendipitously in a video survey of the area

during Dive #148 of the *Isis* ROV on February 7th 2010, as part of Voyage 42 of the *RRS James Cook*.

The ROV followed a grid of survey lines at an altitude of 3.5 m above the seafloor to obtain overlapping video images of the whale skeleton from its downward-looking 3-chip CCD video camera during *Isis* Dives #148 (February 7th 2010) and #151 (February 10th 2010). The Doppler control facility of the ROV enabled precise movements of the vehicle relative to the seafloor during video surveys, and a gyrocompass was used to maintain constant vehicle heading. Two parallel lasers mounted 0.1 m apart provided a scale in images. Frames from the downward-looking video camera were extracted and compiled into an overall digital mosaic image of the whale skeleton (Fig. 2.2). A high-definition video camera and digital-stills camera on separate adjustable pan-and-tilt mounts were also used to obtain supplementary close-up observations of bones and fauna. Three bone samples were retrieved from the skeleton with the ROV manipulators (Fig. 2.2). These bones were placed in individual bioboxes on the ROV until recovery on the deck of the ship, thereby preventing unnecessary washing of the bone samples.

Video footage from the ROV cameras was analysed as follows: (1) each bone in the skeleton was numbered and where possible, its anatomical type identified from bone morphology; (2) fauna visible on bones in video images were identified to the lowest taxon possible; (3) abundances of organisms were quantified on bones from digital still and video images, using four groups to classify organisms present: (i) bacterial mats; (ii) *Osedax*; (iii) peracarids (Amphipoda and Isopoda); and (iv) gastropods (*Pyropelta* sp.). Only bone-encrusting fauna that could be resolved were quantified. Amphipoda and Isopoda were grouped into peracarids because the two orders could not be distinguished from each other in the footage. Visible bone surface areas were calculated using the scaling lasers in the ROV imagery and 3D-geometric models of bone shape, to provide numbers of individuals per m^2 for each faunal group and percentage coverage for bacterial mats.

The skeleton was revisited on February 9th 2011 during Dive #4 of SHRIMP (Seabed High Resolution Imaging Platform) as part of Voyage 55 of the *RRS James Cook.* During this research voyage, the skeleton was due to be observed and surveyed again using high-definition cameras on the *Isis* ROV in a similar pattern to as was done in 2010. Unfortunately, the ROV suffered a collision with the propeller of the *RRS James Cook* during the first dive and so could not be used for the remainder of the cruise. Hence, SHRIMP was used and as this is a passively-towed vehicle, high resolution imagery could not be achieved. Observations on the placement of the bones and limited faunal presence were possible however.

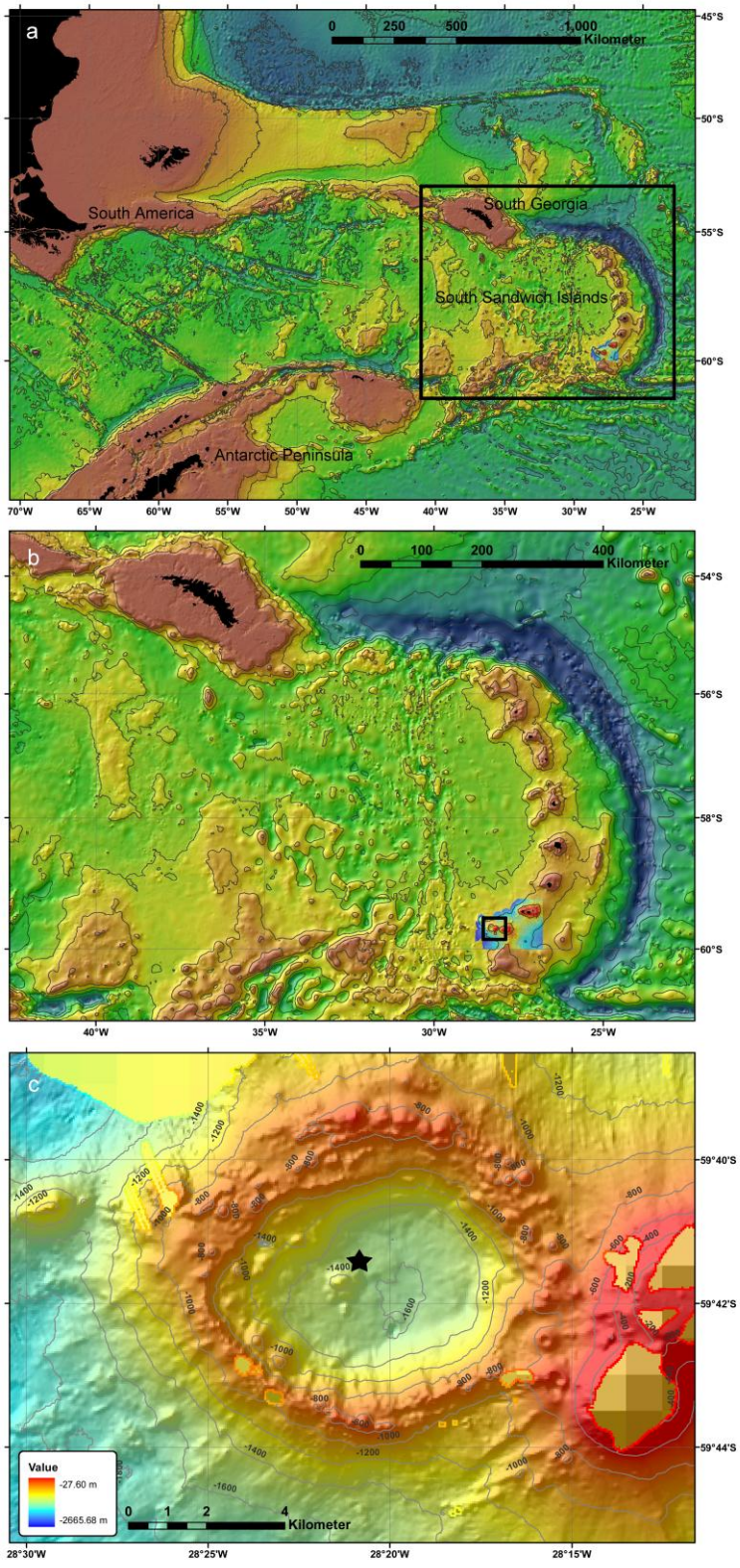

Figure 2.1 Location of the whale fall. (a) Bathymetry of the Scotia Sea and surrounding region. Contour lines are 1000 m with land above sea level indicated in black. (b) Bathymetry of the South Sandwich Islands. Contour lines are 1000 m with land above sea level indicated in black. (c) Bathymetry of the Kemp Caldera with the specific location of the whale fall denoted by ✯. Contour lines are 200 m. Bathymetry data for Figs. 2.1a and 2.1b is from Smith and Sandwell (1997). Fig. 2.1c was constructed from unpublished ship-based bathymetry data from the British Antarctic Survey.

2.2.2 DNA barcoding of the whale fall

DNA was isolated from the caldera whale bone using liquid nitrogen to freeze the bone so that it could be ground into powder. Approximately 0.1 g of bone powder was placed in each of three vials. Extraction was done using Qiagen DNeasy Blood and Tissue Extraction Kit following the protocol: Purification of Total DNA from Animal Tissue. Qiagen extractions were stored at -20°C. The primers used were made for a segment approximately 873 bp in length, containing the complete cytochrome b gene and part of the tRNA of the *Orcinus orca* Linnaeus, 1758 complete mitochondrion genome: Whale892F and Whale892R (Foote *et al.*, 2011)(NC_014682). The primer sequences were as follows: 5'-GTTATAGCCACCGCATTCGT-3' and 5'-AATTCCAGCTTTGGGTGTTG-3'. The DNA extracted from one vial of bone material (0.1 g) was used for PCR using PureTaq Ready-To-Go PCR beads (GE Healthcare). The PCR was performed in 25 µL reactions, consisting of 1 µL of each primer, 2 µL DNA template and 21 µL dH_2O. The PCR amplification profile consisted of initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 2 minutes and a final extension at 72°C for 10 minutes. Products of the PCR were confirmed by electrophoresis in a 1.5% agarose gel. Purification of the PCR products was achieved using a Qiagen PCR Purification Kit. Sequencing was performed on an ABI 3730XL DNA Analyser (Applied Biosystems) at the Sequencing Facility at the Natural History Museum, London using the primers described above.

Overlapping sequence fragments were merged into consensus sequences using Geneious (Drummond *et al.*, 2011) and aligned using MUSCLE (Edgar, 2004) provided as a plug-in in Geneious with default settings. The mtDNA sequences for all of the whales used in the phylogenetic analysis were obtained from GenBank (Table 2.1). Bayesian phylogenetic analyses (BA) were conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Analyses were run three times with the combined dataset with four chains for 2 000 000 generations, with 400 000 generations discarded as burn-in. The evolutionary models used for the molecular data in BA were obtained by running the two separate datasets in MrModelTest (Nylander, 2004), and for tRNA the optional model was K80+G. For cytochrome b, the data were partitioned into codon positions with position 1 following K80+G, position 2 following HKY, while HKY+G was used for position 3. In the combined BA, the data were partitioned into the two parts (cytochrome b and tRNA) and the evolutionary models mentioned above were applied to each partition and corresponding codon position respectively.

Table 2.1 Specimen information for the mysticete and outgroup mt-genomes analysed in this study.

Scientific name	English name	Accession no.	References
Hippopotamus amphibious	Hippopotamus	AP003425	Yasue *et al*., unpublished
Caperea marginata	Pygmy Right whale	NC_005269	Arnason *et al*., 2004
Balaena mysticetus	Bowhead whale	NC_005268	Arnason *et al*., 2004
Eubalaena australis 1	Southern Right whale	NC_006930	Sasaki *et al*., 2005
Eubalaena australis 2		AP006473	Sasaki *et al*., 2005
Eubalaena japonica	Northern Right whale	NC_006931	Sasaki *et al*., 2005
Balaenoptera acutorostrata 1	North Atlantic Minke whale	NC_005271	Arnason *et al*., 2004
Balaenoptera acutorostrata 2		AP006468	Sasaki *et al*., 2005
Balaenoptera acutorostrata 3		AJ554054	Arnason *et al*., 2004
Balaenoptera bonaerensis 1	Antarctic Minke whale	NC_006926	Sasaki *et al*., 2005
Balaenoptera bonaerensis 2		AP006466	Sasaki *et al*., 2005
Eschrichtius robustus 1	Gray whale	NC_005270	Arnason *et al*., 2004
Eschrichtius robustus 2		AJ554053	Arnason *et al*., 2005
Eschrichtius robustus 3		AP006471	Sasaki *et al*., 2005
Megaptera novaeangliae 1	Humpback whale	AP006467	Sasaki *et al*., 2005
Megaptera novaeangliae 2		NC_006927	Sasaki *et al*., 2005
Balaenoptera physalus 1	Fin whale	NC_001321	Valverde *et al*., 1994
Balaenoptera physalus 2		X61145	Arnason *et al*., 1991
Balaenoptera omurai	Omura's whale	NC_007937	Sasaki *et al*., 2006
Balaenoptera edeni	Bryde's whale	NC_007938	Sasaki *et al*., 2006
Balaenoptera borealis	Sei whale	AP006470	Sasaki *et al*., 2005
Balaenoptera musculus	Blue whale	NC_001601	Arnason & Gullberg, 1993
Physeter catodon	Sperm whale	AJ277029	Arnason *et al*., 2000
Orcinus orca	Orca whale	NC_014682	Foote *et al*., 2010

2.2.3 ^{210}Pb/^{226}Ra analysis of the whale fall

The isotopic pair (^{210}Pb/^{226}Ra) has been used previously to establish the time since cetacean death and deposition on the seafloor by Schuller *et al*. (2004). We tried to analyse the age since death of the caldera skeleton using the method below but were unsuccessful after two attempts. Seven other bones from skeletons of known varying ages were also analysed during this study to show the degree of error with the methodology (Table 2.2). The detailed methodology can be found below. Despite taking great care to avoid contamination, ratios of the isotopic pair ^{210}Pb/^{226}Ra were greater than unity on both occasions. Attempts are however, ongoing. As a result, only the state of decomposition of the bones could be used to gauge the age of the skeleton.

2.2.3.1 Sample Preparation

The known bone samples used in this study were collected from various museums, research institutes and whale strandings (Table 2.2). The samples were drilled using an electric hand drill and the shavings were collected, weighed and placed in plastic vials. At the National Oceanography Centre, Southampton, 36 mL of 2:1 chloroform:methanol were added to the entire whale-bone samples, which were left overnight on a tumbler to remove lipids. The vials were centrifuged at 2000 rpm for two minutes each to allow the bone matter to settle and the lipid solute was decanted carefully to prevent loss of any bone material. This lipid-removal

process was repeated three times but samples were only left for two hours on a tumbler rather than overnight. After the lipid separations, the solutions were replaced with methanol and left overnight. The samples were then individually filtered using Whatman glass microfibre filter papers. The filtrates were then placed in a drying oven (65°C) for two hours. After, the samples were filled with 35 mL 30 % hydrogen peroxide to remove organic deposits. After 'fizzing' (O_2 generation) had stopped, vials were then centrifuged, emptied and filled with deionised MilliQ water, gently shaken, centrifuged, and excess water decanted. The samples were then freeze-dried overnight. Each was reduced to powder form using an agate mortar and pestle and then weighed the following morning. Each powdered sample was placed in an airtight glass vial of fixed geometry with the sample number, sampling location and weight written on the vials. These were then left to allow ingrowth of Rn daughters for 20 days before being counted on a Canberra (HPGe-ULB) Ultra Low Background High Resolution Gamma Spectrometer for 200,000 seconds.

2.2.3.2 ^{210}Pb analysis

After gamma counting, 3.5 g of each sample were removed and placed in PTFE beakers. Each was spiked with 10 µL of ^{133}Ba from a stock solution of 1900 Bq/mL in 2M HNO_3, and 20.0 µL of ^{209}Po from a stock solution of 0.2612 Bq/g (Ref date of 14/02/11). Two blank solutions and two standard solutions were prepared using the same spikes, however 20.0 µL of the spike ^{210}Pb was added to each of the standard samples. 2 g of sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) and 2 g of hydrated calcium chloride ($CaCl_2.2H_2O$) were also added as a substrate to each of the blank and standard samples to match the approximate chemical composition of the bone samples. Then approximately 10 mL fuming HNO_3 (100 %) was added to all samples including the blanks and standards. These were left on a 90°C hot plate in a fume cupboard overnight. The following morning, a further 3 mL of fuming HNO_3 was added to each sample and left until the HNO_3 stopped fuming and each had evaporated to dryness. This process was done to remove all organics and carbonates from the samples. To remove the fuming HNO_3 in each mixture, three washes with 3 mL of concentrated HCl were added and then evaporated until dryness. The residues were then dissolved in 10 mL of 6M HCl and filtered into a glass beaker using Whatman cellulose (No. 41) filter papers. Each PTFE container was then washed with 5 mL deionised MilliQ water and poured through the filter paper into the acid fraction. The filter paper was washed with 5 mL deionised MilliQ water. After filtration was complete, the volume of each solution was made up to around 60 mL with deionised MilliQ water diluting the acid to about 1 M plus 0.5 g of ascorbic acid (to ensure reduction of any FeIII to FeII). Silver discs were polished with silver cleaner to remove any residues, labeled and placed in PTFE holders. Each disc was then placed into the respective solutions and left for two days on a warm surface to allow the polonium to plate onto the silver

discs. The silver discs were removed from the solutions, washed in deionised MilliQ water and air-dried for one hour. The discs were placed into a Canberra Quad Alpha Spectrometer Model 7404 and counted for approximately 400,000 seconds until there were approximately 1000 counts in both alpha peaks.

2.2.3.3 ^{226}Ra analysis

The solutions that had their ^{209}Po and ^{210}Po removed were used to determine the ^{226}Ra activity. The solutions were transferred to a 90°C hot plate to evaporate to dryness. The borosilicate beakers were placed in a muffle furnace for four hours at 550°C to ash. The selected temperature allows ashing but avoids melting the glassware. The samples were allowed to cool and then the ash from each sample was leached twice using 10 mL 8M HNO_3 each time. Solid and liquid fractions were separated by centrifuging (3000 rpm, 5 minutes) and the resulting clear liquid was evaporated to dryness in a 20 mL glass scintillation vial. The remaining solid residue was re-dissolved using 5 mL of 2M HNO_3 and the total sample volume was set to 10 mL using MQ water. The chemical recovery for all sample digests and subsequent handlings was monitored through the use of 10 µL ^{133}Ba tracer added (an effective chemical proxy for Ra) to each sample at the beginning of the sample digestion. Any losses were determined by comparing the ^{133}Ba gamma activity (HPGe gamma detector) with that from a reference solution that had not been through any chemical manipulation. Next, 10 mL of PerkinElmer ®High Efficiency Mineral Oil with Scintillator (HEMOS) was added. Each scintillation vial lid was fitted with a Viton seal to prevent any loss of radon gas. The mineral oil cocktail effectively traps Rn (insoluble in the acidic aqueous fraction but highly soluble in the oil) produced in the ^{226}Ra decay chain and after a 20 day radioactive in-growth period secular equilibrium is achieved. The date and time of sealing were marked on the vials, and the radon was allowed to re-equilibriate with the ^{226}Ra for a period of 20 days. The samples were then counted using a PerkinElmer® 1220 Quantulus Ultra Low Level Liquid Scintillation Spectrometer with alpha/beta signal discrimination mode switched on to count the radon and other short-lived radon progeny in the samples. The Rn daughters in the HEMOS provide a sensitive indication of the ^{226}Ra activity.

Table 2.2 Minke-whale bone samples of known and unknown ages dated in this study using $^{210}Pb/^{226}Ra$ disequilibria.

No.	Sample Type	Location Bone Found	Time Since Death
1	Natural whale fall	Kemp Caldera, Scotia Sea F549	Unknown
2	Natural whale fall	Kemp Caldera, Scotia Sea F542	Unknown
3	Natural whale fall	Tjarno, Sweden - 125 m	8 years
4	Natural whale fall	Koster Fjord Sweden (Fjallbacka)	4 years
5	Museum	Bohuslan, Fjallbacka (Gothenburg)	13 years
6	Museum	Bohuslan, Kungshamn (Gothenburg)	19 years
7	Museum	Bohuslan, Goteborgs (Gothenburg)	21 years
8	Museum	Skagen (Gothenburg)	100 years
9	Museum	Trawled (Gothenburg)	52 years

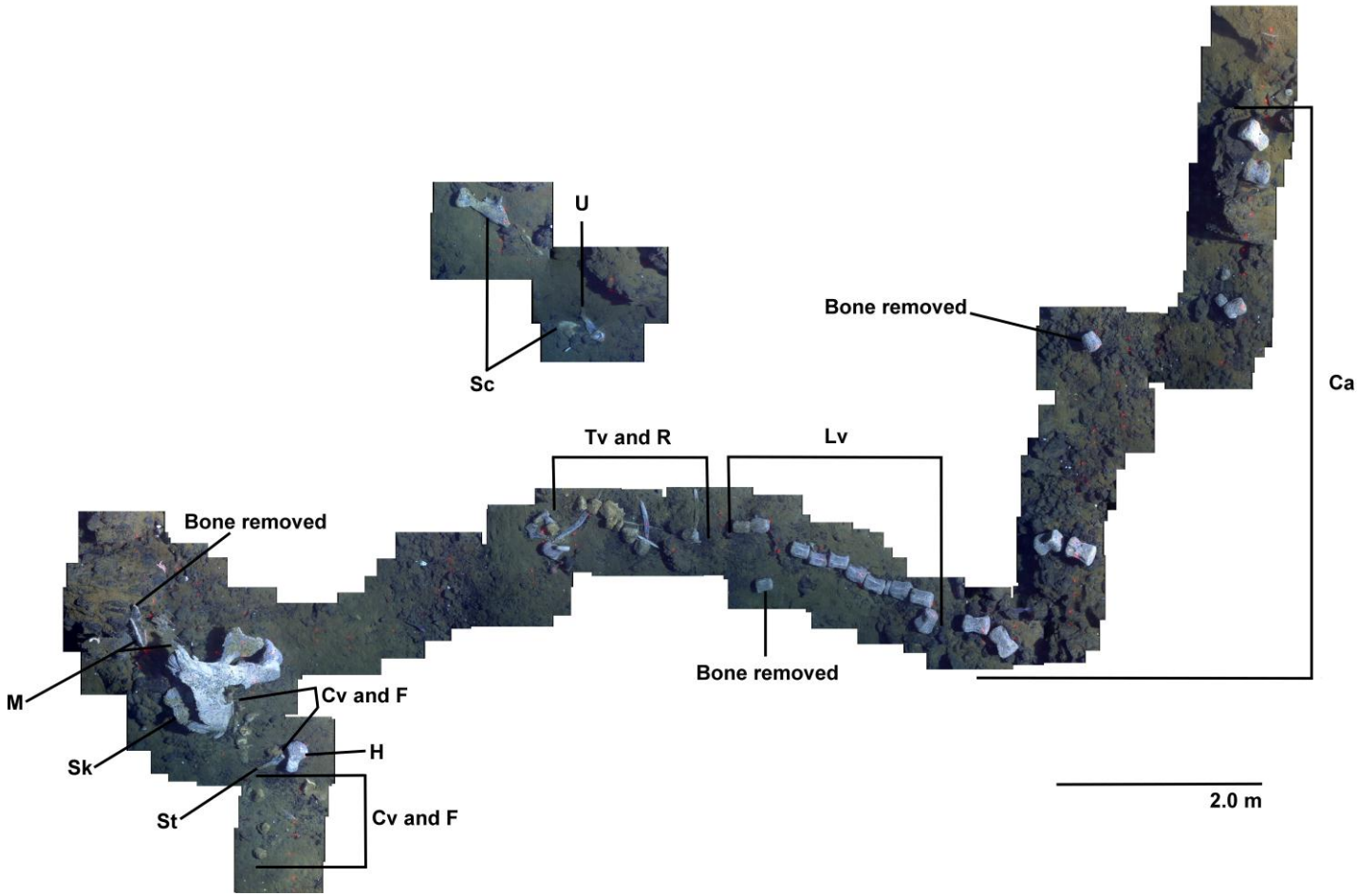

Figure 2.2 Photo mosaic of the whale fall. The three bones removed by the ROV for further analysis are indicated, as well as the anatomical bone types within the skeleton. M mandible, Sk skull, F small unidentified fragment, H humerus, U ulna, Sc scapula, St sternum, R rib, Cv cervical verterbra, Tv thoracic vertebrae, Lv lumbar vertebra, Ca caudal vertebra**.**

2.3 Results

2.3.1 Location and initial observations of the whale fall

The skeleton was found in a seafloor caldera west of Kemp Seamount on the southern tip of the South Sandwich Island arc (Fig. 2.1). The whale fall was on the northeast slope of a small sub-cone in the centre of the caldera at 1444–1447 m (Fig. 2.1). The substratum was rocky with a thin layer of sedimentation. Sediment underneath the whale bones was black in colour.

The whale fall was approximately 10.7 m long and completely skeletonised, lying in a disjointed line with the skull downslope (Fig. 2.2). The dorsal surface of the skeleton was uppermost (evidenced by the positions of the skull and mandibles) (Fig. 2.2). The vertebral column had been displaced from the skull but remained with most bones in order (Fig. 2.2). Some lumbar and caudal vertebrae were spread upslope in a haphazard manner (Fig. 2.2). Several bones appeared to be missing, including many smaller bones such as the pelvic bones, ear complexes, some ribs, chevrons, vertebral and pectoral epiphyses, radii, vorners, hyoid bones, carpals and metacarpals (Fig. 2.2 and Table 2.3). However, there were several unidentified fragments and these may have constituted some of the missing bones (Fig. 2.2 and Table 2.3). Most of the bones were highly degraded, although the degree of damage was variable (Fig. 2.2 and Table 2.3). Spongy bone was exposed on many of the bones following the removal of compact bone and some bones, such as the mandibles, were fragmented in several places. There was no evidence of any of the vertebral processes, with the cervical and thoracic vertebrae the most badly-eroded regions of bones (Fig. 2.2 and Table 2.3). Despite the high degree of erosion on many of the bones, they did still have lipid present. Sawing through sections of the collected bones revealed visual and olfactory confirmation of large amounts of oil. The smaller bones (unidentified fragments and cervical vertebrae) that were present were observed to be almost completely covered with sediment, but the majority of bones were, however, still projecting above the sediment.

Table 2.3 Bones present at the whale fall and their respective conditions.

Bone Type	Quantity Present in Live Minke Whale	Quantity Present at the Caldera Whale	Degree of Erosion	Notes on Erosion
Maxillae	2	-	-	-
Pelvic bones	2	-	-	-
Ear complexes	2	-	-	-
Chevrons	12	-	-	-
Vertebral epiphyses	76	-	-	-
Radii	2	-	-	-
Vorner	1	-	-	-
Hyoid bones	5	-	-	-
Carpals	10	-	-	-
Pectoral epiphyses	8	-	-	-
Metacarpals	8	-	-	-
Phalanges	40	-	-	-
Humeri	2	2	Limited	Some bone surfaces missing
Ribs	22	7	Limited to Severe	Some mostly intact, some fragmented
Small unidentified fragments	-	14	Limited to Severe	-
Skull	1	1	Moderate	Anterior of skull missing
Sternum	1	1	Moderate	Rounded edges
Lumbar vertebrae	12	11	Moderate	No processes
Caudal vertebrae	18	10	Moderate	No processes
Cervical vertebrae	7	7	Moderate to Severe	No processes
Ulnas	2	2	Moderate to Severe	Fragmented, missing large portions
Mandibles	2	2	Severe	Fragmented
Scapulas	2	2	Severe	Fragmented, missing large portions
Thoracic vertebrae	11	9	Severe	No processes, missing large portions
Total	**237**	**68**		

2.3.2 DNA barcoding of the whale fall

The DNA sequence was run in BLAST through Geneious (Drummond *et al.*, 2011) and the analysis (MrBayes) of 13 mysticete sequences (using *Physeter catodon* Linnaeus, 1758, *Orcinus orca* and *Hippopotamus amphibius* as outgroups) identified the species of whale as an Antarctic Minke whale (*Balaenoptera bonaerensis* Burmeister, 1867*)* by 97% DNA sequence similarity of the cytochrome *b* gene (745 bp) and a segment of the tRNA gene (746-873 bp) (Fig. 2.3). The genetic result was in concordance with the examination of some remaining morphological features of the skull region, and the overall size of the carcass; however, the morphology on its own would not have been enough to confirm identification to species level.

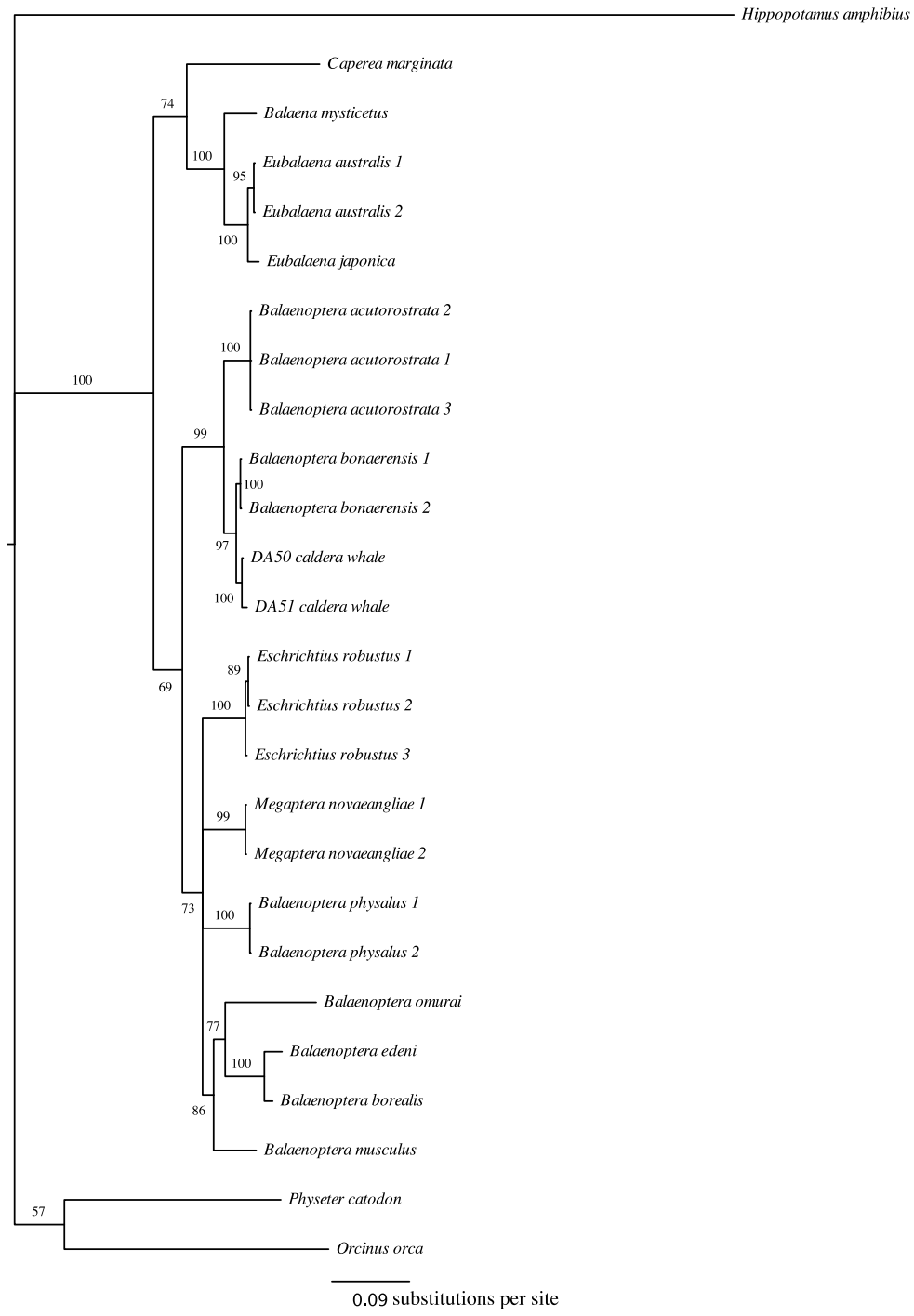

Figure 2.3 Molecular phylogenetic tree from Bayesian analysis showing the placement of the caldera whale. The whale (DA50 and DA51) fit within the species, *Balaenoptera bonaerensis*, using the cytochrome *b* gene (745 bp) and a segment of the tRNA gene (746-873 bp). Duplicates or triplicates of some species are used to show intra-species variation. The small numerals represent Bayesian Posterior Probabilities expressed out of a total of 100.

2.3.3 Whale-fall successional stage and community composition

The bones provided a substratum for nine taxa of abundant encrusting macrofauna (Fig. 2.4 and Table 2.4). Gastropods included an undescribed species of *Lepetodrilus*, an undescribed species of Osteopeltidae, and an undescribed species of *Pyropelta* (Fig. 2.4 and Table 2.4). The bones also harboured an undescribed species of amphipod (Lysianassidae sp.) and an undescribed species of isopod (*Jaera* sp.) (Fig. 2.4 and Table 2.4). There were also four undescribed species of polychaetes on the whale skeleton: two *Ophryotrocha* (sp. X and sp. P), an *Osedax* sp. and a Capitellidae sp. (Fig. 2.4 and Table 2.4). White and pink microbial mats covered much of the bone surface. We also observed 21 morphospecies of fauna in the immediate vicinity (<2 m) of the bones including several species of nemerteans, actinarians, echinoderms, poriferans, an unidentified polychaete tubeworm, and unidentified bivalves (Table 2.4). Bivalves were found within 0.2 m of the skeleton and were completely buried, only visible from their siphons. These were not sampled and so could not be positively identified.

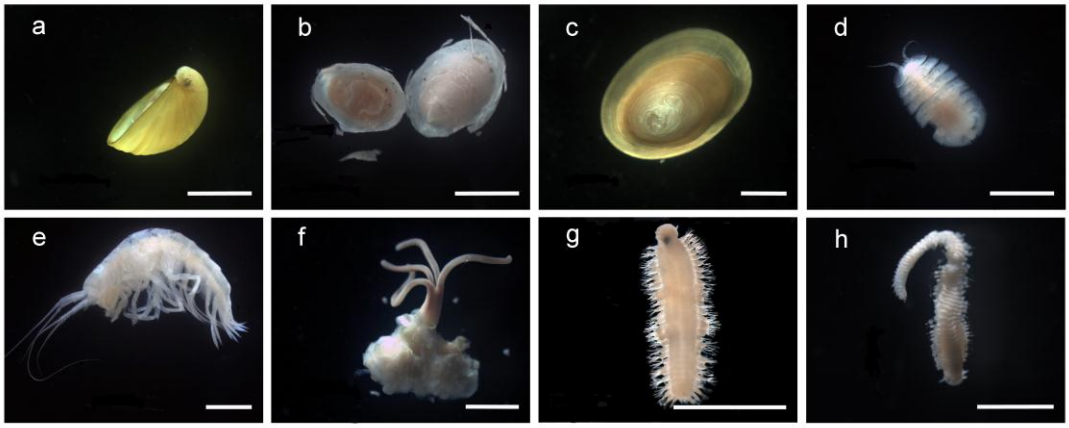

Figure 2.4 Fauna found on the whale bones. (a) *Lepetodrilus* sp., (b) Osteopeltidae sp., (c) *Pyropelta* sp., (d) *Jaera* sp., (e) Lysianassidae sp., (f) *Osedax* sp., (g) *Ophryotrocha* sp. P, (h) *Ophryothrocha* sp. X. There was also Capitellidae sp. (not shown). Scale for (a-h) is 2000 µm.

Table 2.4 Megafaunal and macrofaunal taxa with their respective locations with regard to the whale skeleton as observed by the ROV *Isis*.

Phylum	Class	Order	Family	Genus		Location
Porifera	indet.	indet.	indet.	indet.	2 spp.	Surrounding
Cnidaria	Anthozoa	Actinaria	indet.	indet.	4 spp.	Surrounding
Nemertea	Anopla		Cerabratulidae	*Parbolasia*	1 sp.	Surrounding
		indet.	indet.	indet.	1 sp.	Surrounding
Mollusca	Cephalopoda	Teuthida	indet.	indet.	2 spp.	Surrounding
	Bivalvia	indet.	indet.	indet.	1 sp.	Surrounding
	Gastropoda		Lepetodrillidae	*Lepetodrilus*	1 sp.	Bone epifaunal
			Osteopeltidae	indet.	1 sp.	Bone epifaunal
			Pyropeltidae	*Pyropelta*	1 sp.	Bone epifaunal
Sipuncula	indet.	indet.	indet.	indet.	1 sp.	Surrounding

Annelida	Polychaeta	Sabellida	indet.	indet.	1 sp.	Surrounding
			Siboglinidae	*Osedax*	1 sp.	Bone infaunal
		Eunicida	Dorvilleidae	*Ophryotrocha*	2 spp.	Bone epifaunal
			Capitellidae	indet.	1 sp.	Bone epifaunal
Arthropoda	Malacostraca	Decapoda	Nematocarcinidae	*Nematocarcinus*	1 sp.	Surrounding
		Isopoda	Munnopsidae	*Ilyarachna*	1 sp.	Surrounding
			Janiridae	*Jaera*	1 sp.	Bone epifaunal
		Amphipoda	Lysianassidae	indet.	1 sp.	Bone epifaunal
	Pycnogonida	Pantopoda	Ammotheidae	*Sericosura*	1 sp.	Surrounding
Echinodermata	Asteroidea	Forcipulatida	Stichasteridae	indet.	1 sp.	Surrounding
	Opiuroidea	indet.	indet.	indet.	1 sp.	Surrounding
	Echinoidea	Camarodonta	Echinidae	*Sterechinus*	1 sp.	Surrounding
Chordata	Actinopterygii	Gadiformes	Macrouridae	indet.	1 sp.	Surrounding
		Perciformes	Nototheniidae	Dissostichus	1 sp.	Surrounding

2.3.4 Variation in community composition along the skeleton

Many of the bone epifauna were distributed differentially along the skeleton (Fig. 2.5). Mean bacterial-mat cover was less than 5% of surveyed bone surface area on the cervical and thoracic vertebrae (Fig. 2.5). Bacterial mats were also absent on some of the heavily-sedimented small unidentified fragments of bone (Fig. 2.5). The highest mean bacterial-mat percentage cover was on the humerus, with 72% of the surveyed bone surface area covered (Fig. 2.5). The lumbar- and caudal-vertebral region of the skeleton also had high mean bacterial-mat percentage cover (54 and 55% respectively) (Fig. 2.5). The bacterial mats appeared to be thickest on the anterior and posterior ends of these vertebrae.

Pyropelta sp. were most dense on the sternum (1034 m^{-2}), the mandibles (mean 803 m^{-2}) and the ulna (858 m^{-2}) (Fig. 2.5). The humerus (445 m^{-2}), skull (322.21 m^{-2}), lumbar vertebrae (mean 282 m^{-2}) and caudal vertebrae (mean 288 m^{-2}) also had high densities of *Pyropelta* sp. (Fig. 2.5). *Pyropelta* sp. had the lowest mean densities on the cervical and thoracic vertebrae (20 and 60 m^2 respectively), and the small unidentified fragments of bone (62 m^{-2}) (Fig. 2.5). These low mean densities may have been due to the fact that *Pyropelta* sp. were absent from some of the individual bones of these anatomical types (Fig. 2.5). *Osedax* sp. were most dense on the mandibles and the thoracic vertebrae (mean 1176 m^{-2} and 765 m^{-2} respectively) (Fig. 2.5). *Osedax* sp. were least prevalent on the ribs, sternum, humerus, lumbar vertebrae, and caudal vertebrae with all those bone regions having less than mean 220 m^{-2} (Fig. 2.5). Peracarids were most dense on the mandibles (mean 13427 m^{-2}) and skull (7338 m^{-2}) (Fig. 2.5). They were least abundant on the cervical vertebrae (mean 693 m^{-2}) (Fig. 2.5). Otherwise the peracarids showed a fairly uniform mean presence on surveyed bones with between 1497 m^{-2} and 3652 m^{-2} (Fig. 2.5). *Jaera* sp. were frequently observed on the trunk or palps of specimens of *Osedax* sp.

The densities of bacterial mats ($p = 0.001$), *Pyropelta* sp. ($p < 0.0006$) and *Osedax* sp. ($p < 0.003$) did differ significantly between the twelve anatomical types of bones. There was however no significant difference for peracarid densities between different anatomical types of bones. The densities of *Pyropelta* sp. and the percentage covers of bacterial mats were strongly positively correlated on surveyed bones ($r = 0.579$, $p < 0.00001$). Peracarid and *Pyropelta* sp. densities were also strongly positively correlated ($r = 0.713$, $p < 0.0002$). *Osedax* sp. showed the opposite trend, decreasing in densities with increasing coverage of bacterial mats ($r = -0.508$, $p < 0.0002$). On closer inspection of this trend, it appeared that *Osedax* sp. were found only growing on areas of bone devoid of white bacterial mats (Fig. 2.6).

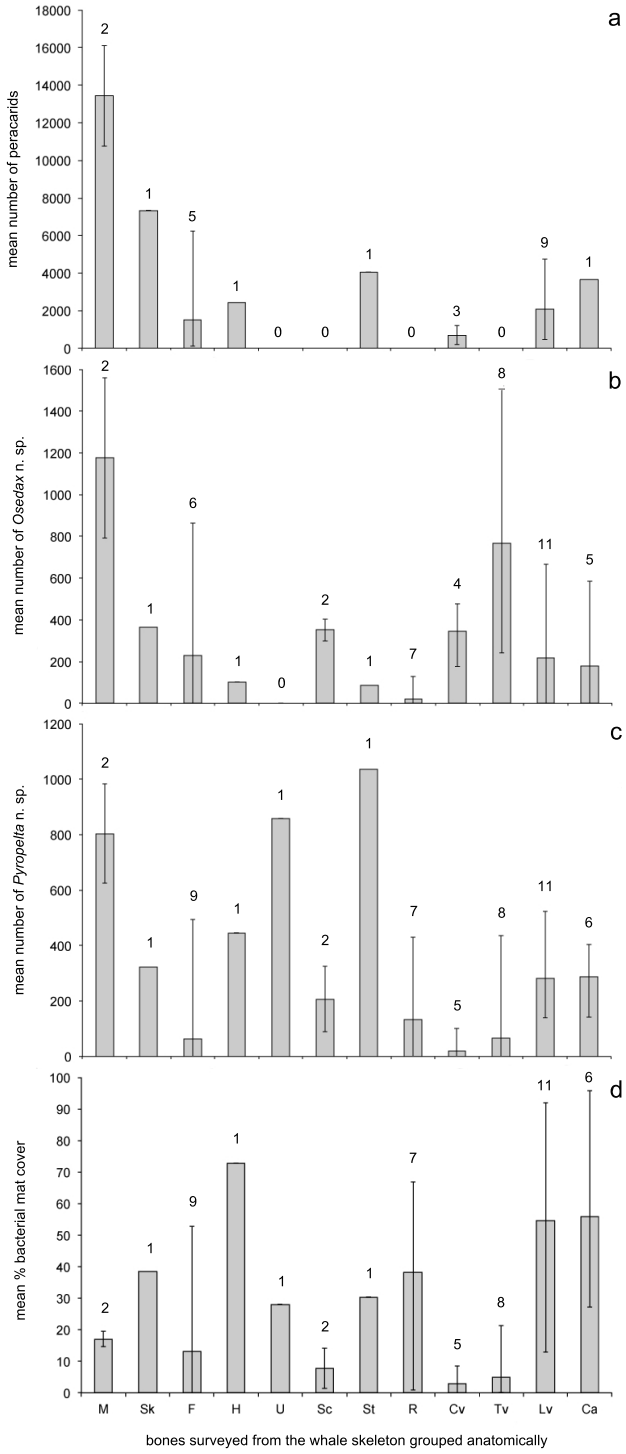

Figure 2.5 The microdistribution of fauna on the bones of the whale fall. (a) The mean number of peracarids (amphipods – Lysianassidae sp., and isopods – *Jaera* sp.) per metre squared exposed surface area of bone (b) the mean number of *Osedax* sp. per metre squared exposed surface area of bone (c) the mean number of *Pyropelta* sp. per metre squared exposed surface area of bone (d) the mean percentage of bacterial-mat cover of exposed surface area of bone. Bones were grouped together according to their anatomical types as follows: M mandible, Sk skull, F small unidentified fragment, H humerus, U ulna, Sc scapula, St sternum, R rib, Cv cervical verterbra, Tv thoracic vertebrae, Lv lumbar vertebra, Ca caudal vertebra. Numbers above each column are the number (n) of bones of that anatomical type where that type of fauna could be quantified. Means ± SD are indicated by bars on each column.

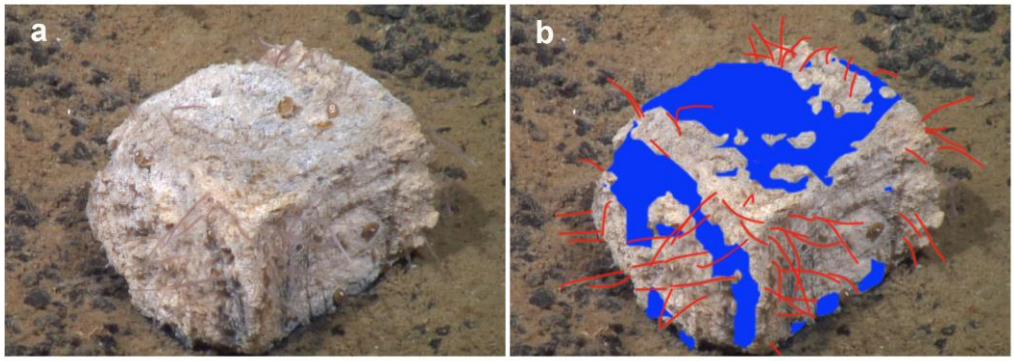

Figure 2.6 *Osedax* sp. distribution with regard to bacterial mats. A lumbar vertebra from the whale skeleton showing the surface area of the bone covered by bacterial mats and the location individuals of *Osedax* sp.

2.3.5 Further observations of the whale fall in 2011

It should be noted that due to the height above seabed of SHRIMP, the imagery obtained was of poor resolution. Most of the bones were in the same locations as seen in 2010 apart from half a rib and a vertebra which had been moved to a location approximately one metre away, and another rib previously present in 2010 is now missing. The bones did not appear to be more eroded than in 2010 (Fig. 2.7). It was very difficult to resolve the fauna on skeleton, however some echinoids were noted amongst the bones and limpets (possibly *Pyropelta* sp. noted in 2010) were seen on some bones (Fig. 2.7). Overall there was very little change to the skeleton observed in the year between the two visits in 2010 and 2011.

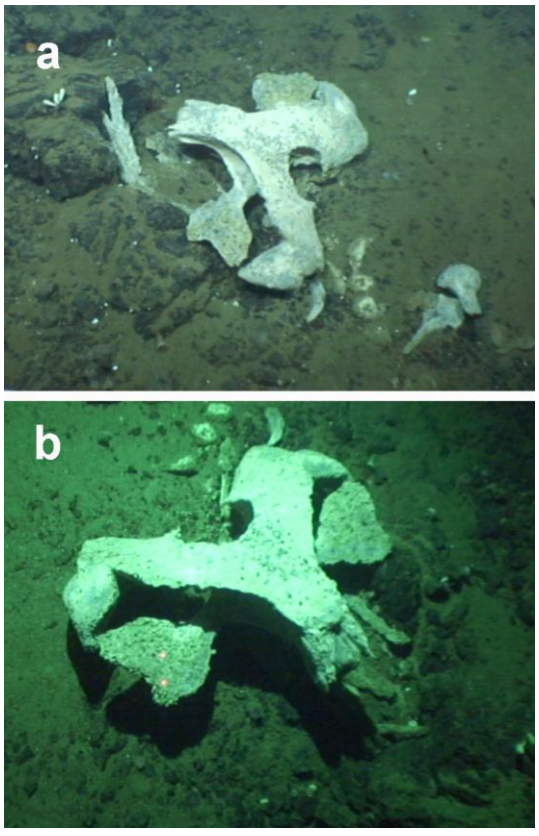

Figure 2.7 Time series of a natural whale fall. (a) The skull and nearby bones of the Antarctic whale fall during the 2010 visit. (b) The same skull and bone in 2011.

2.4 Discussion

2.4.1 Species, age and successional processes of the whale skeleton

The large size (approximately 10.7 m) of the spread-out skeleton suggested that this was an adult, possibly female, specimen. Average adult lengths for Antarctic Minke whales are 8.5 m for males and 9.0 m for females, with a maximum size given as 10.7 m (R. Sabin, personal communication). The size of this whale resulted in the organic enrichment of the sediment and the subsequent creation of anoxic conditions from high microbial oxygen consumption, denoted by blackened sediments below the whale bones and as seen previously at other natural whale falls and emplacement experiments (Allison *et al.*, 1991; Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Jones *et al.*, 1998; Smith *et al.*, 1998; Smith and Baco, 2003; Glover *et al.*, 2005b; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Glover *et al.*, 2008; Treude *et al.*, 2009; Lundsten *et al.*, 2010a).

No mobile scavengers were observed at the remains, and all soft tissue had been consumed. The whale fall was thus in either the enrichment-opportunist or sulfophilic stage of decomposition (Smith and Baco, 2003). The sulfophilic stage has been observed to last from six years to several decades, and is typified by the presence of chemosynthetic sulfide-oxidising microorganisms (e.g. *Beggiatoa* spp.) covering the bones (Smith *et al.*, 1989; Allison *et al.*, 1991; Bennett *et al.*, 1994; Deming *et al.*, 1997; Baco and Smith, 2003; Smith and Baco, 2003; Schuller *et al.*, 2004).

Thin long bones, like the mandibles, were fragmented in several places and the cervical and thoracic vertebrae were the most badly-eroded regions of bone. The missing bones tended to be smaller bones, which may have been carried away by currents, or scavenging animals, or these bones may have become completely buried in the sediment or eroded.

The whale fall, despite the advanced successional stage, was still inhabited by a diverse assemblage of organisms, including many species that we have confirmed are as of yet, undescribed to science (being formally described elsewhere). The abundance of undescribed species at this whale fall was not surprising given a historical lack of deep-sea exploration in the region, and the high endemism of fauna in the Southern Ocean resulting from hydrographic isolation by the Polar Front (Orsi *et al.*, 1995; Rogers, 2012; Rogers *et al.*, 2012b).

Many of the bone-encrusting fauna observed at this whale fall, however, were from families or genera known to inhabit whale falls and other chemosynthetic environments. The sulfidic conditions found at whale falls can be similar to those found at other chemosynthetic environments such as hydrothermal vents and cold seeps, and consequently some clades and

even some species show overlap between these habitats (Tunnicliffe *et al.*, 1996; Sibuet and Olu, 1998; Smith and Baco, 2003; Tunnicliffe *et al.*, 2003; Treude *et al.*, 2009). For example, *Lepetodrilus* sp. from our whale-fall site, was also found on a variety of substrates at the nearby hydrothermal vents, both within the caldera where the whale fall was found (J. Copley, K. Linse, L. Marsh, A. Rogers, personal observation) and at newly-discovered vent fields on the East Scotia Ridge during this research voyage (Rogers *et al.*, 2012b). Rogers *et al*. (2012) observed that this limpet was grazing epizoic microbes at the vent sites, which allows speculation that these limpets were consuming microbes on the whale bones here. *Lepetodrilus* spp. have been found previously at vents, seeps, and whale falls (Desbruyeres *et al.*, 2006; Johnson *et al.*, 2008). The genus *Pyropelta* has also been found previously at vents, seeps and whale falls, and is a known bacterial grazer, while the family Osteopeltidae is comprised of whale-fall specialists (Marshall, 1987; Bennett *et al.*, 1994; Smith *et al.*, 2002; Desbruyeres *et al.*, 2006)

The dorvilleid genus *Ophryotrocha* has been observed in abundance at reducing environments (Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Deming *et al.*, 1997; Baco *et al.*, 1999; Van Dover, 2000; Smith *et al.*, 2002; Baco and Smith, 2003; Smith and Baco, 2003; Rouse *et al.*, 2004; Glover *et al.*, 2005a; Dahlgren *et al.*, 2006; Pleijel *et al.*, 2008; Wiklund *et al.*, 2009a; Lundsten *et al.*, 2010a). Wiklund *et al*. (2009) noted that they depend on the bacterial mats covering the whale bones rather than the whale bones themselves, and thus could live at other chemosynthetic environments if bacterial mats are present. Members of the amphipod family Lysianassidae are frequently found at food falls and are known to be opportunistic scavengers (Jones *et al.*, 1998; Smith and Baco, 2003; Lundsten *et al.*, 2010b). The isopod genus *Jaera* has only previously been recorded in estuaries and shallow intertidal waters, so its occurrence here in the deep Southern Ocean is remarkable (K. Linse, personal communication).

The other fauna in the immediate vicinity of the bones could not all be identified to generic level but the clades observed were consistent with those seen at other late-stage whale falls (carideans, bivalves, isopods, actinarians and echinoderms (Marshall, 1987; Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Deming *et al.*, 1997; Baco *et al.*, 1999; Van Dover, 2000; Smith and Baco, 2003; Rouse *et al.*, 2004; Lundsten *et al.*, 2010a). As a result of the lack of positive identifications, it was not clear which of the surrounding fauna were whale-fall specialists or 'background' deep-sea fauna using the whale fall opportunistically as an additional food source (Wiklund *et al.*, 2009a). The high species richness during the sulfophilic stage of whale falls is thought to be due to high trophic diversity, with bacterial-mat grazers, species with chemoautotrophic endosymbionts, bone specialists, organic-enrichment respondents, predators and suspension feeders (Bennett *et al.*, 1994; Baco and Smith, 2003).

Fauna with endosymbionts such as bivalve molluscs are typical of the sulfophilic stage in whale-fall ecology (Smith *et al.*, 1989; Bennett *et al.*, 1994; Feldman *et al.*, 1998; Baco *et al.*, 1999; Fujiwara *et al.*, 2009; Lorion *et al.*, 2009; Lundsten *et al.*, 2010a). The unidentified bivalves may have arrived from nearby hydrothermal vents to take advantage of the reducing conditions under the bones. There have, however, been few records of siboglinids other than the bone specialist *Osedax* spp., *Escarpia spicata* and *Lamellibrachia barhami* at whale falls (Feldman *et al.*, 1998; Rouse *et al.*, 2004; Glover *et al.*, 2005b; Lundsten *et al.*, 2010a). Whale falls, with their combination of sediment, sulfide and hard substratum (bone) may offer an intermediate-habitat type between hard-substratum vents and sedimented-hosted seeps in the evolution of some deep-sea chemosynthetic taxa (Baco *et al.*, 1999; Smith and Baco, 2003).

It can therefore be confidently stated that the caldera whale fall was in the sulfophilic stage of decomposition from several characteristics; a) there was no evidence of soft tissue present, b) the bones were significantly eroded with mandibles and ribs fragmented, and vertebral processes missing, c) there was sustained chemoautotrophic production fuelled by sulfides indicated by abundant bacterial mats growing on the bones and surrounding sediment, d) the sediments below the bones were black in colour indicating anoxia, e) the assemblage of fauna present on and around the skeleton resembled those seen at previous whale falls in the sulfophilic stage (Smith and Baco, 2003).

The caldera skeleton's condition was compared with other known late-stage whale falls that had sufficient information. Several of the previous whale falls seemed younger than the caldera whale fall based on bone-erosion observations. The carcasses studied by Jones *et al*., (1998) and Dahlgren *et al*., (2006) had been on the seafloor for less than a year. Whale-385 and whale-1018 still had vertebral processes after 1.1 years on the seafloor (Braby *et al.*, 2007), as did whale-382 and whale-633 after 2.4 years and 2.2 years respectively (Lundsten *et al.*, 2010b). The most similarities existed between the Santa Catalina Basin whale fall (Allison *et al.*, 1991; Bennett *et al.*, 1994; Smith and Baco, 2003), the Vancouver whale fall (Lundsten *et al.*, 2010a), the implanted sperm-whale carcasses off Japan (Fujiwara *et al.*, 2007), the Torishima whale fall (Naganuma *et al.*, 1996), whale-2893 (Lundsten *et al.*, 2010b), and the caldera whale fall. All these carcasses shared most of the sulfophilic-stage characteristics (a-e) above. The Vancouver whale fall, the implanted Japanese sperm-whale carcasses and the Torishima whale fall had bones of a similarly-degraded manner but mandibles were intact suggesting that our caldera skeleton may be slightly older (Naganuma *et al.*, 1996; Fujiwara *et al.*, 2007; Lundsten *et al.*, 2010a; Lundsten *et al.*, 2010b). Bacterial mats were abundant at all six whale-carcass groups and the diverse faunal assemblages were comprised of similar animals e.g. *Ophryotrocha* spp., ophiuroids, amphipods, isopods, *Pyropelta* spp. and other limpets (Bennett *et al.*, 1994; Smith and Baco, 2003; Lundsten *et al.*, 2010a). It was also taken into account that there seemed to be

very little change in the bone conditions (in terms of erosion and sedimentation) between 2010 and 2011.

The Vancouver whale skeleton was estimated to be at least 6-10 years old and the Torishima whale skeleton, 1-16 years old, based on bivalve-shell size (Naganuma *et al.*, 1996; Lundsten *et al.*, 2010a). The implanted Japanese sperm-whale carcasses had been implanted for three years (Fujiwara *et al.*, 2007) and whale-2893 was on the seafloor for 3.9 years although Lundsten *et al*., (2010a) had noted that this carcass had degraded extremely rapidly. The Santa Catalina Basin whale fall was aged by Schuller *et al*., (2004) to 1948 (64 years since death). Therefore, by comparison with other whale-fall observations and data, the caldera whale fall may have been on the deep-sea floor between 4 and 64 years.

2.4.2 The 'oil-gradient' hypothesis

The estimated age of 4 to 64 years for this whale skeleton allowed for the testing of the 'oil-gradient hypothesis', which proposes that differences in lipid content in different parts of whale skeleton and between different whale species may result in variations in the assemblage structure of whale falls and the rate of degradation of different parts of the skeleton (Higgs *et al.*, 2011b). Although lipid-content data for Antarctic Minke whale bones are not available, data from other mysticete whales can be used as a proxy (Higgs *et al.*, 2011b). The most lipid-rich bones in the great whales are the lumbar and caudal vertebrae (40-50% depending on the species), and the humeri (approximately 64%) (Higgs *et al.*, 2011b). The mandibles and skull are estimated to have 20-45%, and the bones of the chest region (scapula, sternum and ribs) 15-30%. The cervical vertebra usually contain less lipid than the lumbar and caudal regions, and the thoracic vertebra contain an even lower lipid content of ~10%.

The bacterial mats on whale bones tend to be sulfophilic (Deming *et al.*, 1997) and are therefore found growing most heavily on the lipid-rich regions of bone. They require sulfide as an energy source for carbon-dioxide fixation and oxygen for the oxidation of the sulfide (Deming *et al.*, 1997). In our study, the mean percentage cover by sulfophilic bacterial mats corresponded to predicted bone-lipid content. The bacterial mats were less prevalent on bones predicted to have lower lipid content, such as the cervical vertebrae, thoracic vertebrae and small unidentified bone fragments. The lumbar and caudal vertebrae, and the humerus (bones with high lipid content) exhibited the highest percentage coverage of bacterial mats, specifically on the anterior and posterior ends of these vertebrae. The skull and mandibles tended to have more bacterial mats than the cervical and thoracic vertebrae, but less than the other bones. Trends such as these have been seen previously: Bennett *et al*. (1994) reported that bacterial mats were largely absent from buried or highly-degraded ribs and thoracic vertebrae, with heaviest coverage seen on

anterior and posterior ends of vertebrae. In addition, regions of the skull and caudal vertebrae were the most covered in bacterial mats (Bennett *et al.*, 1994). Lundsten *et al*. (2010a) observed bacterial mats covering at least small portions of the lower vertebrae as well as the skull and jaws. Treude *et al.* (2009) witnessed large changes in bacterial-mat coverage over a one-year period for a six- to seven-year-old carcass. At first, the skull, thoracic vertebrae and ribs were most covered but one year later the skull and ribs showed decreases in bacterial-mat coverage but still retained the most cover. Bones like the thoracic- and caudal-vertebral regions, however, showed large decreases (approximately 30%) in cover.

The genus *Pyropelta* is comprised of bacterial grazers and so it follows that these limpets would be most abundant on the bones with the most bacterial-mat coverage, as was seen at this skeleton. There may have been other bacterial grazers, such as dorvilleids, that were overlooked, as they were too small to be seen in the video footage. The *Osedax* sp. had the greatest abundance on bones with predicted lower lipid content, such as the mandibles, and the cervical and thoracic vertebrae, and were rarest on the high-lipid bones; the lumbar and caudal vertebrae, and the humerus. The densities of *Osedax* sp. on the bones was negatively correlated with the percentage cover by bacterial mats with *Osedax* sp. found only growing on areas of bone devoid of white bacterial mats. This may have been as a result of (1) competition for space with the bacterial mats; (2) ecosystem engineering by the burrowing activity of *Osedax* sp., which may facilitate the influx of oxygen into bones and thus limit the anaerobic decomposition of bone lipids. However, burrowing by *Osedax* may also promote escape of sulfide (Higgs *et al.*, 2011a) or influx of seawater sulfate for increased sulfate reduction (Treude *et al.*, 2009). Conversely, measurements of the microenvironment between individual *Osedax* roots and their bone matrix indicate anoxic conditions (Huusgaard *et al.*, 2012); (3) the anaerobic breakdown of bone lipids creating a high-sulfide environment that is not conducive to *Osedax* sp. growth or settlement; (4) *Osedax* sp. may secrete a substance inhibiting bacterial-mat growth in the immediate vicinity of the worm, although no evidence of this has been found thus far. Overall, a similar trend for macrofauna in general was previously reported by Bennett *et al*. (1994), where there was a near-total absence of all macrofauna from yellow and white microbial mats occurring on the anterior and posterior ends of vertebrae.

The occurrence of *Osedax* sp. also corresponded with the high levels of erosion seen in the thoracic and cervical vertebrae. *Osedax* bore into bones in high densities contributing to their rapid degradation and this can have huge taphonomic significance (Braby *et al.*, 2007; Higgs *et al.*, 2010; Kiel *et al.*, 2010; Higgs *et al.*, 2011b). Lundsten *et al.* (2010b) noted that the skulls of whales off California were slow to be colonized by *Osedax* and also took the most time to degrade, highlighting the potential influential effect *Osedax* can have on bones. This bone erosion can be as high as 6% per year and possibly even higher depending on the species and

the location (Higgs *et al.*, 2011a). The collagen and proteinaceous areas that the *Osedax* rely on for nutrition via bacterial endosymbionts (Goffredi *et al.*, 2005; Goffredi *et al.*, 2007; Higgs *et al.*, 2011a) may be more plentiful or easier to access in bones with lower lipid content - the 'oil-protection theory' (Higgs *et al.*, 2011b). Higgs *et al*. (2010b) states "it may be that the bioeroding organisms in the seawater are excluded from the bones by the hydrophobic oils, or that the breakdown of the lipids creates an environment that is not conducive to bioeroding micro-organisms (e.g. high sulfide levels)". Even though information in the literature regarding differential patterns of erosion at whale falls was sparse, Dominici *et al*. (2009) observed increased levels of degradation of thoracic vertebrae compared with lumbar and caudal ones in fossil skeletons. This has also been seen at recent whale falls (Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Lundsten *et al.*, 2010a). Allison *et al*. (1991) and Naganuma *et al*. (1996) specifically mentioned rib degradation and Bennett *et al*. (1994) noted vertebrae and rib degradation.

Goffredi *et al*. (2005) and Braby *et al*. (2007) noted that *Osedax rubiplumus* were most prevalent on enamel-covered bones like the ribs and mandibles whereas *O. frankpressi*, *O. roseus*, *O.* 'yellow collar' and *O.* 'orange collar' preferred vertebral processes. *Osedax* 'spiral' was observed growing on degraded and sedimented bone fragments (Braby *et al.*, 2007). *O. japonicus* have been observed growing on bone, blubber and spermaceti (Fujikura *et al.*, 2006; Fujiwara *et al.*, 2007). *Osedax* spp. have even been observed growing on quadruped bones (Jones *et al.*, 2008; Vrijenhoek *et al.*, 2008a) and teleost vertebrae (Rouse *et al.*, 2011). This evidence suggests that even though *Osedax* spp. do feed on the organic content at whale skeletons, the exact preference of nutritional material taken from the bone may differ between species and so the occurrence of *Osedax* sp. may be as a result of species preference also. There may be other influential factors such as succession of *Osedax* spp. (Braby *et al.*, 2007), competition for resources, and environmental parameters such as sedimentation also (Glover *et al.*, 2008).

2.5 Conclusions

This was the first natural whale fall to be observed in the Southern Ocean, despite this area harbouring an abundance of large cetaceans. The presence of large amounts of lipids and/or collagen in the bones showed that the carcass was still able to support life on the seafloor for a considerable time, and the diverse faunal assemblage found on this skeleton included several undescribed species. The study of this whale skeleton has shown that there is evidence for the 'oil-gradient hypothesis'. Further exploration of the deep sea in this area is necessary for the taxonomy of fauna and the biogeographic patterns of chemosynthetic ecosystems in this ocean to be clarified.

3. Three new species and an expansion of range for the genus *Osedax* (Annelida: Siboglinidae) in the deep Antarctic

A modified version of this chapter is in review with the journal 'Zoologica Scripta' as:

Amon, D. J., J. T. Copley, T. G. Dahlgren, A. J. Jamieson, C. R. Smith, H. Wiklund and A. G. Glover (2013). "Three new species and an expansion of range for the genus *Osedax* (Annelida: Siboglinidae) in the deep Antarctic".

We report the expansion of diversity in the genus *Osedax*, by the discovery of three new species found in the deep Antarctic Southern Ocean. *Osedax rogersi* sp. nov. and *Osedax crouchi* sp. nov. were found on a whale skeleton at 1444 m in the Kemp Caldera in the East Scotia Sea during a research cruise that was part of the ChEsSo (Chemosynthetic Ecosystems of the Southern Ocean) project. The recently-described species, *Osedax antarcticus*, found on whale bones implanted at a depth of 550-650 m off Smith Island in the Bransfield Strait, was also found inhabiting the whale skeleton in the Kemp Caldera. The new species, *O. crouchi* as well as another new species, *Osedax nordenskioeldi* sp. nov. have been found on these implanted whale bones off Smith Island. These two localities are approximately 1800 km apart demonstrating the wide distribution of species within the genus. We describe the three new species, *O. rogersi, O. crouchi* and *O. nordenskioeldi*, and report the second record of *O. antarcticus*. A new phylogenetic analysis including data examining genetic connectivity between the Scotia Arc and the Bransfield Straight for these unusual vertebrate-bone endemic species is also presented.

3.1 Introduction

The deep sea is a food-limited environment, and organisms remote from local sources of production such as hydrothermal vents or cold seeps, must sustain themselves on food that sinks to the seafloor from surface waters. The most extreme example of this is the carcass of a whale, and since the first discovery of a natural whale carcass on the seafloor in 1987 (Smith *et al.*, 1989), a number of rather unique species have been described that are able to consume these remains, termed 'whale-falls' (Smith and Baco, 2003). Possibly the most remarkable group are the 'bone-eating' worms in the genus *Osedax* (Annelida: Siboglinidae), with two species in the genus first described in 2004 from a whale carcass at approximately 3000 m depth in Monterey Canyon, California, USA (Rouse *et al.*, 2004). Since then, a further five species have been described from a shelf-depth site in the North Sea (Glover *et al.*, 2005b), a shelf-depth site off Japan (Fujikura *et al.*, 2006), mid-bathyal depths in the Monterey Canyon (Rouse *et al.*, 2008) and most recently, from shelf-depth in the Antarctic (Glover *et al.*, 2013). In addition, there are a further 16 undescribed taxa for which unique DNA sequences have been submitted to the online repository GenBank (Benson *et al.*, 2012), which we treat here as operational taxonomic units (OTUs).

The Southern Ocean exhibits several characteristics that should make it one of the more hospitable ocean regions for this unique bone-boring genus of polychaetes. Given the high abundance of large marine mammals, there should be large quantities of bones on the seafloor, which could be used for nutrition and as a substrate by *Osedax* species (Laws, 1977; Tonnessen and Johnsen, 1982). The isolation of the Antarctic water mass by the Antarctic Polar Front and the circulation induced by the Antarctic Circumpolar Current may provide the opportunity for *Osedax* to disperse within the Southern Ocean and have broad biogeographic ranges around the Antarctic as has been reported in molluscs (Griffiths *et al.*, 2008). *Osedax* species thus far have never been found in tropical shallow waters suggesting that perhaps the genus is limited by temperature (Rouse *et al.*, 2004; Glover *et al.*, 2005b; Dahlgren *et al.*, 2006; Fujikura *et al.*, 2006; Vrijenhoek *et al.*, 2009; Schander *et al.*, 2010). The low temperature, isothermal water column of the Southern Ocean is likely to allow for the intrusion of *Osedax* species at shallow depths, as has recently been reported for *Osedax deceptionensis* Taboada *et al*., 2013 which is recorded from just 21 m depth (Glover *et al.*, 2013). The ability to colonise shallow sub-tidal habitats in high latitudes may greatly increase available *Osedax* habitat. Taking these criteria into account, it may be surprising to some that it has taken this length of time for scientists to search for *Osedax* species in the area but exploration has been slow given the inaccessibility of the waters surrounding Antarctica and especially of the deep sea. In addition, it is only now that

we have a better idea of the morphology of *Osedax* that researchers are able to look for it in likely habitats.

In February 2009, samples of experimentally implanted whale bone were recovered from a free-vehicle lander at shelf depths in the Bransfield Strait which were colonised by the species *Osedax antarcticus* (Glover *et al.*, 2013). In January 2010, the deep sea Remotely Operated Vehicle (ROV) *Isis* was diving at a hydrothermal vent site in the Scotia Sea when the team happened across a natural whale fall: the remains of an Antarctic Minke whale (*Balaenoptera bonaerensis* Burmeister, 1867) in approximately 1400 m water depth (Amon *et al.*, 2013). In Chapter 2, one new species of *Osedax* was reported from this whale fall, however it was found to actually be three species during this study. Here we describe three new species of the siboglinid genus *Osedax* found from these sites in the Southern Ocean and undertake a study of their phylogenetic relationships and population connectivity from sites 1800 km apart and separated by depths of approximately 1000 m. We test the hypothesis that *Osedax* species in the Southern Ocean have shared common ancestry, exhibit high degrees of eurybathy and their populations are connected by the formation of the Antarctic Circumpolar Current (ACC).

3.2 Methods

3.2.1 Study areas and sampling in the Southern Ocean

The species discussed in this study were recovered from two sites: a natural whale fall in Kemp Caldera and implanted whale bones off Smith Island (Fig. 3.1). The skeleton at the Caldera site was an Antarctic Minke whale (*Balaeonoptera bonaerensis*) and was found serendipitously in the East Scotia Sea during ROV *Isis* Dive #148 on February 7th 2010, on the *RRS James Cook* cruise JC42 (Amon *et al*., 2013). The whale fall was located at a depth of 1444-1447 m in the seafloor caldera at 59°41.671'S, 28°21.089'W (Fig. 3.1). This area is home to a number of chemosynthetic environments and associated ecosystems such as white smoker vent fields and extensive areas of diffuse flow, which were being investigated as part of the UK-funded ChEsSO (Chemosynthetic Ecosystems of the Southern Ocean) project (Rogers *et al.*, 2012b). The skeleton was visually surveyed by the ROV and the digital stills and video images captured were used to quantify the visible bone surface areas using scaling lasers in the imagery, to provide numbers of *Osedax* per m^2 (Amon *et al.*, 2013). Three bone samples (a mandible and two vertebrae) were collected by the ROV. Upon recovery, specimens of *Osedax* were photographed while alive and still in the bones. 48 specimens for molecular analysis were stored in 96% ethanol and then frozen at -80°C. Six other specimens for morphological analysis

were fixed in 4% formalin overnight at 4°C. They were then washed three times in MQ water for 10-15 minutes and stored in 70% ethanol at -80°C.

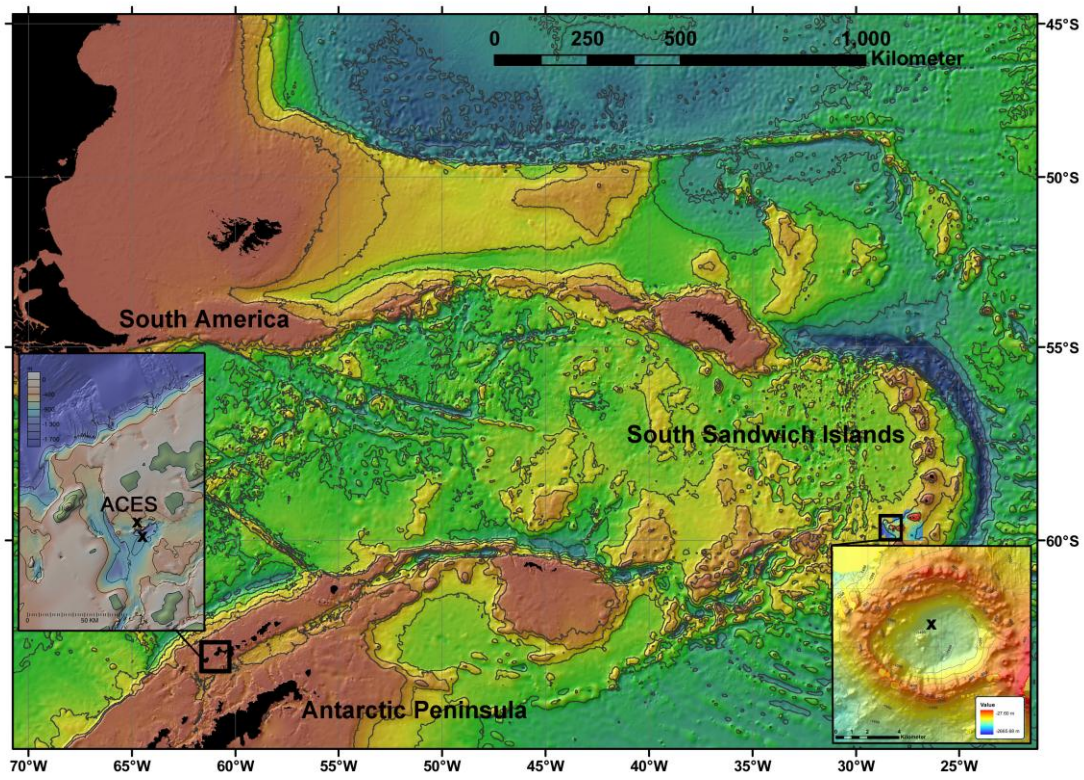

Figure 3.1 Locations in the Southern Ocean where the *Osedax* species were found. Kemp Caldera, the location of the natural whale fall, is denoted by a black box, enlarged in the bottom right, with the exact location of the whale skeleton denoted by X. Contour lines are 200 m in this enlarged section. The locations of the ACES landers off Smith Island in the Bransfield Strait can be seen within another black box. This black box is enlarged in the bottom left and can be distinguished by the word **ACES** with the two lander locations denoted by two **X**s. Contour lines are 200 m in this enlarged section. In the main map, contour lines are 1000 m with land above sea level indicated by black. These maps have been reproduced with changes from Amon *et al*. (2013) and Glover *et al*. (2013).

Bones from a Minke whale (*Balaenoptera acutorostrata* Lacepede, 1804*)* were implanted attached to free-falling compact landers from the Antarctic Chemosynthetic Ecosystems (ACES) project at two locations near Smith Island in the Bransfield Strait (Glover *et al.*, 2013) (Fig. 3.1). The whale bones attached to each lander were collected from a dead, stranded carcass in Fjällbacka, Sweden on 4th April 2007. Prior to deployment, the bones were de-fleshed, sterilized in freezers and transported frozen aboard the Swedish Icebreaker *Oden* to Antarctica (Glover *et al.*, 2013). Minke whale bones were chosen as they are naturally abundant in Antarctic waters, particularly *B. bonaerensis* (morphologically almost identical to *B. acutorostrata*). The ACES 1 and 2 experiments were deployed in December 2007 in a relatively flat area of soft-sediment shelf environment at 550-650 m close to the 'FOODBANCS2 Project'

site 'AA' (Glover *et al.*, 2013). The ACES experiments were recovered by acoustic command in February 2009 by the US vessel *RV Laurence M Gould* (Glover *et al.*, 2013).

On recovery, the bones from the ACES experiments were detached from the lander frame and stored in onboard aquarium facilities at ambient temperature of 1-2°C and running sea surface water prior to processing. The substrate surface and fauna were photographed immediately using an Olympus underwater camera. Specimens were then picked and dissected from the whale bone under stereo microscopes and imaged using a Nikon 4500 camera with microscope adaptor before preservation. The majority of the fauna was later preserved in 95% ethanol in individual numbered vials, with some specimens selected for preservation in 10% formalin or 2% glutaraldehyde in sodium cacodylate buffer for more detailed morphological study.

3.2.2 Morphological analyses

All of the *Osedax* specimens were examined and imaged at the Natural History Museum, London, using a Leica DM5000 compound microscope with DFC 480 camera and a Zeiss V.20 stereomicroscope with AxioCam camera. Thirty-two preserved specimens of *Osedax crouchi* sp. nov. were measured using the Zeiss V.20 stereomicroscope with AxioCam camera. Measurements were taken of the maximum lengths and widths of the trunks, palps and oviducts. Eggs were counted and the maximum diameter measured of each. Photographs of nine live specimens of *Osedax* morphospecies B (hypothesised to be *O. crouchi* sp. nov.*) in situ* were examined and the maximum lengths and widths of the trunk and palps were measured. Twelve preserved specimens of *O. rogersi* sp. nov. and ten preserved specimens of *Osedax nordenskioeldi* sp. nov. were also measured as above. Measurements were taken of the maximum length and width of the trunks and palps. Eggs were also counted and the maximum diameter measured of each. Four specimens were prepared for scanning electron microscopy using a Phillips XL-30 FEG scanning electron microscope by dehydrating in ethanol, critical point drying and gold-coating.

3.2.3 Molecular analyses

The molecular phylogenetic analysis was made with datasets from fragments of the mitochondrial genes 16S, cytochrome oxidase subunit I (COI) and the nuclear gene 18S. DNA extractions were performed using Qiagen DNeasy Blood and Tissue Extraction Kit following the protocol: Purification of Total DNA from Animal Tissue. The Qiagen extraction was stored at -20°C. The primers used for amplification and sequencing can be seen in Table 3.1. About 400-500 bp of 16S, 1800 bp of 18S and 1250 bp of COI were amplified. The PCR was performed on DNA extracted in 25 µL reactions, consisting of 1 µL of each primer, 2 µL DNA

template and 21 µL Red Taq DNA Polymerase Master Mix (VWR). The PCR amplification profile consisted of initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, extension at 72°C for 2 minutes and a final extension at 72°C for 10 minutes. Products of the PCR were confirmed by electrophoresis in a 1.5% agarose gel. PCR purification was done using a Millipore Multiscreen 96-well PCR Purification System. Sequencing was performed on an ABI 3730XL DNA Analyser (Applied Biosystems) at the Natural History Museum Sequencing Facility using the primers described in Table 3.1.

Table 3.1 Primers used in the *Osedax* molecular studies. (F) and (R) denote the forward and reverse primer respectively.

Gene Targeted	Primers	Primer Sequence (5'-3')	Reference
CO1	OseCO1F (F)	TA(CA)ACTAATCA(CT)ATTGG(ATGC)AC	(Nelson and Fisher, 2000)
CO1	OseCO1R (R)	CC(ATG)CTTAG(TA)CCTA(GA)TGTTG(ATCG)GG	(Nelson and Fisher, 2000)
16S	ann16Sf (F)	GCGGTATCCTGACCGTRCWAAGGTA	(Sjölin *et al.*, 2005)
16S	ann16Sr (R)	TCCTAAGCCAACATCGAGGTGCCAA	(Sjölin *et al.*, 2005)
18S	18SA (F)	AYCTGGTTGATCCTGCCAGT	(Medlin *et al.*, 1988)
18S	1324R (R)	CGGCCATGCACCACC	(Cohen *et al.*, 1998)
18S	620F (F)	TAAAGYTGYTGCAGTTAAA	(Nygren and Sundberg, 2003)
18S	18SB (R)	ACCTTGTTACGACTTTTACTTCCTC	(Nygren and Sundberg, 2003)

Overlapping sequence fragments were merged into consensus sequences using Geneious (Drummond *et al.*, 2011) and aligned using MUSCLE (Edgar, 2004) for CO1 and MAFFT for 16S and 18S, provided as plug-ins in Geneious with default settings. In total, 51 terminal taxa were included in the analyses, 29 from *Osedax*, 29 from other genera within Siboglinidae, and three outgroup taxa of which a spionid, *Malacoceros fuliginosus* Claparède, 1870, was used as root. In the Sabellidae outgroup, 18S and 16S from *Sabella pavonina* Savigny, 1822 was used together with COI from *Sabella spallanzanii* Gmelin, 1791. In the Oweniidae outgroup, 18S and COI from *Owenia fusiformis* Delle Chiaje, 1844 was used together with 16S from *Myriochele* sp. Accession numbers for the taxa used can be found in Table 3.2. Bayesian phylogenetic analyses (BA) were conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Analyses were run three times with the combined dataset for 10,000,000 generations. 2,500,000 generations were discarded as burn-in.

Table 3.2 NCBI GenBank accession numbers for the siboglinid and outgroup sequences.

Terminal taxa	18S	16S	COI
Alaysia sp. Southward, 1991	FM995545	---	---
Escarpia spicata Jones, 1985	AF168741	AF315041	FJ667537
Galathealinum brachiosum Ivanov, 1961	AF168738	AF315040	U74066
Lamellibrachia barhami Webb, 1969	AF168742	AF315047	AY129146
Lamellibrachia columna Southward, 1991	FJ347679	FJ347646	DQ996645
Lamellibrachia satsuma Miura, 1997	FM995543	---	D38030
Malacoceros fuliginosus Claparède, 1870	AY525632	EF431962	EF432016
Myriochele sp. Malmgren 1867	---	AY340468	---
Oasisia alvinae Jones, 1985	AF168743	AF315052	AY646020
Oligobrachia haakonmosbiensis Smirnov, 2000	AM883186	---	FM178481
Osedax antarcticus	KF444420	KF444418	KF444422
Osedax nordenskioeldi	---	---	---
Osedax rogersi	---	---	---
Osedax crouchi	---	---	---
Osedax deceptionensis	KF444421	KF444419	KF444428
Osedax frankpressi Rouse *et al*., 2004	FJ347682	AY577876	DQ996621
Osedax japonicus Fujikura *et al*., 2006	FM995535	---	FM998111
Osedax mucofloris Glover *et al*., 2005	AY941263	---	AY827568
Osedax roseus Rouse *et al*., 2008	FM995536	FJ347657	EU032469
Osedax rubiplumus Rouse *et al*., 2004	FM995538	AY577878	EU223302
Osedax sp. 'green palp'	FJ347694	FJ347655	FJ347640
Osedax sp. 'nude palp A'	FJ347687	FJ347653	EU223356
Osedax sp. 'nude palp B'	FJ347686	FJ347652	EU236218
Osedax sp. 'nude palp C'	FJ347688	FJ347650	EU267675
Osedax sp. 'nude palp D'	FJ347691	FJ347649	FJ347630
Osedax sp. 'nude palp E'	FJ347692	FJ347648	FJ347634
Osedax sp. 'nude palp F'	FJ347695	FJ347651	FJ347643
Osedax sp. 'orange collar'	FJ347690	FJ347661	EU223340
Osedax sp. ‘pinnules’	---	---	FJ431197
Osedax sp. 'sagami 3'	FM995537	---	FM998078
Osedax sp. 'sagami 4'	FM995541	---	FM998082
Osedax sp. 'sagami 5'	FM995539	---	FM998083
Osedax sp. 'sagami 6'	FM995540	---	FM998091
Osedax sp. 'sagami 7'	FM995542	---	FM998108
Osedax sp. 'sagami 8'	FM995534	---	FM998110
Osedax sp. 'spiral'	FJ347693	FJ347647	FJ347637
Osedax sp. 'white collar'	FJ347684	FJ347659	FJ347611
Osedax sp. 'yellow collar'	FJ347689	FJ347660	EU223323
Osedax sp. 'yellow patch'	FJ347685	FJ347654	FJ347618
Owenia fusiformis Delle Chiaje, 1844	AB106256	---	AY428839
Paraescarpia sp. Southward *et al*., 2002	FM995546	AF315053	D50595
Polybrachia sp. Ivanov, 1952	AF168739	AF315037	FJ480393
Ridgeia piscesae Jones, 1985	AF168744	AF315054	AF022233
Riftia pachyptila Jones, 1981	AF168745	AF315049	FJ667529
Sabella pavonina Savigny, 1822	U67144	AY340482	---
Sabella spallanzanii Gmelin, 1791	---	---	AY436349
Sclerolinum brattstromi Webb, 1964	AF315061	FJ347645	FJ347644
Sclerolinum contortum Smirnov, 2000	AM883187	---	FM178480
Siboglinum ekmani Jägersten, 1956	AF315062	AF315038	---
Spirobrachia sp. Ivanov, 1952	AF168740	AF315036	FJ483547
Tevnia jerichonana Jones, 1985	AF168746	AF315042	AY646000

The evolutionary models used for the molecular data in BA were obtained by running the three separate datasets in MrModelTest (Nylander, 2004). For 18S and 16S we used the optional model GTR+I+G, while for COI the data was partitioned into codon positions, where position 1 followed SYM+I+G and GTR+G was used for positions 2 and 3. In the combined BA, the data

was partitioned into the three parts (18S, 16S and CO1) and the evolutionary models mentioned above were applied to each partition and corresponding codon position respectively. A separate COI dataset was made for haplotype distribution analysis using TCS 1.21 (Clement *et al.*, 2000), with 122 *Osedax* specimens recovered from the sampled whale bones. Of those 122, 20 were from *Osedax rogersi*, 39 from *O. crouchi*, 10 from *O. nordenskioeldi* and 53 from *O. antarcticus* (Table 3.3).

Table 3.3 *Osedax* specimens used for CO1 haplotype distribution analysis. Specimens with * have been also processed for 16S and 18S. All specimens with names beginning with SI were collected from the implanted bones off Smith Island. All other specimens were collected from the natural whale fall in Kemp Caldera.

Osedax rogersi	***Osedax crouchi***	***Osedax antarcticus***	***Osedax nordenskioeldi***
F549-5	F549-1	F549-35*	SI-22
F549-8	F549-2	F549-42	SI-26
F549-11	F549-6	F549-49	SI-35
F549-15	F549-7	SI-1	SI-36
F549-19	F549-9	SI-2	SI-38
F549-21	F549-10	SI-3	SI-40
F549-25	F549-12	SI-4	SI-46*
F549-26	F549-14	SI-5	SI-49
F549-27	F549-23	SI-6	SI-56
F549-31	F549-24	SI-7	SI-58
F549-39	F549-28	SI-9	
F549-40	F549-29	SI-10	
F549-43*	F549-30	SI-11	
F571-1	F549-32	SI-12	
F571-2	F549-33	SI-13	
F571-3	F549-36	SI-15	
F571-6	F549-41	SI-16	
F571-7	F549-44	SI-17	
F571-8	F549-45	SI-18	
F602-a	F549-46*	SI-19	
	F549-47	SI-20	
	F549-48	SI-21	
	F549-50	SI-23	
	F549-51	SI-24	
	F571-9	SI-25	
	F571-10	SI-29	
	F602-b	SI-30	
	F602-c	SI-31	
	F602-d	SI-32	
	F602-e	SI-37	
	F602-g	SI-41	
	SI-14	SI-44	
	SI-28	SI-45	
	SI-33	SI-47	
	SI-34	SI-48	
	SI-60	SI-51	
	SI-63	SI-52	
	SI-68	SI-53	
	SI-242	SI-55	
		SI-57	
		SI-61	
		SI-62	
		SI-64	
		SI-65	
		SI-66	
		SI-67	
		SI-240	

SI-241
SI-243
SI-244
SI-281
SI-282
SI-283

3.3 Results

3.3.1 Systematics

3.3.1.1 *Osedax rogersi* sp. nov.

Annelida Lamarck, 1809, Siboglinidae Caullery, 1914, *Osedax rogersi* sp. nov.

(Figs. 3.2a to 3.2c)

Material examined

Osedax rogersi type material. Kemp Caldera, South Sandwich Arc, East Scotia Sea, Southern Ocean, Antarctica collected aboard *RRS James Cook* research cruise 42 as part of the ChEsSO project on 7th February 2010 from a natural whale fall of an Antarctic Minke whale at 1444-1447 m at 59°41.671'S, 28°21.089'W. Holotype: mature adult female, collected as palps only as the entire animal was impossible to excavate intact from bone tissue.

Diagnosis

Type material observed, four smooth palps of equal length, average 3.07 mm, and equal width average 0.23 mm in ethanol-fixed specimens, under high-power light microscopy (Figs. 3.2a and 3.2c). One row of micropinnules observed on each palp visible only under oil-immersion giving rugose appearance to palp surface at high magnification (Fig. 3.2b). Palps becoming opaque white to light pink after preservation (Fig. 3.2a). Some specimens observed with single thin line of red pigment globules running length of palp (Fig. 3.2a). No oviduct, trunk region or root observed and no trace of any male individuals. Elliptical eggs ranging 0.05-0.09 mm diameter found after specimen dissection. Thin mucous tube surrounds the specimen; larger at base of the palps.

Etymology

Named after Alex Rogers, Professor of Conservation Biology at Oxford University, for his contributions to deep-sea science and as Principal Scientific Officer on Voyage 42 of the *RRS James Cook*.

Figure 3.2 Four species of *Osedax*. (a) The palps of an *Osedax rogersi* sp. nov. specimen preserved in ethanol. A single line of red pigmentation globules can be seen within one of the palps. There are also some remnants of the tube surrounding the palps. The palps may not be inflated as seen; this may be an artifact from keeping the animals alive in tanks at the surface. Scale is 2.0 mm. (b) Micropinnules seen on the inside surface of a palp of *O. rogersi* using oil-immersion. Scale is 0.1 mm. (c) An SEM image of the four palps of *O. rogersi*. Scale is 0.5 mm. (d) An *Osedax crouchi* sp. nov. specimen. The tips of the palps were lost during dissection and preservation in ethanol. Scale is 2.0 mm. (e) An SEM image of the palp surface of *O. crouchi*. Scale is 0.05 mm (f) An SEM image showing the trunk, collar and beginning of the palps and oviduct of an *O. crouchi*. Scale is 1.0 mm. (g) A palp from *O. crouchi* showing double lines of red pigment globules viewed using oil immersion. Scale is 0.4 mm. (h) An intact specimen of *O. crouchi* imaged using light microscopy. Scale is 2.0 mm. (i) A damaged specimen of *Osedax nordenskioeldi* sp. nov. imaged using light microscopy. The trunk region can be seen to the right of the palps of the specimen. Scale is 1.0 mm. (j) An intact specimen of *O. antarcticus* Glover *et al*., 2013 from the whale skeleton in Kemp Caldera. Scale is 2.0 mm.

3.3.1.2 *Osedax crouchi* sp. nov.

Annelida Lamarck, 1809, Siboglinidae Caullery, 1914, *Osedax crouchi* sp. nov.

(Fig. 3.2d to 3.2h)

Material examined

Osedax crouchi Type material. Kemp Caldera, South Sandwich Arc, East Scotia Sea, Southern Ocean, Antarctica collected aboard *RRS James Cook* research cruise 42 as part of the ChEsSO (Chemosynthetic Ecosystems of the Southern Ocean) project on 7 February 2010 from a natural whale fall of an Antarctic Minke whale at 1444-1447 m at 59°41.671'S, 28°21.089'W. Holotype: mature adult female, dissected from vertebra collected from the skeleton. Paratypes: mature adult females, dissected from vertebra from skeleton (locality as above), mature adult females, dissected from implanted Minke whale bone (locality: 63°10.98'S 61°38.16'W, Bransfield Strait, West Antarctic Peninsula, Southern Ocean, 568 m, implanted Minke whale bones). Two locations in close proximity: dissected from Minke whale rib bone collected from 'ACES 2 Lander' at 63°10.98'S 61°38.16'W deployed on the seafloor in 568 m of water and from Minke whale bones of 'ACES 1 Lander' at 63°9.87'S 61°41.34'W deployed on the seafloor in 650 m of water.

Diagnosis

Type material observed, four light-pink palps due to colour loss on fixation, average length 6.17 mm and average width 0.23 mm in ethanol-fixed specimens (Fig. 3.2d). Palps fused for approximately 80% of length, smooth without visible pinnules under light microscopy (Fig. 3.2d). Some specimens observed to have two thin lines of red pigment globules with decreasing globule size towards tips of the palps (Fig. 3.2g). SEM giving rugose appearance to palp surface at high magnification (Fig. 3.2e). Oviduct similar length to palps (average 5.54 mm), sometimes longer, free to base and adjoined to trunk at collar region (Figs. 3.2d and 3.2f). Elliptical eggs ranging 0.05-0.13 mm diameter found after specimen dissection. Mean of measured trunk in ethanol-fixed specimens, 1.06 mm width, 1.34 mm length, ridged appearance under SEM (Fig. 3.2f). Roots compact, lobate, pigmented green in fixed specimens. No data on male morphology available. Female tubes consist of thin, mucous sheaths.

Etymology

Named after Leslie M. and Judy A. Crouch, founders of the Leslie. M and Judy A. Crouch Educational Trust, who provided funding enabling myself to undertake my PhD.

3.3.1.3 *Osedax nordenskioeldi* sp. nov.

Annelida Lamarck, 1809, Siboglinidae Caullery, 1914, *Osedax nordenskioeldi* sp. nov.

(Figs. 3.2i)

Material examined

Osedax nordenskioeldi type material. Bransfield Strait, West Antarctic Peninsula, Southern Ocean. Two locations in close proximity: dissected from Minke whale rib bone collected from 'ACES 2 Lander' at 63°10.98'S 61°38.16'W deployed on the seafloor in 568 m of water and from Minke whale bones of 'ACES 1 Lander' at 63°9.87'S 61°41.34'W deployed on the seafloor in 650 m of water. Holotype: mature adult females, dissected from implanted Minke whale bone (locality: 63°10.98'S 61°38.16W, Bransfield Strait, West Antarctic Peninsula, Southern Ocean, 568 m, implanted Minke whale bones). Paratypes: mature adult females same locality as holotype as well as from Minke whale bones of 'ACES 1 Lander' at 63°9.87'S 61°41.34'W deployed on the seafloor in 650 m of water.

Diagnosis

Type material observed, four smooth palps of equal length without pinnules, average 2.52 mm, and equal width average 0.17 mm in ethanol-fixed specimens (Fig. 3.2i). Palps become opaque white to light pink after preservation (Fig. 3.2i). Mean of measured trunk in ethanol-fixed specimens, 0.57 mm width, 1.07 mm length. Data on root morphology lacking, no trace of any male individuals. Thin mucous tube surrounds the specimen.

Etymology

Named after Otto Nordenskjöld, professor at the University of Gothenburg and leader of the Swedish Swedish Antarctic Expedition 1901-1904.

3.3.1.4 *Osedax antarcticus* Glover, Wiklund and Dahlgren 2013

(Fig. 3.2j)

Material examined

Kemp Caldera, South Sandwich Arc, East Scotia Sea: 07.02.2010, 59°41.671'S, 28°21.089'W, 1444-1447 m, Minke whale skeleton, 3 specimens (Fig. 3.2j).

New Record

This species has previously been recorded only once from the Bransfield Strait, West Antarctic Peninsula, Southern Ocean (Glover *et al.*, 2013). This record extends the distributional range of *O. antarcticus* to the South Sandwich Arc (a distance of approximately 1800 km).

3.3.1.5 Taxonomic Remarks

The bones of the Kemp-Caldera natural whale fall had two dominant morphospecies of *Osedax* worm present, initially identified by examination of ROV video (Figs. 3.3a and 3.3b). These

two morphospecies are presumed to belong to the two new species of *Osedax* identified by molecular analysis and described as *Osedax rogersi* and *O. crouchi* here. However we were unable to say with full certainty which description when alive belonged to the preserved specimens discriminated later by molecular analyses (polychaete taxonomists were not on the recovery vessel, and detailed notes and images of each fixed specimen were not taken owing to time constraints). From video analysis, 'Morphospecies A' (Fig. 3.3a) is thought to be *O. rogersi*. When observed *in situ* alive, the whole individual is dark pink to red in colour (Figs. 3.3a, 3.3c and 3.3d). There are four distinct palps, fused for just under half of the total body length, which emerge from the bone (Figs. 3.3a, 3.3c and 3.3d).

'Morphospecies B' is thought to belong to *Osedax crouchi*, observed *in situ* alive on the bones collected from the skeleton from Kemp Caldera. There are four palps fused together along most of length, light pink in colour when viewed at a distance and narrowing towards the unfused tips (Figs. 3.3b, 3.3c and 3.3d). Palps extend for over 60% of the entire length of the animals emerging from the bone and average 15.21 mm in length and 0.27 mm in width (Figs. 3.3a, 3.3c and 3.3d). The trunk is easily distinguished upon close inspection, as the only pigmentation is two thin lines of red colour on opposite sides of the trunk of each specimen (Fig. 3.3d). The rest of the trunk appears translucent and is 4.40 mm long and 0.53 mm wide on average (Fig. 3.3d). The pigmentation pattern on the trunk is the same as for each palp; two lines of red pigmentation the entire length of each translucent palp (Fig. 3.3d). Some individuals are completely enclosed in a translucent ridged mucous tube but others are seen with tube bunched where the specimens emerge from the bone.

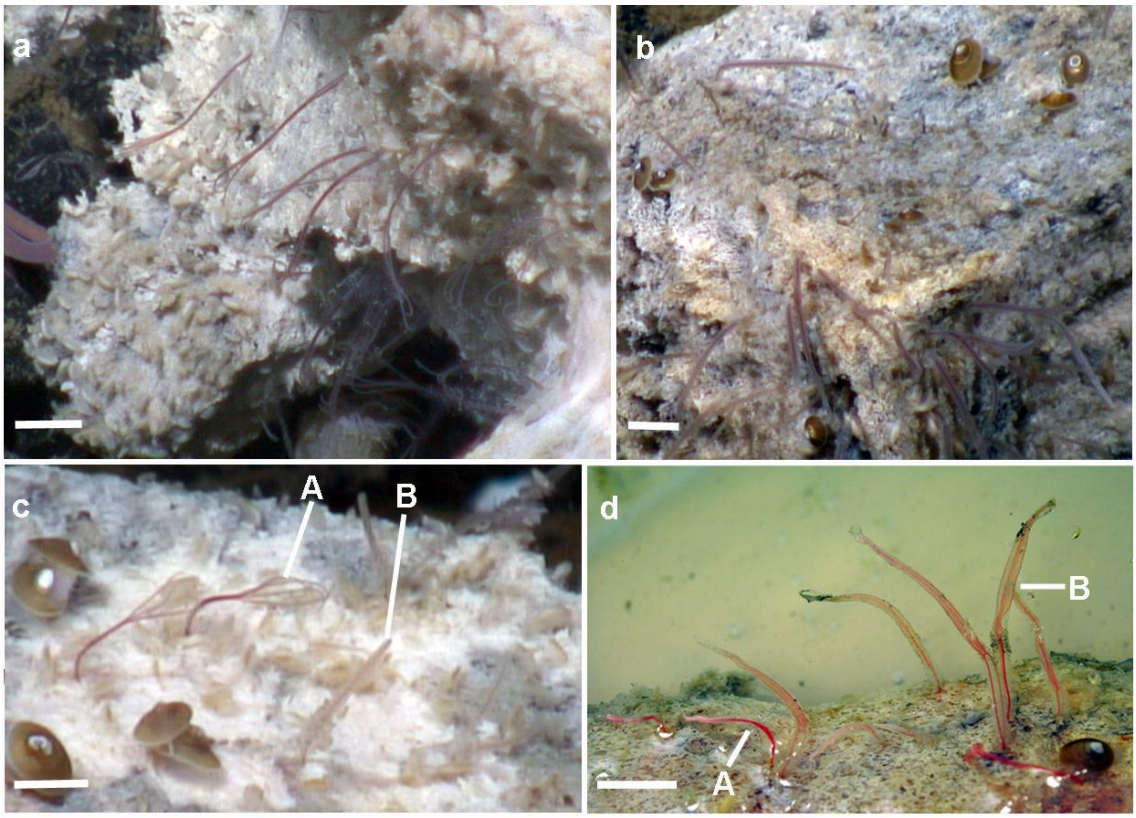

Figure 3.3 Live specimens of *Osedax* 'morphospecies A' and 'morphospecies B' on the whale fall. (a) Live specimens of *Osedax* 'morphospecies A' emerging from the skull bone *in situ*. Scale is 20.0 mm. (b) Live specimens of *Osedax* 'morphospecies B' emerging from a vertebra *in situ*. Scale is 10.0 mm. (c) Live specimens of *Osedax* 'morphospecies A and B' emerging from the skull bone *in situ*. Scale is 6.0 mm. (d) Live specimens of '*Osedax* morphospecies A and B' emerging from a vertebra collected and placed in a tank on board on the *RRS James Cook*. Scale is 10.0 mm. In photos a – d, *Pyropelta* sp., *Jaera* sp. and Lysiannassidae sp. can also be seen on the bones (Amon *et al.*, 2013).

In addition to *Osedax rogersi* and *O. crouchi*, a third species was identified based almost purely on molecular characters: *O. nordenskioeldi*. This species was discovered based on molecular data after preservation and therefore a description of the characteristics when alive was not possible. A new record of *O. antarcticus* is also presented, with specimens recovered from the Kemp-Caldera whale fall. In summary, *O. rogersi*, *O. crouchi* and *O. antarcticus* were all present at the Kemp whale fall. At the Smith-Island site in the Bransfield Strait, DNA barcode-identified *O. nordenskioeldi* and *O. crouchi* have now also been recovered from the collected specimens at that site along with the previously described *O. antarcticus*.

Morphologically, it is difficult to separate the species given the lack of corroboration between observed characters in living specimens and those identified later based on molecular evidence. A barcoding approach will be needed to confirm future species identifications until further material is forthcoming that can be used to improve distinctive morphological knowledge. Nevertheless, some comparisons with previously-described material can be made. All three new species correspond most closely with *Osedax antarcticus* in the presence of 'smooth' palps

rather than the heavily-pinnulated palps seen in some of the other species. In this regard, they also correspond with *O. deceptionensis*, although very limited morphological information is available for that species (for example, the type material lacks evidence of oviduct). If we assume that 'Morphospecies A' identified from video is in fact *O. rogersi*, and 'Morphospecies B' identified from video is *O. crouchi*, then we can delineate them from one and other by the quite different palp coloration (completely red in *O. rogersi*, striped in *O. crouchi*). No information is available on the palp coloration of *O. nordenskioeldi*, however we can assume that as it was not observed at the time of collection as being considerably different from the more abundant *O. antarcticus*, that it has similar palp coloration. *O. antarcticus* itself has red palps with thick stripes, but are closer in appearance to the assumed *O. rogersi*. An additional useful character may be the degree of palp fusion, although this is also hard to study in fixed specimens where the ends of the palps may be lost. In *O. antarcticus*, *O. rogersi* and *O. nordenskioeldi*, the palps are fused for a portion of their length, probably at least 50% in all the species except for *O. antarcticus* where it is slightly less and *O. crouchi*, where it appears nearly totally fused in some cases. Further examination of fresh material will be needed to confirm the utility of this character to discriminate species.

The Antarctic *Osedax* species differ in size. The smallest is *Osedax deceptionensis* which has a preserved trunk/palp maximum length of 1.2 mm, *O. nordenskioeldi* is also quite small with an estimated maximum length of 6 mm, *O. rogersi* and *O. crouchi* are larger with a maximum palp length of 7 mm and a maximum trunk/palp length of 17 mm respectively, and *O. antarcticus* the largest, with maximum of 25 mm. The size of *O. antarcticus* is similar to *O. roseus*, but still almost half the size of the largest species, *O. rubiplumus*. With a reasonable sample size, adult body size may be a useful species-discriminating character amongst some *Osedax* species.

Dwarf males that have been characterised in *Osedax rubiplumus*, *O. frankpressi*, *O. roseus* and *O. antarcticus* were not found on any of the specimens, however it is possible that they had fallen off during collection or sorting in petri dishes at sea. As such it is impossible to further delineate the species on the nature of the males and in particular the opisthosomal chaetae, which have only been found on the males.

3.3.2 Phylogenetics and population genetics

Phylogenetic analyses were conducted for the family Siboglinidae using the genes 16S, 18S and CO1 (Fig. 3.4). These analyses used the ten described species of *Osedax*, including the three new species, *Osedax rogersi*, *O. crouchi* and *O. nordenskioeldi*, and an additional 19 undescribed taxa for which sequences are available on GenBank (Table 3.2). Several of these

undescribed taxa are, informally, synonyms based on our molecular analyses (listed in Table 3.4), reducing the total number of undescribed OTUs to 16.

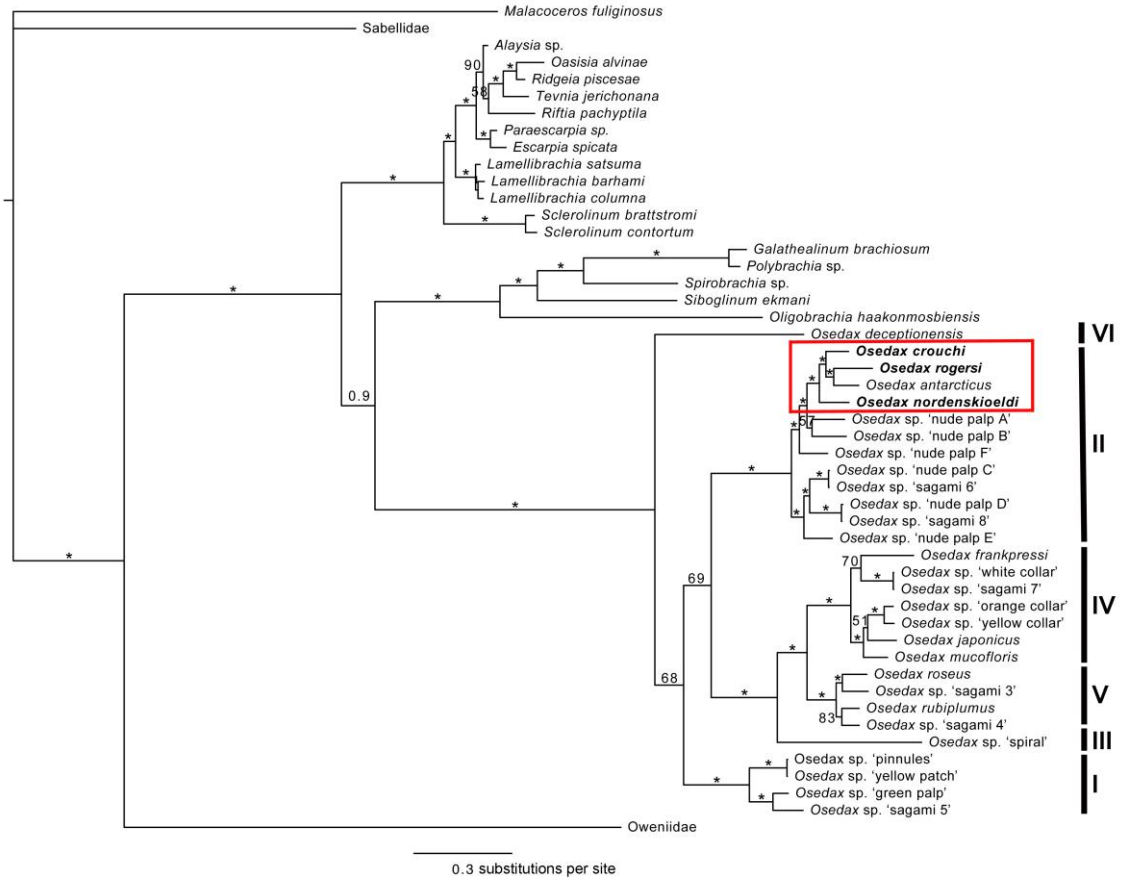

Figure 3.4 Phylogeny of the three new species of Antarctic *Osedax*. All species described during this study are indicated by bold text. This analysis used Bayesian analysis of all sampled siboglinid polychaetes and *Osedax* including undescribed OTUs on Genbank based on 18S, 16S and CO1 genetic markers. Numbers indicate the posterior probability values out of 100. Values of 95 and over are marked by asterisks. Clades from Table 3.4 are also indicated by bold lines and text. The 'Antarctic clade' of *Osedax* is indicated by a red box.

The three new species of *Osedax* described in this paper form a well-supported monophyletic clade that includes *Osedax antarcticus*. This clade, which we refer to here informally as the 'Antarctic clade' is nested within 'clade II', a group that contains several undescribed OTUs that share a similar morphological characteristic – smooth palps (Vrijenhoek *et al.*, 2009; Glover *et al.*, 2013). The phylogenetic position of an additional Antarctic species, *O. deceptionensis*, recovered from a very shallow depth in Deception Island, is still not well resolved, but it appears to lie outside the clade of the other Antarctic species.

Ten CO1 sequenced specimens of *Osedax nordenskioeldi* belonged to seven haplotypes: H1 (n=3) and H2, H3, H4, H5, H6, H8 (n=1) (Fig. 3.5a). 53 specimens of *O. antarcticus* belonged to 40 haplotypes: H1 (n=8), H25 (n=7) and the other 38 haplotypes came from individual

specimens (Fig. 3.5b). For *O. rogersi*, 20 specimens were sequenced, yielding ten haplotypes (Fig. 3.5c): H1 (n=9), H7 (n=3) and the other eight haplotypes from individual specimens. From 39 individuals of *O. crouchi*, there were 37 haplotypes; haplotypes H2 to H37 (n=1) excluding H1 and H7 (n=2) (Fig. 3.5d).

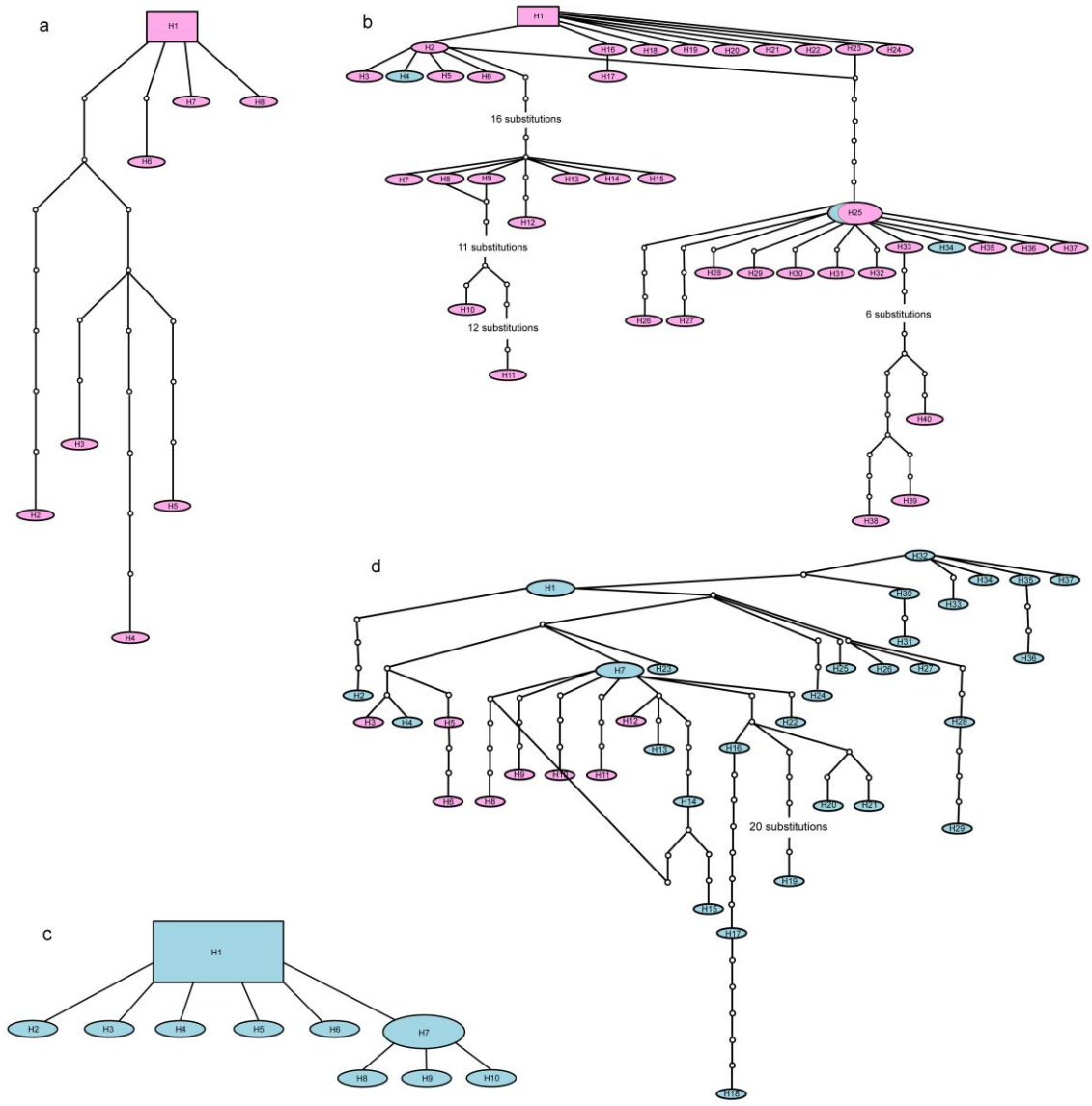

Figure 3.5 Population genetics for four species of Antarctic *Osedax*. (a) CO1 haplotype network for *Osedax nordenskioeldi* from ten individuals, haplotype H1 (n=3) and H2, H3, H4, H5, H6, H8 (n=1); (b) CO1 haplotype network for *O. antarcticus* from 53 individuals, haplotype H2 to H40 (n=1) excluding H1 (n=8) and H25 (n=7); (c) CO1 haplotype network for *O. rogersi* from 20 individuals, haplotype H1 (n=9), H2, H3, H4, H5, H6, H8, H9, H10 (n=1) and H7 (n=3); (d) CO1 haplotype network for *O. crouchi* from 39 individuals, haplotypes H2 to H37 (n=1) excluding H1 and H7 (n=2). Each blue haplotype indicates that individual came from Kemp Caldera; all pink haplotypes came from bones off Smith Island.

3.4 Discussion

Our study brings the total number of described species in the genus *Osedax* to ten. Of the additional 19 'taxa' listed on GenBank, at least 16 of them appear to be separate species based purely on DNA evidence, thus we estimate the current diversity within the genus to be 26 species. The total number of *Osedax* species described from the Antarctic region is now five; *Osedax antarcticus*, *O. deceptionensis*, *O. rogersi*, *O. crouchi* and *O. nordenskioeldi.* With the current total number of described polychaetes in the family Siboglinidae at an estimated 167 (Appeltans *et al.*, 2013), the genus *Osedax* is rapidly becoming one of the more species-rich groups in the clade.

Morphologically, the Antarctic species differ from all other previously-described species in having both smooth and partially-fused palps (Fig. 3.2). Whilst it would seem a reasonable hypothesis that this is an adaptation to the oxygen-rich Antarctic waters (Glover *et al.*, 2013), it must be noted that several of the undescribed OTUs that are mentioned in the literature also have smooth palps (although electron microscopy has not yet been published) and have been recorded from the NE Pacific (Monterey Canyon) and NW Pacific (off Japan). Owing to poor preservation of material, we have been unable to improve our knowledge of the morphology of the new species, but it would appear that palp coloration, the degree of translucency in the palps and trunk, the degree of palp fusion and body size are characters that are highly variable between species. For example, the maximum adult body size of *Osedax* (Table 3.4 and references therein) now varies from less than 2 mm (*Osedax deceptionensis*) to 60 mm (*O. rubiplumus*), however caution must be exercised given that *O. deceptionensis* was described from only a single specimen. Further taxonomic work must be undertaken on the basic morphology of the species, and this can only be done with careful examination of live specimens, not an easy task when the material is recovered from such remote locations and often opportunistically without expert taxonomists present at recovery.

As seen in previous phylogenetic studies, *Osedax* species seem to be grouped into clades according to morphology and to some extent location rather than bathymetry or other obvious environmental factors (Vrijenhoek *et al.*, 2009; Glover *et al.*, 2013). All the new species of *Osedax,* as well as *Osedax antarcticus,* fit into 'clade II' referred to as the 'nude-palp' clade by Vrjienhoek *et al*. (2009); these species lack the long lateral pinnules as has been seen on many of the *Osedax* species (Vrijenhoek *et al.*, 2009; Glover *et al.*, 2013). However, it should be noted that although the palps appear smooth under light microscopy, electron microscopy reveals that they are in fact with a rugose appearance from the presence of numerous micropinnules (Fig. 3.2). Four of the Antarctic *Osedax* species are found to form a well-supported monophyletic ‘Antarctic clade’ within the nude-palp clade (Fig. 3.4). This is

suggestive of some speciation within *Osedax* occurring south of the Polar Front. However, an additional Antarctic species, *O. deceptionensis*, is not well resolved with regards to its phylogenetic position and does not currently fit within the Antarctic clade. Analysis of further taxa or genes in this species would help, as would a better understanding of its morphology. It is interesting to note that *O. deceptionensis* is found in a much shallower littoral setting (21 m) and within an isolated embayment (Deception Island); as such it may represent a divergent lineage from the rest of the *Osedax* clade.

Two of the species of *Osedax* discussed in this paper show an apparent broad distribution of their populations: *Osedax antarcticus* and *O. crouchi* were found at both the Smith-Island site (500-600 m) and Kemp Caldera (1400 m). *Osedax rogersi* was found only at Kemp Caldera and *O. nordenskioeldi* was found only at the Smith-Island site. These sites differ in depth by approximately 890 m, are 1800 km apart and exhibit remarkably different local habitat conditions: the site off Smith Island is a sedimented Antarctic shelf habitat whereas Kemp Caldera is a very hydrothermally-active caldera (Fig. 3.1). Our population genetic data (Fig. 3.5) reveal that the sampled populations of *O. antarcticus* from Kemp Caldera and the Smith-Island site are well-embedded in each other with potential exchange between populations. However, with only three samples from Kemp Caldera, this must be treated with caution. The network also shows high diversity with divergence into five main groups that are not representative of sampling location. For the other species, haplotype networks show high diversity in *O. crouchi* and *O. nordenskioeldi* (the latter albeit with a restricted sample size of just 10 individuals) and low diversity in *O. rogersi* from its population in Kemp Caldera. The high numbers of haplotypes for all four species of *Osedax* within one or two populations suggests a large population size with no signs of recent bottlenecks or other processes, which could have reduced diversity.

Our data are supportive of the notion of high degrees of eurybathy in Southern-Ocean species, as a result of a cold isothermal water column (Kaiser *et al.*, 2013), although it must be noted that several of the NE-Pacific species and undescribed OTUs have also shown bathymetric ranges of at least 1000 m (Braby *et al.*, 2007). We can also say that for the Antarctic *Osedax* species, *Osedax crouchi* and *O. antarcticus* have broad dispersal capabilities and possibly habitat tolerances. Further sampling will show whether the Antarctic Circumpolar Current acts to aid the dispersal of these species around the continent, as has been found in other Antarctic species (Griffiths *et al.*, 2008).

In total, both studied sites, despite being relatively small habitats on the seafloor, had three species of *Osedax* on them (Amon *et al.*, 2013; Glover *et al.*, 2013). The presence of multiple species at the same whale fall has been previously observed off California (Braby *et al.*, 2007;

Vrijenhoek *et al.*, 2009). Other types of fauna were also found inhabiting both the whale fall and the implanted whale bones confirming that whale bones create unique habitats for many animals (Smith and Baco, 2003; Amon *et al.*, 2013; Glover *et al.*, 2013). It should also be noted that the number of individuals of *Osedax* found on the skeleton in Kemp Caldera varied according to whale-bone type, with low-lipid bones such as the mandibles and thoracic vertebrae having densities of up to 1176 per m^2 and 765 per m^2 and the ribs, sternum, humerus, caudal and lumbar vertebrae having much lower densities (less than 220 per m^2) (Amon *et al.*, 2013). This shows that *Osedax crouchi*, *O. rogersi*, *O. nordenskioeldi* and *O. antarcticus* may have preferences for certain bone types, but unfortunately it was not resolved whether those preferences were species specific.

Table 3.4 List of known *Osedax*. This table is a modified version of Electronic Supplementary Table 3 in Glover *et al*. (2013). [a]Clade numerals are in the same order as in the phylogenetic tree in Figure 3.4, and build upon Vrijenhoek *et al*. (2009). [b]OTU's based on sequences deposited at GenBank and names mentioned in the literature using informal taxonomy. [c]Maximum length of trunk and crown (mm) after preservation. [d]OTU's are synonyms based on data provided on GenBank. [e]Depth estimated from Google Earth using position data reported under GenBank accession number. [f]Based on data on GenBank, this OTU may be a synonym of 'nude palp D'. [g]None of the specimens collected showed evidence of a trunk; this measurement is therefore only palp length.

Clade[a]	Described species	Undescribed OTUs[b]	Depth range (m)	Type/voucher locality	Size[c]	Palps	Pinnule	Roots	Tube	References
VI	*O. deceptionensis*	none	21	Antarctica	1.2	pale	absent	robust, lobulate	gelatinous, hemispherical	(Glover *et al.*, 2013)
II	*O. crouchi*	none	568-1446	Antarctica	17.3	red, striped	absent	robust, lobate	transparent, cylindrical	This study
II	*O. rogersi*	none	1444-1446	Antarctica	7[g]	red	absent	?	thin mucous sheath	This study
II	*O. antarcticus*	none	568-1446	Antarctica	25	red, striped	absent	robust, lobate	thin mucous sheath	(Glover *et al.*, 2013), this study
II	*O. nordenskioeldi*	none	568-650	Antarctica	6	pink	absent	?	thin mucous sheath	This study
II	none	'nude palp A'	1820	NE Pacific	25	red	absent	?	?	(Jones *et al.*, 2008; Vrijenhoek *et al.*, 2009)
II	none	'nude palp B'	2893	NE Pacific	25	red	absent	?	?	(Jones *et al.*, 2008; Vrijenhoek *et al.*, 2009)
II	none	'nude palp F'	2893	NE Pacific	18	red	absent	robust, lobate	?	(Vrijenhoek *et al.*, 2009)
II	none	'nude palps' & 'nude palp C'[d] & 'sagami 6'[d]	113[e]-1018	NE & NW Pacific	12	red	absent	?	?	(Rouse *et al.*, 2009), Pradillon *et al.* (unpub. GenBank FM995540)
II	none	'nude palp D'	1018–1820	NE Pacific	12	red	absent	?	?	(Vrijenhoek *et al.*, 2009)
II	none	'sagami 8'[f]	113[e]	NW Pacific	?	?	?	?	?	Pradillon *et al.* (unpub. GenBank FM995534)
II	none	'nude palp E'	1018	NE Pacific	12	red	absent	robust, lobate		(Vrijenhoek *et al.*, 2009)
IV	*O. frankpressi*	none	1820–2893	NE Pacific	23	red, striped	present	robust, lobate	gelatinous, hemispherical	(Rouse *et al.*, 2004)

IV	none	'white collar' & 'sagami 7'[d]	113[e]-1018	NE & NW Pacific	6	red, striped	present	robust, lobulate	?	(Vrijenhoek *et al.*, 2009) Pradillon *et al*. (unpub. GenBank FM995542)
IV	none	'orange collar'	385–1018	NE Pacific	18	red	yes	robust, lobulate	?	(Braby *et al.*, 2007)
IV	none	'yellow collar'	385	NE Pacific	18	red	yes		?	(Braby *et al.*, 2007)
IV	*O. japonicus*	none	224–250	NW Pacific	12	white to pink	present	robust, lobulate	gelatinous, cylindrical	(Fujikura *et al.*, 2006)
IV	*O. mucofloris*	none	30–125	North Sea	15	white to pink	present	robust, lobulate	gelatinous, hemispherical	(Glover *et al.*, 2005b)
V	*O. roseus*	'rosy'	633–1820	NE Pacific	24	bright red	present	long, branched	transparent, cylindrical	(Rouse *et al.*, 2008)
V	none	'sagami 3'	113[e]	NW Pacific	?	?	?	?	?	Pradillon *et al*. (unpub. GenBank FM995537)
V	*O. rubiplumus*	none	1820–2893	NE Pacific	59	red	present	long, branched	rigid, cylindrical	(Rouse *et al.*, 2004)
V	none	'sagami 4'	113[e]	NW Pacific	?	?	?	?	?	Pradillon *et al.* (unpub. GenBank FM995541)
III	none	'spiral'	2893	NE Pacific	25	absent	absent	thin filaments	thick? gelatinous	(Braby *et al.*, 2007; Vrijenhoek *et al.*, 2009)
I	none	'yellow patch' & 'pinnules'[d]	633–1018	NE Pacific	5	pale	present		?	(Vrijenhoek *et al.*, 2009)
I	none	'green palp'	1820	NE Pacific	3	red/green	present	robust, lobate	?	(Vrijenhoek *et al.*, 2009)
I	none	'sagami 5'	113[e]	NW Pacific	?	?	?	?	?	Pradillon *et al*. (unpub. GenBank FM995539)

4. Faunal assemblages of wood- and bone-colonisation experiments from two seamounts on the Southwest Indian Ridge

The Southwest Indian Ridge is an ultraslow-spreading mid-ocean ridge with numerous poorly-explored seamounts. The benthic fauna of seamounts is thought to be highly endemic due to reproductive isolation but this hypothesis is rarely tested. We report the results from colonisation experiments, which comprised two packages of mango wood and whale bones. One was deployed at 732 m on Coral Seamount and the other at 750 m on Atlantis Bank. These packages mimic organic falls, large parcels of food on the deep seafloor that are important in fulfilling the nutritional needs and providing shelter and substratum for many deep-sea animals. A large number of species colonised the deployments; 53 species at Coral Seamount and 38 species at Atlantis Bank. The two colonising assemblages were different however, with only 11 species in common. This is suggestive of both differing ecological conditions and some barriers to dispersal between these seamounts. Apart from Xylophaginae and *Idas* bivalves, few organic-fall specialists were present but there were many genera that have previously been found at other chemosynthetic habitats. Several putative new species have been observed, and three new species have been described from the experiments. It is not clear, however, whether this is indicative of high degrees of endemism or simply as a result of undersampling.

4.1 Introduction

The deep sea is an ecosystem primarily dependent on the supply of food from surface waters. This may consist of both animal or plant matter. Large animal falls from surface waters to the deep seafloor can include the carcasses of marine mammals such as whales, squids, large fish and also swarms of gelatinous animals such as salps (Stockton and DeLaca, 1982; Smith and Baco, 2003; Billett *et al.*, 2006). A range of plant matter can also sink to the deep seafloor including seagrasses, macroalgae and terrestrial plant material such as wood (Wolff, 1979; Harrold *et al.*, 1998). Large food falls are thought to play an important role in the ecology of the ocean floor by providing shelter and substratum for fauna, and by fulfilling the nutritional needs of many deep-sea species that feed either directly on the substrates or on smaller fauna that have come to also feed on or be sheltered by the falls (Turner, 1977; Wolff, 1979; Smith and Baco, 2003).

Whilst their origin may be very different, the remains of wood and whale on the seafloor share some interesting ecological similarities. For example, whales, once the flesh has been removed in the early decompositional stages (Smith & Baco 2003), can persist on the seabed as a pile of bones for months to several years. They thus present an organic-rich hard substrate, in some cases, of a similar size and nature to a pile of wood on the seafloor. Both wood and whale bone are consumed by a range of organisms, including specialist biodegraders such as wood-eating Xylophaginae bivalves (Turner, 1955; Knudsen, 1961; Turner, 1973, 1977) and bone-eating *Osedax* worms (Rouse *et al.*, 2004; Glover *et al.*, 2005b; Fujikura *et al.*, 2006). In addition, the anaerobic degradation of sunken wood and the bacterial decomposition of lipids from whale bones can also support chemosynthetic communities in a similar way as at deep-sea hydrothermal vents and cold seeps (Smith, 1992; Duperron *et al.*, 2008; Samadi *et al.*, 2010). There are many genera and families of fauna that are found at whale falls, wood falls and other reducing habitats, suggesting that whale falls and wood falls may actually act as evolutionary 'stepping stones' along the seafloor for the dispersal of a subset of fauna between vents or seeps and vice versa (Smith *et al.*, 1989; Distel *et al.*, 2000).

Despite the fact that the deep seafloor close to terrigenous sources of wood (e.g. tropical and temperate forested zones) is likely to be hugely influenced by this food source, there have been remarkably few detailed studies of wood falls beyond the work undertaken in the tropical west Pacific (Pailleret *et al.*, 2007a; Pailleret *et al.*, 2007b; Samadi *et al.*, 2010) and experimental work off the US west and east coasts (Turner, 1973; Romey *et al.*, 1994; Bernardino *et al.*, 2010). Almost nothing is known with regard to the nature of organic falls in the Indian Ocean, the subject of this study; two species of *Xylophaga* have been recorded but there was no record of plant matter in either record (Knudsen, 1961, 1967).

Research was conducted along the Southwest Indian Ridge (SWIR), a seafloor spreading centre transitioning between slow and ultraslow spreading, along its axis from the Bouvet Triple Junction in the South Atlantic to the Rodriguez Triple Junction in the central Indian Ocean (Fig. 4.1a) (Dick *et al.*, 2003). Intermittency of magma supply at this ultraslow-spreading ridge has resulted in the formation of seamount features such as Oceanic Core Complexes in many ridge segments (Escartin *et al.*, 2008). The Sub-Tropical Front runs through this region, separating the Agulhas Front (which marks the southern boundary of the Agulhas Return Current) to the north and the Antarctic Circumpolar Current to the south. This results in the bodies of water on either side of the Sub-Tropical Front having very different physical, chemical and biological characteristics (Read *et al.*, 2000).

Despite being the site of many geological investigations, very little has been observed biologically on the SWIR (Rogers *et al.*, 2012a). Deep-sea research in the Indian Ocean has been concentrated in the Arabian Sea and is quite limited elsewhere (Banse, 1994; Ingole and Koslow, 2005). A prevailing theory has been that seamount fauna are, in general, characterised by high levels of endemism due to the geographic isolation of clusters of seamounts (Richer de Forges *et al.*, 2000), and as a result, many of the seamounts on the Southwest Indian Ridge have been declared Benthic Protected Areas (BPAs). This view has been challenged in the last ten years however (McClain, 2007; Clark *et al.*, 2010). The fauna inhabiting seamounts in the Indian Ocean are particularly poorly known with the main source of information thus far coming from scientific reports from past Soviet expeditions related to exploratory fishing (Romanov, 2003). It is only with further studies, that a complete picture of the biodiversity and productivity of the region can be completed (Demopoulos *et al.*, 2003).

To date, there have been no studies of natural- or experimental-organic falls in the SWIR area; probably not due to lack of their natural occurrence but rather to the poor exploration of the seafloor in this region. The main aim of this experiment was to characterise the faunal assemblages of bone and wood falls in terms of taxonomy and functional characteristics in the deep South-West-Indian-Ridge area for the first time. The assemblages found colonising the bone and wood were compared to test whether faunal presence was driven more by site or substrate type, thus evaluating the relative degrees of connectivity at a species level between both the seamounts and the different types of organic fall. These assemblage compositions were also related to the physical, chemical and biological characteristics of the surrounding environments. The colonising fauna found within this region of the deep Indian Ocean were then compared with other wood- and bone-colonising assemblages from other parts of the deep ocean to ascertain the similarity between them. The presence of specialist fauna such as the bone-boring siboglinid genus, *Osedax* and wood-boring bivalves, *Xylophaga* was also investigated.

4.2 Methods

4.2.1 Mooring deployments in the SWIR

Two moorings with wood and whale-bone packages attached to each were deployed at two sites in the SWIR as part of the 410th voyage of the RV *Dr. Fridtjof Nansen* (Rogers *et al.*, 2009) (Fig. 4.1). This was a research expedition aimed at investigating the pelagic biology and physical-oceanographic setting of the seamounts on the SWIR and the first of two cruises hoping to gather information to contribute to ecosystem-based management of fisheries on the high seas of the Southern Indian Ocean (Rogers *et al.*, 2009; Rogers *et al.*, 2012a). Each mooring was comprised of 150 kg concrete-filled tyres as ballast, connected to a 15 m double mooring line with a 20 mm rope (Fig. 4.2). The rope was the weak point in the mooring, which the Remotely Operated Vehicle (ROV) cut during recovery (Fig. 4.2). The double mooring line was shackled to a string of eight floats and fitted with a Sonardyne Transponder Type 7832 (Fig. 4.2). Whale bones used were collected from dead stranded whales, frozen and transported by surface shipment to the waiting vessel. The whale bones were individually drilled and fitted with loops of 8 mm polypropylene line (Fig. 4.3). They were then sewed into coarse-net bags with the loops of polypropylene line protruding through the mesh (Fig. 4.3). These lines were spliced onto a single lifting ring that was connected directly to the ballast by a single 14 mm polypropylene line (Fig. 4.2). A separate parcel was prepared in a similar way for the mango wood (*Mangifera indica*) (Fig. 4.3). The mango wood was collected from a recently-cut tree in the departure port on Reunion Island prior to the deployment cruise.

The first mooring was deployed on 18th November 2009 at 32°42.71'S, 57°16.31'E at a depth of ~750 m (Figs. 4.1a and 4.1c). This deployment site was located on the summit of the seamount, Atlantis Bank, a BPA (Fig. 4.1c). This seamount is located north of the SWIR and the Sub-Tropical Front (Fig. 4.1a). The whale bones attached, some of which still had flesh present, included ½ Sperm whale (*Physeter macrocephalus* Linnaeus 1758) vertebra, a Minke whale (*Balaenoptera acutorostrata* Lacepede 1804) vertebra, several Humpback whale (*Megaptera novaeangliae* Borowski 1781) ribs, and some Northern Bottlenose whale (*Hyperoodon ampullatus* Forster, 1770) ribs. There were also several large pieces of *Mangifera indica* (wood) attached separately.

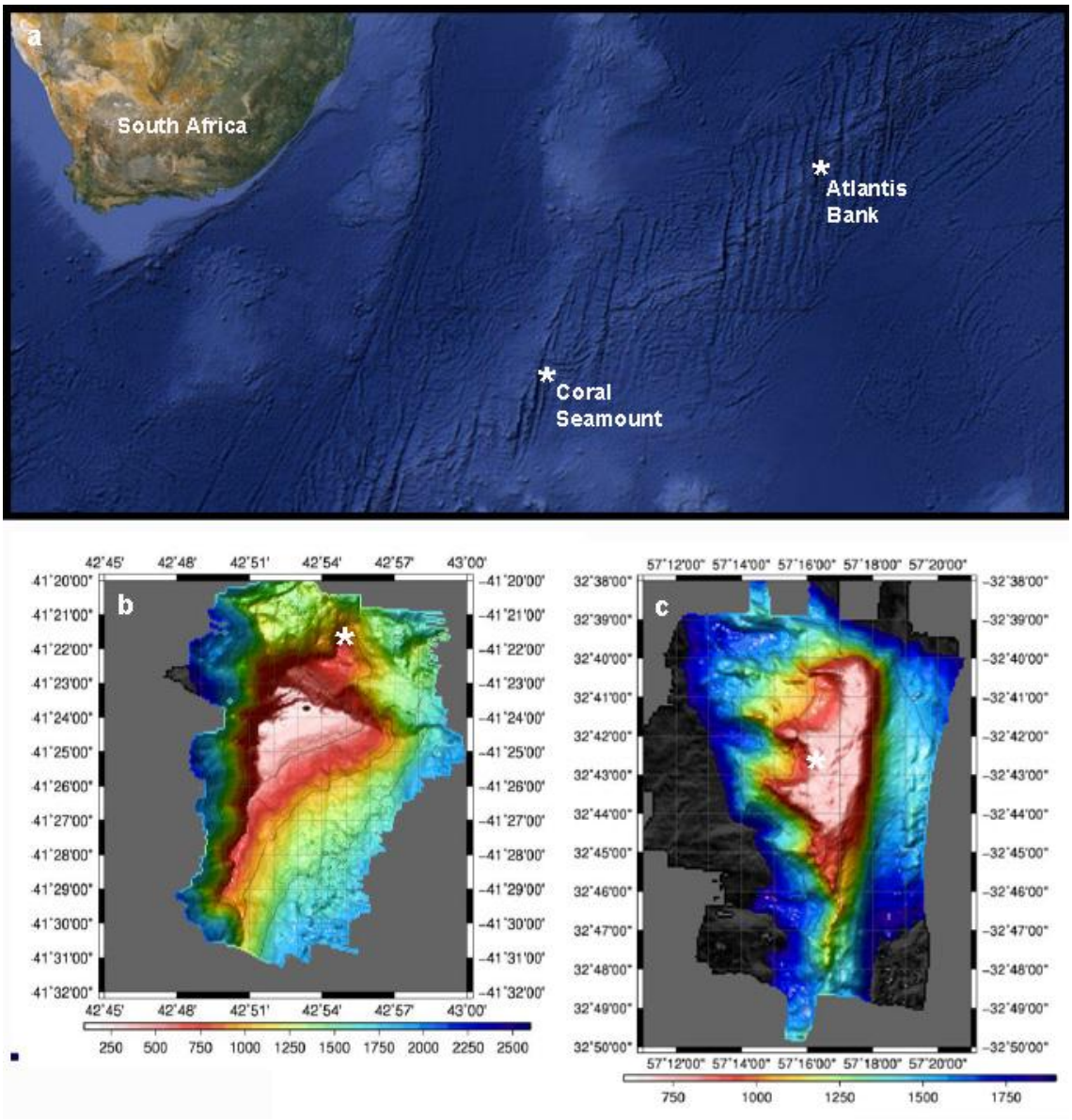

Figure 4.1 The Southwest Indian Ridge (SWIR) and deployment sites. (a) Regional bathymetry map of the SWIR showing the locations of the two mooring-deployment sites, Coral Seamount and Atlantis Bank. (b) Bathymetry of Coral Seamount with the location of the mooring deployment indicated by *. (c) Bathymetry of Atlantis Bank with the location of the mooring deployment indicated by *. The bathymetry data used to construct (a) is © Google Earth - 2013 Cnes/Spot Image, Image IBCAO, Data SIO, NOAA, U.S. Navy, NGA, GEBCO, 2013 TerraMetrics. Bathymetry for (b) and (c) was acquired using the shipboard multibeam echo sounder on the *RRS James Cook*. All depth measurements are in metres.

The second mooring was deployed on 4th December 2009 at 41°22.38'S, 42°54.64'E at a depth of 732 m (Figs. 4.1a and 4.1b). This deployment was on the slope of Coral Seamount, also a BPA and is located to the south of the SWIR and Sub-Tropical Front (Fig. 4.1). The whale bones attached to this mooring included two Minke whale (*Balaenoptera acutorostrata*) vertebrae, a Minke whale (*B. acutorostrata*) vertebral cap, several Humpback whale (*Megaptera novaeangliae*) ribs, and some Northern Bottlenose whale (*Hyperoodon ampullatus*)

ribs. Some of these bones still had flesh present. There were also several *Mangifera indica* logs attached separately.

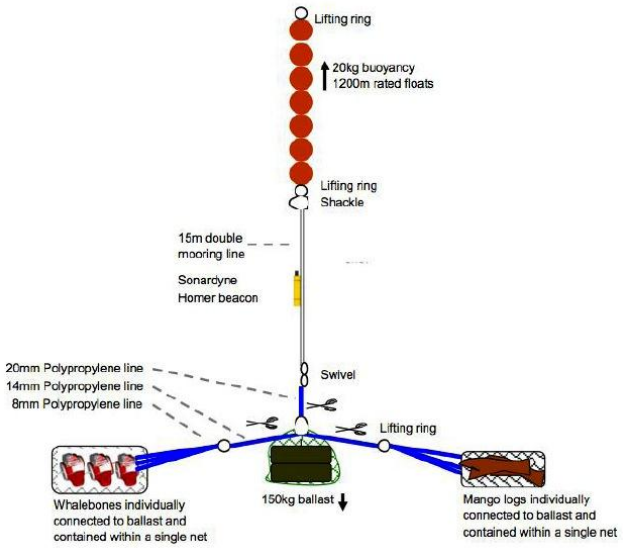

Figure 4.2 Mooring construction. The scissor symbol designates where the ROV cut during the recovery. Image reproduced from Rogers *et al*., (2009).

Figure 4.3 Preparation of the bone and wood packages. Bones (a-b) and wood (c-d) were drilled and fitted with individual rope loops before being sown into netted bags. Images reproduced from Rogers *et al*. (2009).

4.2.2 Mooring recoveries in the SWIR

Mooring recovery took place during the 66th voyage of the *RRS James Cook* using the ROV *Kiel 6000*. This was the follow-up cruise to that of the *RV Dr. Fridtjof Nansen* mentioned previously. The aim of this cruise was to ground truth models of habitat suitability for deep-sea stony corals which are associated with vulnerable marine ecosystem formation and also to analyse the fauna and oceanography of five seamounts of the SWIR. Once the moorings had been located on exploratory dives, dedicated mooring-recovery dives were undertaken. Each package was filmed in detail prior to any disturbance by the ROV. The mooring from Coral Seamount was collected on the 20th November 2011 (Station 4 Event 38). The mooring from Atlantis Bank was collected on the 14th December 2011 (Station 8, Event 29). The packages were recovered by cutting the polypropylene lines, which attached each package directly to the ballast. Each was then placed in a sealed biobox on the ROV. However some fauna may have unavoidably fallen from the packages during collection, so richness and faunal counts must be considered a lower limit for these samples.

4.2.3 Analysis of the fauna recovered from the wood and bone packages

Once on the ROV was on deck, the netted bags were removed from the bioboxes and opened so the bones, wood and fauna could be transferred to containers containing chilled seawater. Biobox residues were washed through a 250 μm sieve. Examination followed in the 4°C cold room. All fauna was then removed from the bones and preserved in 70% ethanol, 100% ethanol or 4% formalin depending on the specimen. Bones were preserved in either 70% ethanol or 4% formalin. The wood was too large to be preserved in this manner and so was frozen to -20°C. All of the water that the bone and wood packages had been sitting in was also sieved for macrofauna and meiofauna, which were then preserved in 100% ethanol or 4% formalin.

When samples arrived at the Natural History Museum, London, the morphology of each specimen was examined for taxonomic identification. All the wood and bone sievings were also sorted into species of fauna. Light microscopy was undertaken for every specimen using a Zeiss V.20 stereomicroscope with AxioCam camera. Once samples were identified, either to a named species or a species-level morphotype, one of the following functional groups were assigned to each: Autotrophs, Mixotrophs, Xylophagous, Grazers, Deposit Feeders, Suspension Feeders, Predators/Scavengers and Other. The functional group ‘Grazers’ refers to both grazers of bacterial mat and plant material (excluding wood), and ‘Other’ refers to species that could not be categorised by one functional group but rather a combination thereof. These groups were assigned using existing knowledge of the ecological functions of those fauna (or closely related) from whale-fall and wood-fall environments. When that information was not available,

examples were drawn from other chemosynthetic habitats (i.e. hydrothermal vents and cold seeps) or if that was not possible, from any environments these fauna were known from.

The approximate volumes of each type of substrate deployed at each site were calculated using the weight and an average density of each substrate type to assess whether the deployment sizes would have significantly impacted the number of fauna present. The assumed density of *Mangifera indica* used was 0.555 g/cm^3, an average of 0.52 g/cm^3 and 0.59 g/cm^3 (Brown, 1997). 1.173 g/cm^3 and 1.161 g/cm^3 were the assumed densities used to calculate the volume of all whale vertebrae and whale ribs deployed respectively (Tont *et al.*, 1977). The number of *Xylophaga* present in each wood deployment also had to be approximated as the wood samples would have had to been fully destroyed to recover all specimens of this boring genus. Instead one subsample equalling approximately 1/5 of the total sample was taken for three pieces of wood at each seamount. These subsamples were imaged using a Micro-CT scanner at the Imaging and Analysis Centre at the Natural History Museum, London. The number of *Xylophaga* specimens present in each subsample was counted from the images produced and then multiplied by five to create an approximate total sample. The total values for each of the three pieces of wood at each site were then summed and doubled as at each site there were six samples of wood. Univariate and multivariate statistical analyses were performed using PRIMER v6.

4.3 Results

4.3.1 Observations of the bone and wood moorings recovered on Coral Seamount

The deployment was located on the slope of the seamount where the substrate appeared to be comprised of broken calcareous coral, some small live coral, sponges and pennatulids (Fig. 4.4a). Temperature data were collected close to the deployment indicating the average temperature was 4.3°C. The netted bags were intact when found, after approximately two years on the seafloor (Fig. 4.4a). The approximate volumes of the experimental substrates deployed at this seamount were 11713.1 cm^3 of bone and 32612.6 cm^3 of wood. There were eight species of epifaunal animals growing on the mooring line including one *Neopilumnoplax heterochir* Studer, 1883, one *Tanystylum* sp. A, two species of hydrozoan, one Actinaria sp. A, two cf. *Perissasterias* sp. A (also found on living on the wood at this site) and one specimen of *Gorgonocephalus pustulatum* Clark, 1916. In total, there were 5490 individuals from 53 species comprising 10 classes recovered from this wood and bone deployment (including the epifauna

on the mooring line) (Table 4.1 and Fig. 4.4). 42 of the 53 (79.25%) species observed were found only on Coral Seamount and not on Atlantis Bank during this study.

4.3.1.1 Coral-Seamount whale-bone package

The whale bones showed evidence of grazing but this was not extensive. There were 4270 individuals present from 34 species (Fig. 4.6). This package was the most species-rich of all four packages but this was derived from the fewest classes (six classes) (Table 4.1 and Fig. 4.6a). The most species-rich phylum and class was the Annelida: Polychaeta with 18 species but the class with the highest number of individuals was Malacostraca with 81.83% of the total individuals (Fig. 4.6). This high number of individuals was as a result of the number of the amphipod *Seba* sp. A. individuals present (78.31% of total individuals or 3344 individuals) (Table 4.1 and Figs. 4.4d and 4.6b). The dorvilleid annelid *Ophryotrocha* sp. AA and mytilid mollusc *Idas* sp. A were also abundant (269 and 148 individuals respectively) (Table 4.1 and Figs. 4.4b and 4.6b). Other notable fauna observed on the whale bones included two species of shrimp described from this deployment, *Eualus oreios* and *Lebbeus ketophilus* Nye, 2013 (Nye, 2013) and several species of pycnogonids, amphipods and isopods (Table 4.1 and Figs. 4.4 and 4.6). No echinoderms, nemerteans or *Osedax* were found on this deployment and only one species of mollusc and cnidarian each occurred (Table 4.1 and Figs. 4.4 and 4.6).

Fauna that colonised this deployment were allocated to six functional groups (Table 4.1 and Fig. 4.7a). There were no fauna with a wood diet present ('Xylophagous') (Table 4.1 and Fig. 4.7a). 16 species comprising of 3395 individuals (79.51% of the total) were assigned to the 'Predators/Scavengers' group making it the most species-rich and abundant (Table 4.1 and Fig. 4.7). 'Grazers' (of plant material and bacterial mat) had the second highest number of individuals present with only 297 individuals (or 6.96% of the total) but 'Deposit Feeders' had the second highest number of species at this package with six species present (Table 4.1 and Fig. 4.7).

4.3.1.2 Coral-Seamount wood package

After two years on the seafloor, the wood was still very solid despite being bored by *Xylophaga murrayi* Knudsen, 1967 (Table 4.1 and Fig. 4.4g). The openings to some burrows were visible and once the wood was opened, some live specimens were found. There were 32 species (from eight classes) of fauna found inhabiting the wood (Table 4.1 and Fig. 4.6a). The most species-rich phylum and class was the Annelida: Polychaeta (fourteen species), as was seen at the whale bones at this site also (Table 4.1 and Fig. 4.6a). The total number of individuals present was much lower than that seen at the whale-bone deployment at this site however at just 1211 individuals (Table 4.1 and Fig. 4.6b). *X. murrayi* was present in the largest numbers on this

wood deployment (870 individuals or 71.84% of the total) followed by the amphipod species, *Seba* sp. A (227 individuals or 18.74% of the total) (Table 4.1 and Fig. 4.6b). There were several species of pycnogonid, mollusc, echinoderm and nemertean among others found on the wood at this site (Table 4.1 and Figs. 4.4 and 4.6).

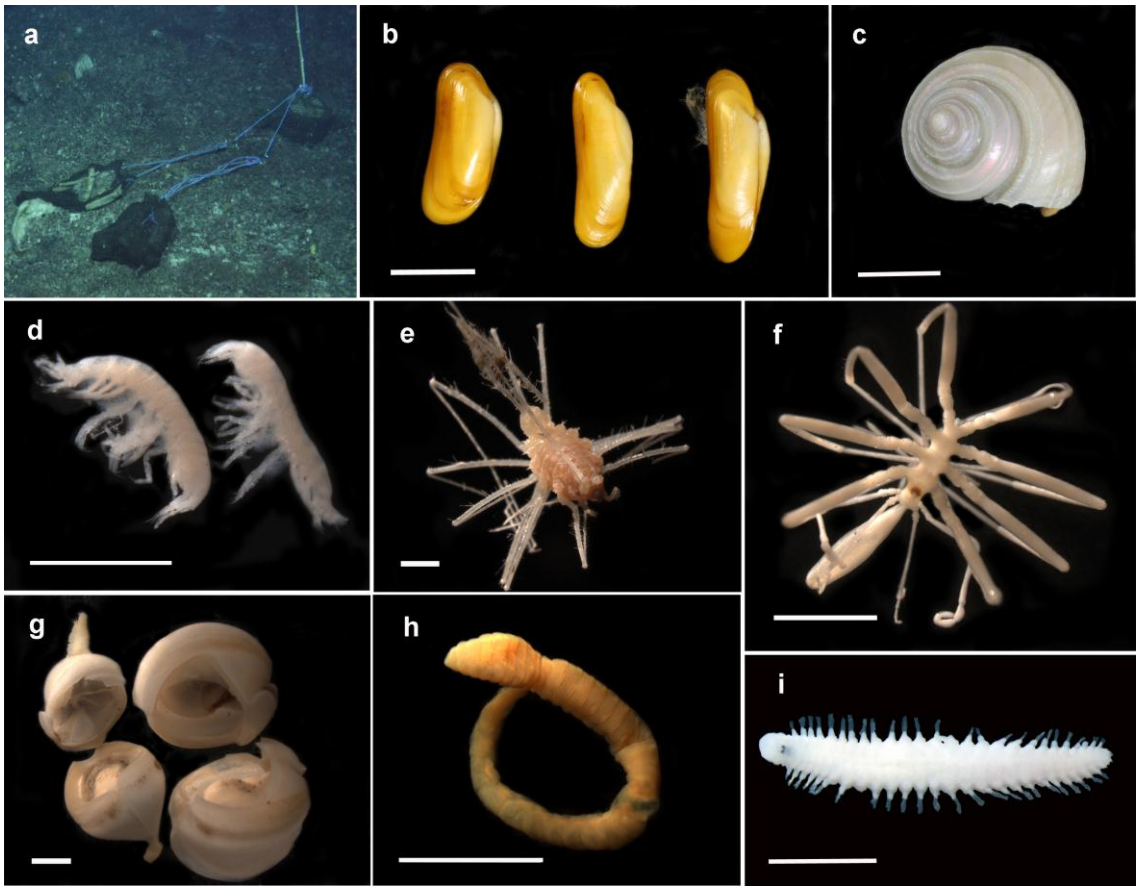

Figure 4.4 Fauna observed on the recovered bones and wood from Coral Seamount. (a) The deployment *in situ* on the seafloor. The wood and bone packages are on the left and the ballast is on the right of the photo. (b) *Idas* sp. A – scale is 10 mm (c) Calliostomatidae sp. A – scale is 10 mm. (d) *Seba* sp. A – scale is 1 mm. (e) Munnopsidae sp. A – scale is 2 mm. (f) *Hedgpethia* sp. A – scale is 2 mm. (g) *Xylophaga murrayi* – scale is 2 mm. (h) *Capitella* sp. C – scale is 3 mm. (i) *Ophryotrocha* sp. AB – scale is 1 mm.

This wood deployment was colonised by fauna from six functional groups (Table 4.1 and Fig. 4.7). The most species-rich functional group was the 'Predators/Scavengers' (15 species), as was seen on the whale bones at this site also (Table 4.1 and Fig. 4.7a). However the most dominant group was comprised of those that were wood eaters ('Xylophagous') with 71.84% of the total individuals present (870 individuals) (Table 4.1 and Fig. 4.7b). This group was made up entirely by *Xylophaga murrayi* (Table 4.1). 'Deposit Feeders' had the second highest species richness (eight species) and 'Predators/Scavengers' had the second highest number of individuals (20.40% of the total individuals). There were no 'Mixotrophs' or individuals of 'Other' feeding strategies present on this deployment (Fig. 5.7).

4.3.2 Observations of the bone and wood moorings recovered on Atlantis Bank

This deployment appeared to be sitting on a flat, lightly-sedimented, hard substrate resembling a pavement (Fig. 4.5a). Temperature was collected close to the deployment; on average, it was 10.1°C. The approximate volumes of the experimental substrates deployed on this seamount were 15254.0 cm^3 of bone and 36216.2 cm^3 of wood. After two years on the seafloor, the wood bag was intact but the bag containing the whale bones had several large holes through which three large Echinothurioida sp. A were feeding on the bones (Fig. 4.5a). One *Polyechinus agulhensis* Doderlein, 1905 and three *Projasus parkeri* Stebbing, 1902 could be seen in the video on the mooring, as well as three *Centriscops humerosus* Richardson, 1846 hovering immediately above the deployment (Fig. 4.5a). Epifauna on the mooring line consisted of one Actinaria sp. B, one Antipatharia sp. A, one *Heliometra* sp. A crinoid and one species of Hydrozoa. A eulimid gastropod was recovered from one of the bioboxes but the package it came from could not be identified. These gastropods are known to live on echinoderms however. In total, there were 8124 individuals from 38 species comprising 12 classes recovered from this deployment (including the epifauna on the mooring) (Table 4.1). 29 of the 38 (76.31%) species observed were found only on Atlantis Bank during in this study.

4.3.2.1 Atlantis-Bank whale-bone package

The whale bones did not show much evidence of grazing but there were many individuals attached *in situ* representing three species of *Idas* (Table 4.1 and Figs. 4.5h and 4.5i). *Idas* sp. A had the highest number of individuals present (468 or 48.64% of the total), then *Idas* sp. C (260 or 27.02%) (Table 4.1 and Fig. 4.6b). *Idas* sp. C were only found on the large ½ Sperm-whale vertebra. There was also a third species of *Idas* present, *Idas* sp. B (Table 4.1). Overall, this bone package had the lowest species richness (15 species from eight classes) and the lowest number of individuals of all four deployments (962 individuals) (Table 4.1 and Fig. 4.6). The most species-rich and dominant phylum was the Mollusca with six species from three classes and 778 individuals present (80.87%) (Table 4.1 and Fig. 4.6). The classes Echinoidea and Bivalvia each had three species present (Table 4.1 and Fig. 4.6a). The number of individuals of *Seba* sp. A was also high (120 individuals or 12.47% of the total) (Table 4.1). There was a notable absence of the bone-eating annelid *Osedax* on these bones. Fauna from only five of the seven functional groups colonised this whale-bone deployment. 'Predators/Scavengers' had six species assigned, making it the most species-rich at this package (Fig. 4.7a). It also had the second highest number of individuals present (154 individuals or 16.01% of the total) (Fig. 4.7b). The most dominant and second most species-rich functional group was the 'Mixotrophs' (three species comprising 79.94% of the total or 769 individuals) (Table 4.1 and Fig. 4.7). There

were no individuals assigned to either the ‘Suspension Feeders’ or ‘Xylophagous’ functional groups (Table 4.1 and Fig. 4.7).

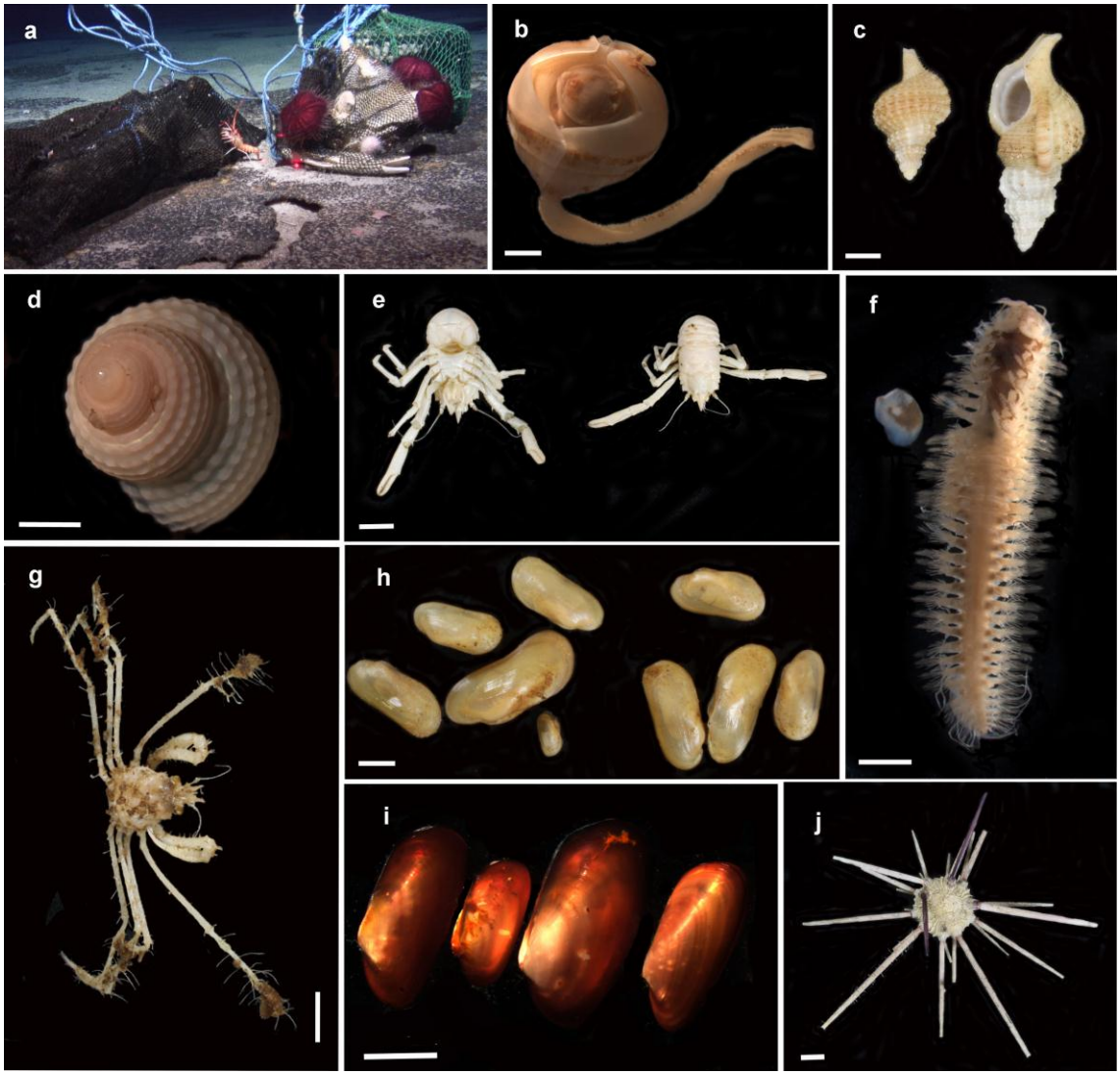

Figure 4.5 Fauna observed on the recovered bones and wood from Atlantis Bank. (a) The deployment *in situ* on the seafloor. Three Echinothurioida sp. A, one *Polyechinus agulhensis* and one *Projasus* sp. A can be seen on the deployment. (b) *Xylophaga* cf. *indica* – scale is 2 mm. (c) *Sassia nassariformis* - scale is 5 mm. (d) Colloninae sp. A – scale is 2 mm. (e) *Munidopsis mandelai* – scale is 5 mm. (f) *Austrolaenilla* sp. B – scale is 2 mm. (g) *Dorhynchus* sp. A – scale is 5 mm. (h) *Idas* sp. B – scale is 5 mm. (i) *Idas* sp. C – scale is 2 mm. (j) Cidaridae sp. A – scale is 10 mm.

4.3.2.2 Atlantis Bank wood package

27 species from nine classes of fauna were present amounting to 7155 individuals (Table 4.1 and Fig. 4.6). The wood was completely consumed by *Xylophaga* cf. *indica* Smith, 1904 as a result of the large numbers of this species present equivalent to 95.74% of the total number of individuals present at this wood package (6850 individuals) (Table 4.1 and Figs. 4.5b and 4.7b). The interior of the wood was so heavily bored that it was disintegrating and crushed by hand. The most species-rich phyla were the Annelida and the Arthropoda (nine species each) making

Polychaeta the most speciose class (Table 4.1 and Fig. 4.6a). The phylum Mollusca and the class Bivalvia, had the highest number of individuals present (7036 individuals or 98.34% and 6998 individuals or 97.81% respectively) (Table 4.1 and Fig. 4.6b). Other fauna observed on the wood at this site included two species of Amphinomidae (sp. A and sp. B) that were found in *Xylophaga* burrows, one specimen of *Dorhynchus* sp. A, one crinoid specimen *Thysanometra* sp. A and a new species of *Munidopsis*, *Munidopsis mandelai* Macpherson *et al*., (In press), that was described from these deployments (Macpherson *et al.*, In press) (Table 4.1 and Fig. 4.5). Fauna from all seven functional groups colonised this wood package and as a result, this deployment had the highest functional richness. The functional group 'Xylophagous' was comprised entirely of one species, *X.* cf. *indica* (Table 4.1 and Fig. 4.7). All other functional groups had between one and three species assigned to them.

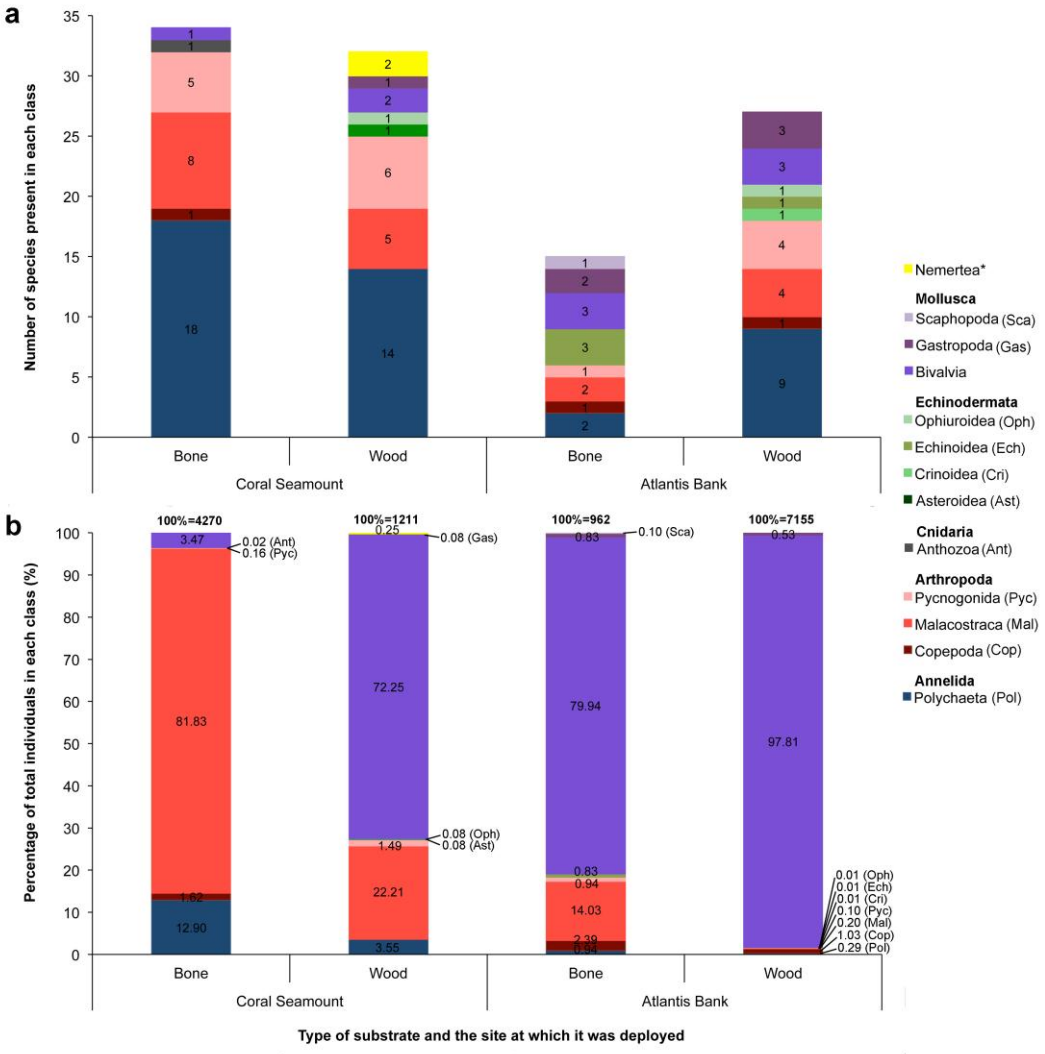

Figure 4.6 Classes of fauna on the recovered bone and wood deployments. (a) The number of species in each class present on each deployment. (b) The percentage of the total number of individuals in each class present on each deployment. The total number of individuals (100%) found on each deployment can be found in bold type at the top of each column.

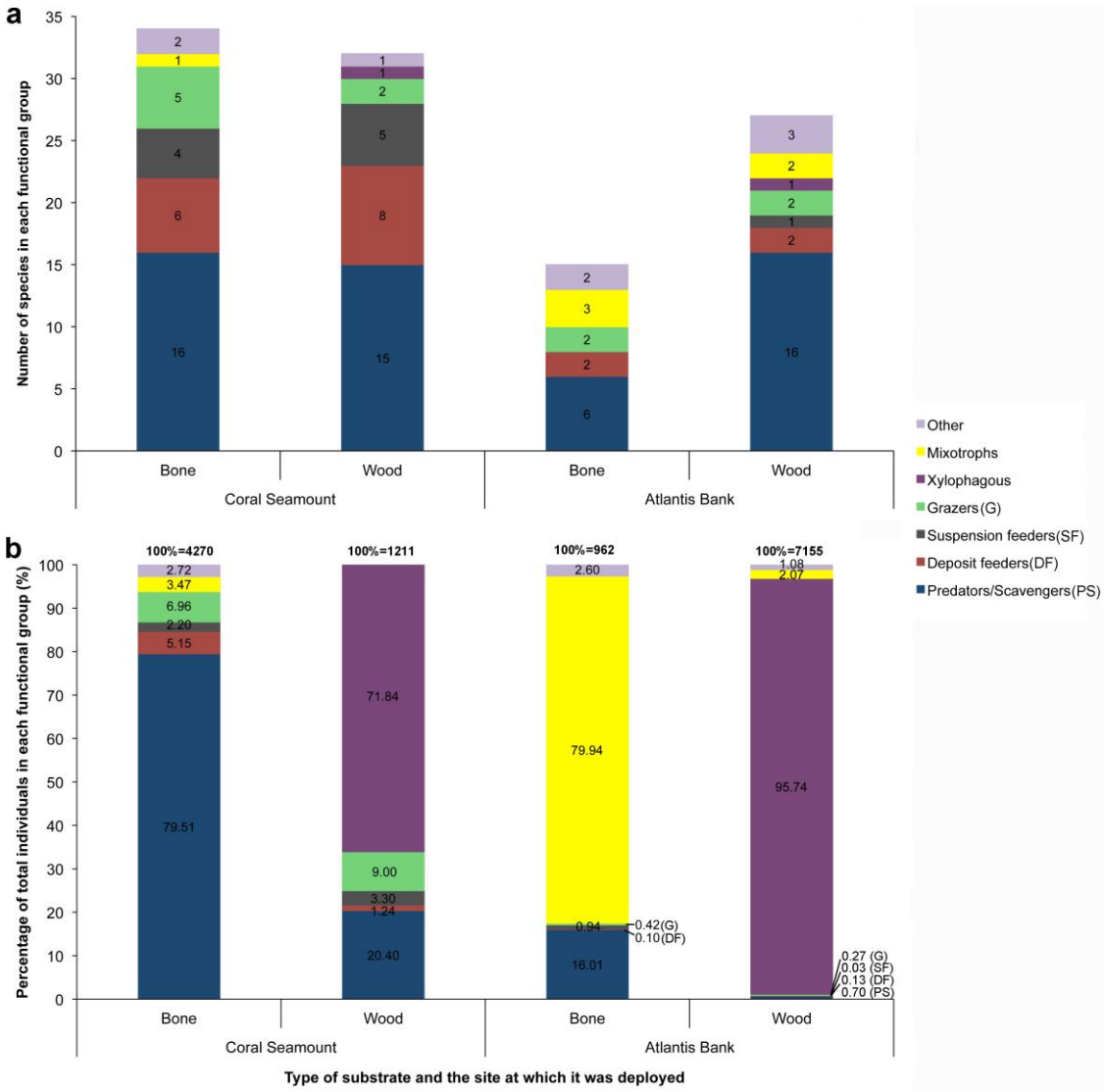

Figure 4.7 Functional groups of fauna on the recovered bone and wood deployments. (a) The number of species in each functional group present on each deployment. (b) The percentage of the total number of individuals in each functional group present on each deployment. The total number of individuals (100%) found on each deployment can be found in bold type at the top of each column.

4.3.3 Statistical analyses of the colonising fauna

As stated in the methodology, we have directly compared the numbers of fauna colonising the packages even though the packages were calculated to have slightly different volumes. As a result of this assumption, results will only be considered significant if $p < 0.001$. The number of individuals (classified into classes and phyla) recovered from the deployments were compared between sites and also between substrates. The total numbers of individuals found on the two substrates on Coral Seamount were significantly different ($\chi^2 = 1706.14$, df = 1, $p < 0.001$) as well as on each substrate on Atlantis Bank ($\chi^2 = 4723.53.18$, df = 1, $p < 0.001$). The total number of individuals on bone differed significantly between Coral Seamount and Atlantis Seamount ($\chi^2 = 2090.26$, df = 1, $p < 0.001$) and also on wood ($\chi^2 = 4211.76$, df = 1, $p < 0.001$). Total faunal

numbers differed between substrates with wood having more fauna than bone ($\chi^2 = 721.85$, df = 1, $p < 0.001$), and also between sites with Atlantis Bank having more fauna than Coral Seamount ($\chi^2 = 510.61$, df = 1, $p < 0.001$).

All arthropod, bivalve, malacostracan and molluscan numbers were significantly different between sites and substrate types ($p < 0.001$). All numbers of copepods differed significantly except the total numbers on wood and bone substrate. The numbers of polychaetes were all significantly different except between bone and wood at Atlantis Bank, and between wood on Coral Seamount and Atlantis Bank. For gastropods numbers, all were significantly different except between substrates on Coral Seamount, and between the bone deployments on the two seamounts. All the numbers of asteroids, cnidarians (anthozoans), crinoids, echinoderms, echinoids, nemerteans, ophiuroids, pycnogonids and scaphopods were not significantly different between the two sites and on both substrate types.

The numbers of individuals assigned to each functional group were significantly different ($p < 0.001$) between sites and substrate types. This however excluded the numbers of 'Deposit Feeders', 'Suspension Feeders' and 'Grazers' on bone and wood at Atlantis Bank, and also the number of 'Deposit Feeders' and 'Grazers' on wood between the Atlantis Bank and Coral Seamount. The number of individuals in the 'Other' functional group was also not significantly different between wood and bone at both sites surveyed and both substrates at each site surveyed.

Sorenson's coefficient was used to compare the similarity at species level between presence/absence data of the faunal assemblages of the four deployments, bone from Coral Seamount, wood from Coral Seamount, bone from Atlantis Bank and wood from Atlantis Bank. This test showed that the faunal assemblages at the bone and wood deployments from Coral Seamount had the least difference with 59% Sorensen's similarity followed by the wood and bone from Atlantis Bank, which had 30% similarity. The assemblages colonising bone samples from the two sites had 21% similarity and the two wood assemblages had 13%. The bone from Coral Seamount and the wood from Atlantis Bank had 23% similarity and the wood from Coral Seamount and the bone from Atlantis Bank had 6% similarity. A cluster analysis using the group average of these results was plotted into a dendrogram, which can be seen in Figure 4.8.

Figure 4.8 Similarity between the faunal assemblages at the four deployments. A cluster dendrogram using the group averages of results from Sorenson's similarity tests performed on the species presence/absence data from the four deployment assemblages: bone from Coral Seamount (Coral Bone), wood from Coral Seamount (Coral Wood), bone from Atlantis Bank (Atlantis Bone) and wood from Atlantis Bank (Atlantis Wood).

Table 4.1. Fauna colonising bone and wood deployments on two seamounts in the Southwest Indian Ridge. Fauna were identified to the lowest taxonomic level, counted and assigned a functional group related to diet from information found in the literature indicated. The numbers for each species may be underestimates as some individuals may have been lost during collection from the seafloor.

					Coral Seamount		Atlantis Bank			
Phylum	**Class**	**Order**	**Family**	**Genus and Species/Morphotype**	**Bone**	**Wood**	**Bone**	**Wood**	**Functional group**	**Reference**
Annelida	Polychaeta	Amphinomida	Amphinomidae	sp. A	-	-	-	5	Predators/Scavengers	(Fauchald and Jumars, 1979; Ward *et al.*, 2003)
				sp. B	-	-	-	1	Predators/Scavengers	(Fauchald and Jumars, 1979; Ward *et al.*, 2003)
		Capitellida	Capitellidae	*Capitella* sp. A	56	1	-	7	Deposit feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
				Capitella sp. B	53	1	-	-	Deposit feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
				Capitella sp. C	69	4	-	-	Deposit feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
				Capitella sp. D	40	2	-	-	Deposit feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
		Eunicida	Dorvilleidae	sp. A	5	-	2	1	Other	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
				Ophryotrocha sp. AA	269	6	-	-	Grazers	(Wiklund *et al.*, 2009b)
				Ophryotrocha sp. AB	17	-	-	-	Grazers	(Wiklund *et al.*, 2009b)
				Ophryotrocha sp. AC	7	2	-	-	Grazers	(Wiklund *et al.*, 2009b)
				Ophryotrocha sp. AE	3	-	-	-	Grazers	(Wiklund *et al.*, 2009b)
		Phyllodocida	Glyceridae	*Glycera* sp. A	-	-	-	1	Predators/Scavengers	(Fauchald and Jumars, 1979; Boggemann *et al.*, 2012)
			Hesionidae	sp. A	-	-	-	1	Predators/Scavengers	(Fauchald and Jumars, 1979; Desbruyeres *et al.*, 1985)
				sp. B	7	1	-	-	Predators/Scavengers	(Fauchald and Jumars, 1979; Desbruyeres *et al.*, 1985)
				sp. C	4	-	-	-	Predators/Scavengers	(Fauchald and Jumars, 1979; Desbruyeres *et al.*, 1985)
			Lacydoniidae	sp. A	-	1	-	-	Deposit feeders	(Fauchald and Jumars, 1979)
			Phyllodocidae	sp. A	1	-	-	-	Predators/Scavengers	(Fauchald and Jumars, 1979; Blake, 1985)
			Pilargidae	sp. A	1	-	-	-	Predators/Scavengers	(Fauchald and Jumars, 1979)
			Polynoidae	*Austrolaenilla* sp. A	5	1	-	-	Predators/Scavengers	(Fauchald and Jumars, 1979; Pettibone, 1993)
				Austrolaenilla sp. B	-	-	-	2	Predators/Scavengers	(Fauchald and Jumars, 1979; Pettibone, 1993)
				Austrolaenilla sp. C	-	-	7	2	Predators/Scavengers	(Fauchald and Jumars, 1979; Pettibone, 1993)

			Syllidae	sp. A	6	-	-	-	Predators/Scavengers	(Fauchald and Jumars, 1979)
		Sabellida	Sabellidae	sp. A	-	1	-	-	Suspension feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
				Jasmineira sp. A	6	17	-	-	Suspension feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
		Terebellida	Acrocirridae	*Flabelligella* sp. A	1	4	-	-	Deposit feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
				Flabelligella sp. B	1	1	-	-	Deposit feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
			Trichobranchidae	*Octobranchus* sp. A	-	1	-	-	Deposit feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
			Cirratulidae	sp. A	-	-	-	1	Deposit feeders	(Fauchald and Jumars, 1979; Kukert and Smith, 1992)
Arthropoda	Copepoda	Harpacticoida		sp. A	69	-	23	74	Grazers	(Geptner and Ivanenko, 2002; Galkin and Goroslavskaya, 2008)
	Malacostraca	Amphipoda		sp. A	-	-	15	-	Other	(Ruppert and Barnes, 1996)
			Calliopiidae	sp. A	42	22	-	-	Predators/Scavengers	(Shoemaker, 1930)
			Podoceridae	*Podocerus* sp. A	85	17	-	-	Suspension feeders	(Barnard *et al.*, 1988)
			Sebidae	*Seba* sp. A	3344	227	120	-	Predators/Scavengers	(Kunzmann, 1996)
		Decapoda	Hippolytidae	*Eualus oreios*	1	-	-	-	Predators/Scavengers	(Squires *et al.*, 2000; Birkely and Gulliksen, 2003)
				Lebbeus ketophilus	1	-	-	-	Predators/Scavengers	(Butler, 1980; Jensen, 2006)
			Inachidae	*Dorhynchus* sp. A	-	-	-	1	Predators/Scavengers	(Duineveld *et al.*, 2007)
			Mathildellidae	*Neopilumnoplax heterochir*	-	-	-	3	Predators/Scavengers	(Turner, 1977; Hoyoux *et al.*, 2009; Hoyoux *et al.*, 2012)
			Munidopsidae	*Munidopsis mandelai*	-	-	-	7	Other	(Personal observation; Parin *et al.*, 1997)
			Palinuridae	*Projasus parkeri*	-	-	-	3	Predators/Scavengers	(Ruppert and Barnes, 1996)
		Isopoda		sp. A	2	2	-	-	Other	(Barnes and Conlan, 2012)
			Arcturidae	sp. A	1	1	-	-	Suspension feeders	(Svavarsson *et al.*, 1993; Gudmundsson *et al.*, 2000)
			Munnopsidae	sp. A	18	-	-	-	Predators/Scavengers	(Arnaud and Bamber, 1987; Sweeting *et al.*, 2013)
	Pycnogonida	Pantopoda	Ammotheidae	*Sericosura mitrata*	1	1	-	-	Predators/Scavengers	(Fry, 1965; Arnaud and Bamber, 1987)
			Austrodecidae	*Austrodecus valdiviens*	-	7	-	2	Predators/Scavengers	(Fry, 1965; Arnaud and Bamber, 1987)
				Austrodecus sp. B	1	-	-	3	Predators/Scavengers	(Arnaud and Bamber, 1987)
			Colossendeidae	*Hedgpethia* sp. A	2	-	9	1	Predators/Scavengers	(Stock, 1978; Arnaud and Bamber, 1987)
			Nymphonidae	*Nymphon* sp. A	1	-	-	-	Predators/Scavengers	(Stock, 1978; Arnaud and Bamber, 1987)
				Nymphon sp. B	-	1	-	-	Predators/Scavengers	

				Nymphon sp. E	-	2	-	-	Predators/Scavengers	(Stock, 1978; Arnaud and Bamber, 1987)
				Nymphon sp. F	-	1	-	-	Predators/Scavengers	(Stock, 1978; Arnaud and Bamber, 1987)
			Rhynchothoracidae	*Rhynchothorax* sp. A	2	6	-	1	Predators/Scavengers	(Fry, 1965; Arnaud and Bamber, 1987)
Cnidaria	Anthozoa	Antipatharia		sp. A	1	-	-	-	Suspension feeders	(Wagner *et al.*, 2012)
Echinodermata	Asteroidea	Forcipulatacea	Asteriidae	cf. *Perissasterias* sp. A	-	1	-	-	Predators/Scavengers	(Jangoux, 1982; Ruppert and Barnes, 1996)
	Crinoidea	Comatulida	Antedonidae	*Thysanometra* sp. A	-	-	-	1	Suspension feeders	(Turner, 1977; Ruppert and Barnes, 1996)
	Echinoidea			sp. A	-	-	-	1	Other	(De Ridder and Lawrence, 1982; Ruppert and Barnes, 1996)
		Camarodonta	Echinidae	*Polyechinus agulhensis*	-	-	1	-	Deposit feeders	(De Ridder and Lawrence, 1982)
		Cidaroida	Cidaridae	sp. A	-	-	4	-	Predators/Scavengers	(De Ridder and Lawrence, 1982; Jacob *et al.*, 2003)
		Echinothurioida		sp. A	-	-	3	-	Predators/Scavengers	(Personal observation; De Ridder and Lawrence, 1982)
	Ophiuroidea	Ophiurida	Ophiacanthidae	sp. A	-	-	-	1	Predators/Scavengers	(Warner, 1982; Smith, 1985; Stohr and Segonzac, 2006)
			Ophiomyxidae	*Ophioscolex* sp. A	-	1	-	-	Predators/Scavengers	(Warner, 1982)
Mollusca	Bivalvia	Myoida	Pholadidae	*Xylophaga murrayi*	-	870	-	-	Xylophagous	(Personal observation; Knudsen, 1961; Turner, 1973, 1977)
				Xylophaga cf. *indica*	-	-	-	6850	Xylophagous	(Personal observation; Knudsen, 1961; Turner, 1973, 1977)
		Mytiloida	Mytilidae	*Idas* sp. A	148	-	468	22	Mixotrophs	(Smith *et al.*, 1989; Smith and Baco, 2003)
				Idas sp. B	-	-	41	126	Mixotrophs	(Smith *et al.*, 1989; Smith and Baco, 2003)
				Idas sp. C	-	-	260	-	Mixotrophs	(Smith *et al.*, 1989; Smith and Baco, 2003)
		Pectinoida	Pectinidae	sp. A	-	5	-	-	Suspension feeders	(Le Pennec *et al.*, 2003)
	Gastropoda	Littorinimorpha	Ranellidae	*Sassia nassariformis*	-	-	4	17	Predators/Scavengers	(Morton, 1990)
		Nudibranchia	Aeolidiidae	sp. A	-	-	-	2	Predators/Scavengers	(Todd, 1981; Ruppert and Barnes, 1996)
		Phasianelloidea	Colloniidae	Colloninae sp. A	-	-	4	19	Grazers	(Zuschin *et al.*, 2009)
		Trochoidea	Calliostomatidae	*Venustatrochus georgianus*	-	1	-	-	Predators/Scavengers	(Williams *et al.*, 2010)
	Scaphopoda			sp. A	-	-	1	-	Deposit feeders	(Kukert and Smith, 1992)
Nemertea				sp. A	-	1	-	-	Predators/Scavengers	(Ruppert and Barnes, 1996)
				sp. B	-	2	-	-	Predators/Scavengers	(Ruppert and Barnes, 1996)

4.4 Discussion

Organic falls are known to host distinct assemblages in the deep sea, usually attracting a variety of specialist and opportunistic fauna, which utilise the wood and bone for different purposes such as nutrition and shelter. For the first time, we have characterised the faunal assemblages at Indian-Ocean organic falls, which are remarkable for the diversity and abundance of both opportunists and specialists on what are relatively small 'targets' on the seafloor for larval settlement. In addition, it is notable that the faunal assemblages from the two sites are so different in both diversity and composition, to the extent that the geographical location was a more important determinant of species composition than substrate type, despite bone and wood being quite different in their organic structure and nature of degradation by specialist organisms. At the more southerly Coral Seamount, the organic falls were characterised by generally higher diversity but much lower colonisation by specialist organic-fall molluscs (*Idas* molluscs in the case of bones, and *Xylophaga* molluscs for the wood). In contrast, the warmer waters of the Atlantis Bank hosted a lower diversity organic fall but with much higher abundances of both *Idas* and *Xylophaga* molluscs. It was also notable that the species of *Xylophaga* was different at each seamount, despite being found at similar depths and not separated by any great geographic distance for a species thought to have impressive dispersal capabilities (Voight and Segonzac, 2012). Here we discuss in further detail the two main structuring variables (site and substrate type) and compare our results with other recent studies of organic falls from global locations.

4.4.1 Comparison between sites

Each seamount site had distinct faunal assemblages colonising the deployments. This may be influenced by two main factors: the environmental characteristics of each site and the geographic isolation of each seamount possibly leading to endemism among the fauna.

The seamount deployment sites had very different physical, chemical and biological settings. Substratum type, the geomorphology, the hydrodynamic regime and the water conditions (salinity, oxygen concentration, temperature, productivity) differed between the seamounts and have previously been shown to affect the faunal complement living at seamounts and organic falls (Clark *et al.*, 2010). Coral Seamount is south of the Sub-Tropical Front and thus has colder and less saline water than the more northerly well-oxygenated seamount, Atlantis Bank (Rogers *et al.*, 2009; Rogers *et al.*, 2012a) (Fig 4.1a). This will result in differing biogeochemistry, phytoplankton composition and productivity associated also. As a result, this front may act as a major potential biogeographic barrier (Rogers *et al.*, 2009).

The geomorphologies of the two seamounts were also very different. Coral Seamount has its summit much shallower in the water column (approximately 200 m) and has steep sides, while Atlantis Bank has a very flat summit at height of approximately 700 m. Even though the two deployments were at similar depths, they were at very different locations with respect to each seamount. Some authors have shown that the flanks of seamounts have very different faunal assemblages to the summit for instance, due to different ecological and hydrological conditions (Sautya *et al.*, 2011). Thus the background-fauna communities (and therefore some of the fauna colonising the packages) may have been influenced during this study as the deployment on Coral Seamount was on a northern slope and the one at Atlantis Bank was on the western side of the flat summit.

The two deployment areas also differed in substratum type. Coral Seamount was reported to host extensive coral communities but Rogers *et al*. (2012) found that the northern flank at 700-800 m where the deployment was located, was comprised of a large area of dead coral framework (mainly *Solenosmilia variabilis* Duncan, 1873, and *Desmophyllum dianthus* Esper, 1794). Very few live scleractinian corals were observed, and this could possibly be related to the destructive effects of fishing on this seamount prior to BPA designation. In contrast, at Atlantis Bank, the deployment area was comprised of carbonates overlain in some areas by sheets of basalts thinly draped in sediment. At Coral Seamount, the dead coral framework "was highly cryptic and provided habitat for a wide range of invertebrates, particularly squat lobsters and gastropods…" (Rogers *et al.*, 2012a). This type of substratum is conducive to epifauna as well as infauna and this may account for the higher species richness and numbers of fauna observed at the organic fall at Coral Seamount during this study. It is quite likely that specialist infauna of coral framework are also able to utilise an organic fall with similar internal matrices (the borings of *Xylophaga* or the bone trabeculae for instance). This might explain why polychaetes were much more species-rich and abundant at Coral Seamount than at Atlantis Bank, as the latter was characterised by an impermeable basaltic-carbonate substratum that is far less hospitable for such infaunal animals. This hard substratum however may have been more conducive to echinoderms and molluscs accounting for the much higher number of those phyla at the Atlantis-Bank organic fall. Although further background data are thus far scarce, Rogers *et al*. (2012) also reported flat areas being dominated by soft echinoids, small pink echinoids and cidarid echinoids at Atlantis Bank.

Suspension feeders are also known to inhabit coral framework, as it provides height off the seafloor and into the water column for ease of feeding (Rogers, 1994; Rowden *et al.*, 2010). Nine suspension-feeding species (six on the wood/bone and three on the mooring) were observed on the deployments at Coral Seamount. In contrast with Atlantis Bank where the

substratum was flat, only five were observed in total, with one on the deployments and four on the mooring suspended off the seafloor.

There was not much information on the hydrodynamic regime at these particular seamounts but some authors have proposed that seamounts have higher benthic-invertebrate richness and abundance than surrounding deep-sea areas due to the 'oasis hypothesis' (Samadi *et al.*, 2006; Rowden *et al.*, 2010). This is said to be owing to interactions between the topography and currents that enhance local biomass by mixing nutrients in the euphotic zone creating highly-productive oases (Rogers, 1994; Samadi *et al.*, 2006). In total, the deployments at Coral Seamount and Atlantis Bank had 53 species and 5490 individuals and 38 species and 8124 individuals respectively. This is a higher species richness than seen at some previous late-stage whale falls (Naganuma *et al.*, 1996; Dahlgren *et al.*, 2006; Lundsten *et al.*, 2010a; Amon *et al.*, 2013), on par with others (Bennett *et al.*, 1994; Lundsten *et al.*, 2010b) and much less than some (Baco and Smith, 2003; Smith and Baco, 2003; Fujiwara *et al.*, 2007). However, the whale-fall studies that have had higher species richness and abundance have been on large carcasses with a significantly higher volume of substrate than at the deployments in this study. Species richness on wood deployments tended to be higher than previously found on some (Pailleret *et al.*, 2007a; Bienhold *et al.*, 2013) and on par with some (Turner, 1977; Gaudron *et al.*, 2010). Baco and Smith (2003) noted that mean diversity for hydrothermal-vent sites and seeps is 50-60 species and 11 species respectively, showing that the species richness at the SWIR packages was similar to that of vents worldwide and more than seeps. The combination of an already highly diverse and abundant seamount fauna opportunistically attending the wood and bone packages, coupled with the presence of organic-fall specialists (*Idas*, *Xylophaga* and bacterial-mat grazers) is a likely reason for the high species richness at these deployments.

The other main factor that may have influenced the significant differences between the faunal assemblages that attended the deployments at each seamount during this study is that of geographic isolation (horizontal and vertical) (Richer de Forges *et al.*, 2000). Richer de Forges *et al*. (2000) noted very little overlap between seamount populations only a few kilometres away and thus they hypothesised that larval dispersal was limited by hydrological phenomena resulting from the interactions of currents and topography, promoting larval retention (Rogers, 1994; Mullineaux and Mills, 1997). This could limit the efficiency of larval dispersion for organisms that inhabit seamounts, resulting in isolation and eventually speciation. Between the deployments (including fauna on the mooring lines), there were only eleven species out of 82 (13%) in common between seamounts and at least eight other species are known from locations other than the seamounts observed. Hence, 19 species out of 82 (23%) were definitely not endemic to either seamount in this study. This figure may still be raised by further positive identification of fauna. This however leaves 77% of species attending these organic-fall

deployments as possible endemics. A previous study has shown that some fauna can be effectively dispersed by probable passive larval transport, in an 'island-hopping' pattern across the small distances that separate the seamount summits along the chain (Leal and Bouchet, 1991). The distances in that study were only between 100 and 250 kilometres, but it was also found that the more proximate the seamount summits, the more similar the gastropod communities would be (Leal and Bouchet, 1991). Coral Seamount and Atlantis Bank are approximately 1600 kilometres apart and thus despite having many seamounts between them, this may explain some of the differences in faunal assemblages.

There were several putative new species of pycnogonid and crinoid, and the confirmed new species *Munidopsis mandelai*, *Eualus oreios* and *Lebbeus ketophilus* described from these seamounts based on material from our packages. It is unclear whether this exemplifies endemism on seamounts or undersampling in a poorly-explored area as has been proposed by other authors (McClain, 2007; Clark *et al.*, 2010). This may be true for our study as only one of each substrate type was deployed at each seamount, as well as the size of each deployment was very small when compared with that of the overall seamount area. Other authors have suggested that endemism on seamounts may be a lot lower than previously expected and instead fauna reflects the regional species pool which use the seamounts as ecological stepping stones for dispersal (excluding animals with non-planktotrophic larvae) (Samadi *et al.*, 2006; Rogers *et al.*, 2012a). The results of this study however, can only support the hypothesis that these seamounts harbor 'potentially endemic' fauna. It follows then that the assemblage of fauna at each deployment on each seamount may be different as its composition is to some extent determined by opportunistic background fauna, which are in turn different on each seamount.

4.4.2 Comparison between substrates

Surprisingly, substrate (bone or wood) influenced the faunal assemblages less than the deployment site in this study but it would have still had an impact, especially with regard to specialist fauna. There were few specialist species present on the deployments but they tended to dominate in very large numbers when they were present. This was especially true with the extremely high numbers of the wood-specialist genus *Xylophaga* on the wood deployments making them by far the two most abundant species in the entire study. A different species of *Xylophaga* was found on each package but both had been previously recorded in the Indian Ocean. The species from Atlantis Bank resembles *Xylophaga indica* but there is no siphon description for this species and thus a positive identification was not possible. One specimen of *X. murrayi* (found at the Coral-Seamount organic fall) was previously trawled up off Zanzibar from a depth of 347-384 m (Knudsen, 1967) and *X. indica* off the Andaman Islands at a depth of 339 m (there was no mention of plant matter in either case) (Knudsen, 1961). These records

have significantly expanded the geographic ranges and to a lesser extent, the depth ranges of both species. This genus seems to be found whenever wood is present in the deep sea (with the exception of the Black Sea, the Baltic Sea and possibly the Southern Ocean (Glover *et al.*, 2013) and are keystone species in wood-fall food chains by making the refractory food source available for other organisms (Turner, 1973, 1977; Pailleret *et al.*, 2007a; Bernardino *et al.*, 2010; Gaudron *et al.*, 2010; Samadi *et al.*, 2010; Bienhold *et al.*, 2013). Other organic-fall specialists attending these deployments were the mixotrophic genus *Idas* found on the bone and wood and the grazing genus *Ophryotrocha*. The genus *Idas* was present in high numbers, especially on the bone deployment at Atlantis Bank where three species were present. *Idas* spp. are usually one of the most abundant fauna at wood and whale falls, and have been recorded on many occasions in the neighbouring Pacific (Tebble, 1966; Turner, 1973; Dell, 1987; Marshall, 1987; Smith *et al.*, 1989; Bennett *et al.*, 1994; Marshall, 1994; Dell, 1995; Naganuma *et al.*, 1996; Smith and Baco, 2003; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Pailleret *et al.*, 2007a; Samadi *et al.*, 2007; Bernardino *et al.*, 2010; Gaudron *et al.*, 2010; Lorion *et al.*, 2010; Lundsten *et al.*, 2010a; Samadi *et al.*, 2010; Bienhold *et al.*, 2013).

4.4.3 Global comparison

Organic falls are known to be specific hotspots of diversity in the deep sea, usually attracting a variety of specialist and opportunistic fauna, which utilise the wood and bone for different purposes (nutrition and shelter). This study has shown that at least 16 of the 82 species (20%) of the fauna found attending the deployed substrates were opportunistic fauna common to other areas of the Indian Ocean. These include: *Capitella* sp. A, Dorvilleidae sp. A, Harpacticoida sp. A, *Seba* sp. A, *Austrodecus valdiviens* Turpaeva, 1990, *Austrodecus* sp. B, *Hedgpethia* sp. A, *Rhynchothorax* sp. A, *Neopilumnoplax heterochir,* Hydrozoa sp. A, *Projasus parkeri, Sericosura mitrata, Polyechinus agulhensis, Sassia nassariformes* and *Venustatrochus georgianus* Powell, 1951. This trend has been noted in previous organic-fall studies including those by Lundsten *et al*. (2010), Goffredi *et al*. (2004) and Braby *et al*. (2007). At both sites, there were also high numbers of predators and scavengers at both substrates. Many of these may have been opportunists making use of an easy plentiful food source.

Despite many of the attending fauna being opportunistic background fauna, several of these genera and families have been noted at organic falls and other chemosynthetic habitats such as seeps and/or hydrothermal vents previously. This suggests that these ecosystems may share a close evolutionary history for part of their faunal component (Smith and Baco, 2003). However, none of the species found inhabiting these deployments were found at the closest explored hydrothermal vent field (Dragon Hydrothermal Vent Field on the SWIR) (J. Copley, pers. obs.).

Of the polychaete families and genera, *Ophryotrocha* were found on both substrates during this study but were much more abundant on the whale bones. These dorvilleids are commonly found at whale falls (Bennett *et al.*, 1994; Goffredi *et al.*, 2004; Dahlgren *et al.*, 2006; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Amon *et al.*, 2013) and other organically-enriched areas (Blake, 1985; Desbruyeres *et al.*, 2006; Wiklund *et al.*, 2009a; Bernardino *et al.*, 2010; Gaudron *et al.*, 2010). Although polynoids are diverse in almost all marine environments, several species have been found at hydrothermal vents, cold seeps, whale falls and wood falls (Turner, 1977; Bennett *et al.*, 1994; Goffredi *et al.*, 2004; Desbruyeres *et al.*, 2006; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Bernardino *et al.*, 2010; Lundsten *et al.*, 2010a; Samadi *et al.*, 2010). Species from the families Capitellidae and Cirratulidae are known to frequent organically-enriched habitats such as organic falls (Turner, 1977; Goffredi *et al.*, 2004; Fujiwara *et al.*, 2007; Bernardino *et al.*, 2010; Samadi *et al.*, 2010; Amon *et al.*, 2013). All the hesionids were found on the whale bones from one site, bar one individual found on the wood at the same site. There are more records of hesionids observed at whale falls previously than at wood falls (Goffredi *et al.*, 2004; Braby *et al.*, 2007; Samadi *et al.*, 2010). *Glycera* sp. A and Amphinomidae were found only on the wood at the Atlantis Seamount. Glycerids have been shown to be highly tolerant to sulfide exposure and have been observed inhabiting wood falls and hydrothermal vents previously (Blake, 1985; Gaudron *et al.*, 2010; Boggemann *et al.*, 2012; Bienhold *et al.*, 2013). Amphinomidae tend to be abundant on wood falls, vents and seeps but have also been found rarely at whale falls (Desbruyeres *et al.*, 2006; Braby *et al.*, 2007; Gaudron *et al.*, 2010; Samadi *et al.*, 2010; Borda *et al.*, 2012; Bienhold *et al.*, 2013). Syllids, phyllodocids and accrocirids have previously been recorded at organic falls (Fujiwara *et al.*, 2007; Samadi *et al.*, 2010) and hydrothermal vents (Blake, 1985).

Nine species of pycnogonid were collected on the deployments. It is thought that any associations with the deployment material are unlikely; rather these specimens were adapted to feeding on hydroids found growing on the moorings (D. Staples, personal communication). Pycnogonids have been found associated with organic falls before but they are thought to be opportunistic fauna (Turner, 1977; Braby *et al.*, 2007). *Sericosura mitrata* was recovered from Coral Seamount and is known from hydrothermal vents and cold seeps off the Southwest Indian Ocean and Antarctica (Desbruyeres *et al.*, 2006; Rogers *et al.*, 2012a). Galatheids are said to have increased species richness and abundance on seamounts in the southwest Pacific Ocean and are thought to be opportunists that attend organic falls (Turner, 1977; Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Smith and Baco, 2003; Goffredi *et al.*, 2004; Samadi *et al.*, 2006; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Bernardino *et al.*, 2010; Samadi *et al.*, 2010; Hoyoux *et al.*, 2012). *Munidopsis mandelai* was only found on wood deployments in the SWIR, possibly adding evidence to the xylophagous nature of some species (Hoyoux *et al.*, 2009). Amphipods

and isopods are abundant on organic falls (Turner, 1977; Bennett *et al.*, 1994; Smith and Baco, 2003; Goffredi *et al.*, 2004; Dahlgren *et al.*, 2006; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Bernardino *et al.*, 2010; Gaudron *et al.*, 2010; Lundsten *et al.*, 2010a; Samadi *et al.*, 2010; Amon *et al.*, 2013; Bienhold *et al.*, 2013); *Seba* have been previously recorded from wood falls and hydrothermal vents (Shaw, 1989; Larsen, 2007). Copepods have been noted at organic falls previously (Turner, 1977; Marshall, 1987; Gaudron *et al.*, 2010) and also from vents (Geptner and Ivanenko, 2002; Galkin and Goroslavskaya, 2008). The genus *Eualus*, which was only found on bone, has been observed once before at a whale fall (Fujiwara *et al.*, 2007; Komai and Fujiwara, 2012). Brachyuran crabs have also been observed at chemosynthetic habitats before (Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Ahyong, 2008).

Molluscan fauna have sometimes been reported to be the most abundant and speciose fauna at some organic falls (polychaetes are noted to be the most abundant and speciose in other studies) but this is commonly due to high *Xylophaga* and limpet numbers (Turner, 1977; Marshall, 1987; Pailleret *et al.*, 2007a; Tyler *et al.*, 2007; Gaudron *et al.*, 2010; Amon *et al.*, 2013). It is curious that no limpets were reported on these deployments. Rogers *et al*. (2012) noted that gastropods were relatively common during seamount surveys on the SWIR indicating that many attending the organic deployments may have been opportunistic background fauna. One calliostomatid was observed on the wood deployment at Coral Seamount; these have been found on wood in the southwest Pacific by Samadi *et al*. (2010). Nudibranchs have been previously found on organic falls (Braby *et al.*, 2007; Lundsten *et al.*, 2010a) and they are known to predate on hydrozoans (Todd, 1981), of which there were many on the deployments. Fujiwara *et al*. (2007) observed scaphopods at a whale fall of Japan.

There were several suspension-feeding taxa (hydroids, anemones, crinoids) found on both deployments. These have usually been found in the third and fourth stage of whale falls as the animals use the exposed bones as a hard substrate to settle on (Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Smith and Baco, 2003; Goffredi *et al.*, 2004; Braby *et al.*, 2007; Fujiwara *et al.*, 2007). They are also common on wood falls (Turner, 1977). Echinoderms were mostly present at the bone on Atlantis Bank, but some were also seen on the wood at Atlantis Bank and Coral Seamount. Ophiuroids, asteroids and echinoids have been observed at other organic falls (Turner, 1977; Bennett *et al.*, 1994; Naganuma *et al.*, 1996; Goffredi *et al.*, 2004; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Pailleret *et al.*, 2007a; Samadi *et al.*, 2010; Bienhold *et al.*, 2013). Three nemerteans were noted on the wood at Coral Seamount; Guadron *et al*. (2010) also found nemerteans on wood deployments but Goffredi *et al*. (2004), Braby *et al*. (2007) and Fujiwara *et al*. (2007) have all reported them at whale falls.

There was an absence of the bone-eating genus *Osedax* on the bone deployments at both sites. *Osedax* has so far not been found in the Indian Ocean. The bones were down for approximately two years, well above the length of time needed for *Osedax* to colonise bone (Braby *et al.*, 2007). The depths and temperatures of the deployments are within the range for which *Osedax* has been previously found in (Fujikura *et al.*, 2006; Braby *et al.*, 2007; Schander *et al.*, 2010). Knowledge of cetaceans in the SWIR area is sparse but it is thought that this may be an important migratory area between high-latitude feeding grounds and low-latitude breeding grounds off Madagascar for many species. There have been reports of pilot whales, humpback whales and sperm whales in the areas of deep-water fishing in the Southern Indian Ocean although it is not clear where these were (Shotton, 2006; Rogers *et al.*, 2009). As a result, there should be whale carcasses reaching the seabed in the SWIR area. Therefore time, depth, temperature and lack of regular substrate were not limiting factors in *Osedax* colonisation. Possibilities to explain the absence include limitations on larval settlement at such small 'bone targets' or isolation by distance for the depth that the whale bones were emplaced at. However, unidentified *Osedax* have been observed on extremely small habitats (discarded galley waste bones) in the west Pacific (Vrijenhoek *et al.*, 2008a) and recorded from relatively small (compared to an entire whale) lander deployments in the Antarctic (Glover *et al.*, 2013). Their absence thus remains a mystery for the time being.

4.5 Conclusions

This study has not only helped to clarify the little known fauna colonising organic falls in the Southwest Indian Ocean but also those inhabiting seamounts in this area and the degrees of species overlap and hence presumed connectivity between them. This study also reiterates that there are many more species unknown to science, even in accessible depths of the ocean. The importance of replicate sampling and exploration of these unknown regions is highlighted, especially for scientists to gain a full understanding of ecosystem functioning. Without this basic information, scientists cannot effectively put conservation measures into place.

5. Ecosystem function of the wood-boring genus, *Xylophaga* (Pholadidae, Bivalvia) revealed by x-ray micro-computed tomography

For hundreds of years, humans have known that 'shipworms' bore into wood in the marine environment. More recently, it has been discovered that wood remains often end up in the deep sea, where they form ephemeral chemosynthetic habitats for a variety of fauna to feed upon. *Xylophaga* bivalves are specialist organisms that bore into and consume the wood but very little is known about the nature of the borings, the comparative abundances and population size structures of the species, their rates of growth and most importantly the consumption rates of the wood. To investigate this, three different sets of experimental wood packages were deployed and retrieved: two sets from two seamount sites on the Southwest Indian Ridge (732-750 m) and the other from the Mid-Cayman Spreading Centre in the Caribbean (4773 m). Wood samples deployed at 500 m in the Tongue of the Ocean, Bahamas, and discussed by Tyler *et al.* (2007), were also used in this study. These wood samples were scanned using X-ray micro-computed tomography (Micro-CT). The wood at each deployment site was colonised by a different species of *Xylophaga*. Using the Micro-CT images, it was possible to investigate the morphology of intact *Xylophaga* borings for the first time. It was also possible to gain quantitative data on the rates of degradation of the wood by the different species of *Xylophaga*. The roles of different deployment sites, deployment depths, species of *Xylophaga*, wood types and deployment lengths were also investigated. This novel analysis has given insight into the importance of the genus *Xylophaga* with regard to wood remineralisation in the deep sea and its role as an ecosystem engineer.

5.1 Introduction

With the exception of areas of local production such as hydrothermal vents, the deep seafloor is sustained by a steady rain of organic materials from the upper ocean. Whilst the majority of this material is marine in origin, terrestrially-derived substances such as wood can form major inputs particularly at bathyal depths close to forested regions or major river systems. Upon reaching the deep seafloor, wood creates ephemeral patchy habitats that host distinct assemblages of fauna from the majority of the surrounding seafloor (Turner, 1973). These fauna colonise and congregate around the organic enrichment caused by the wood substrates, using them as a food source, substratum and shelter (Turner, 1973, 1977; Wolff, 1979). Wood falls also have the ability to support chemosynthetic animals that are dependent on sulfide-rich conditions created by anoxia from organic loading of the immediate sediments (Duperron *et al.*, 2008; Bernardino *et al.*, 2010; Bienhold *et al.*, 2013). The anaerobic degradation of sunken wood supports chemosynthetic communities in the same way as the bacterial decomposition of lipids from whale bones in the deep sea (Leschine, 1995; Duperron *et al.*, 2008; Laurent *et al.*, 2009; Fagervold *et al.*, 2012; Bienhold *et al.*, 2013). Globally, the significance of wood falls to the overall energy budget of the deep-sea environment and carbon mineralisation on the seafloor is still obscure (Gage, 2003).

There is little information on the quantities of terrestrial plant matter entering the oceans worldwide but it must have been in sufficient amounts to allow for the evolution of a diverse obligate genus of deep-sea wood-boring molluscs: *Xylophaga* Turton, 1822 (Turner, 1955; Knudsen, 1961). There are more than 50 known species of *Xylophaga* (Pholadidae, Bivalvia) from around the world at depths varying from 0-7250 m (Knudsen, 1961; Turner, 2002; Voight, 2008; Voight and Segonzac, 2012). These ubiquitous opportunists bore into the wood using the toothed-ridged anterior edge of their shells as rasps as well as the mesoplax (a pair of accessory plates which partially covers the hinge of the shell) (Knudsen, 1961; Turner, 1973; Romey *et al.*, 1994; Distel and Roberts, 1997). They are then able to ingest the wood particles and store them in a caecum, before passing them through the stomach and gut where they are consumed with the aid of symbiotic bacteria (Distel and Roberts, 1997). It is expected that some of the symbiotic bacteria cultivated by *Xylophaga* will be capable of synthesising enzymes such as cellulases that can aid in the digestion of wood as has been seen in teredinids (Yang *et al.*, 2009). Wood not only provides *Xylophaga* with nutrition but also shelter (Distel and Roberts, 1997).

Xylophaga is the most important genus involved in converting the energy in relatively refractory deep-sea wood and other plant material into a more accessible form, e.g. faecal matter for detritus feeders and tissue for predators and scavengers (Turner, 1973, 2002; Bienhold *et al.*,

2013). These bivalves are also responsible for helping to create the sulfidic conditions in the sediment that attract chemosynthetic fauna, by producing large amounts of faecal pellets (Bienhold *et al.*, 2013). *Xylophaga* at wood falls are analogous to *Osedax* at whale falls in that they breakdown and aid in the remineralisation of the hard substrate at these habitats while also using them as a source of food (Rouse *et al.*, 2004; Vrijenhoek *et al.*, 2008a).

Despite the ubiquitous nature of this genus at wood falls, very little is still known about their ecology and life history. In fact, for many of the species, the morphological description serves as the only record known thus far. As a result, information is lacking about measurements such as growth rates where only a few studies have been done (Romey *et al.*, 1994; Harvey, 1996; Tyler *et al.*, 2007; Voight and Segonzac, 2012; Romano *et al.*, In press). There have been no studies on the rates of wood degradation by *Xylophaga*.

One of the similarities between *Osedax* and *Xylophaga* is the boring activity in a hard-substrate that also provides nutrition via an endosymbiotic pathway. There are also similarities in the difficulty of observation of the nature of these borings, as to remove the animals necessitates physically destroying the borings. Several recent studies have used the technique of micro-computed tomography (Micro-CT) to study the nature of *Osedax* borings in modern whale bones recovered from the seafloor (Higgs *et al.*, 2010; Higgs *et al.*, 2011a) and also in apparently fossilised *Osedax* borings from the Pliocene (Higgs *et al.*, 2011c) and Oligocene (Kiel *et al.*, 2010). The method has allowed a far more detailed understanding of the 3-D morphology of the borings as well as permitting quantitative data to be collected on rates of bone degradation as the volume of borings can be measured. In wood-boring molluscs, the x-ray method was first pioneered in the 1950s in studies of *Teredo* shipworms with a view to improve the understanding of the nature of their attack on ships and jetties (Crisp *et al.*, 1953). Stereo x-ray photographs have been quite frequently used in studies of shallow-water *Teredo* but only rarely in studies of deep-sea *Xylophaga* (Tyler *et al.*, 2007) (Fig. 5.1). However, even stereo x-ray photographs provide very poor information on the 3-D nature of the borings and their volume in comparison with Micro-CT (Fig. 5.1).

In this study, we make the first comprehensive Micro-CT based investigation of *Xylophaga* borings in four species from two ocean basins. We investigate the 3-D nature of *Xylophaga* borings and visualise the preserved animals *in situ* while gaining quantitative measurements of the growth rates and the rates of degradation of wood by this wood-boring genus. We will test the hypothesis that different species found in varying locations have different growth rates. Wood-degradation rates of *Xylophaga* are also examined for the first time and it is hypothesised that there will be variance between wood types, species of *Xylophaga* and location.

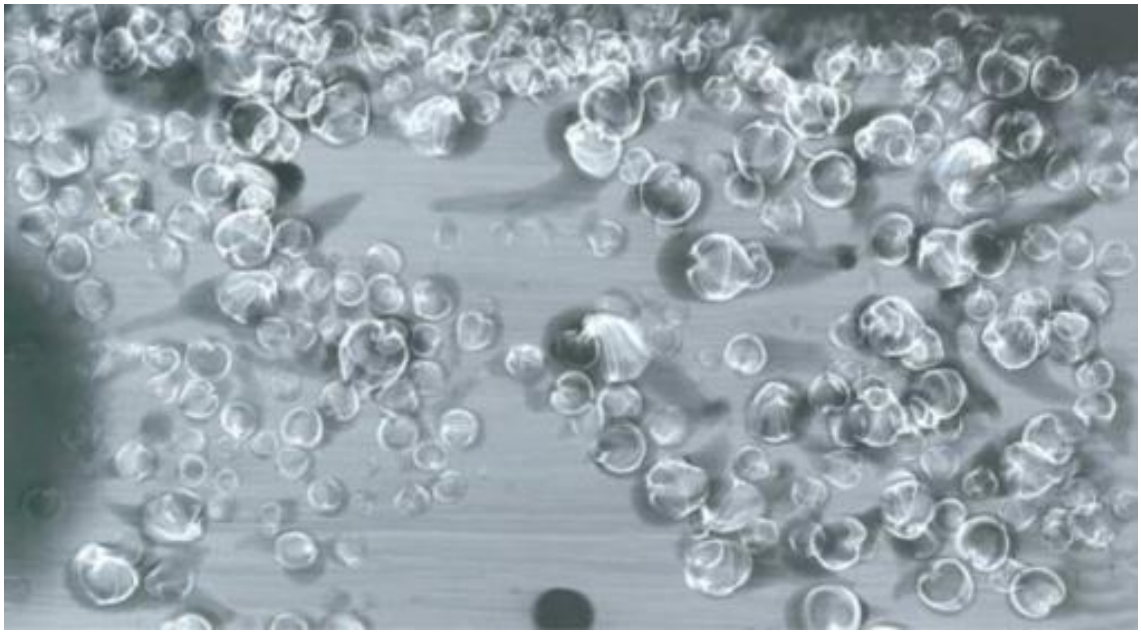

Figure 5.1 X-radiographs of *Xylophaga depalmai* in wood. The calcified shells of *X. depalmai* individuals can be seen in white. Very few borings can be seen effectively. This figure is reproduced with changes from Tyler *et al*. (2007).

5.2 Methods

5.2.1 Experimental deployments and recoveries

Wood samples from three locations were analysed during this study: the Southwest Indian Ridge (SWIR) off the southeast coast of South Africa, the Mid-Cayman Spreading Centre (MCSC) in the Caribbean and the Tongue of the Ocean (TOTO) in the Bahamas, Caribbean. Details of each experimental locality can be found in Table 5.1. Full experimental protocols for the deployments on the SWIR and TOTO can be found in Chapter 4 and Tyler *et al.* (2007) respectively. The deployments from TOTO were kindly donated to the Natural History Museum, London by Professor Paul Tyler. They were contained in buckets in 99% Isopropyl Methylated Spirits (IMS). The SWIR deployments were frozen to -20°C. Temperature was also collected at each deployment site (Table 5.1).

The wood package deployed in the MCSC in the Caribbean was in close proximity to the Beebe Hydrothermal Vent Field at 4773 m. A block of three planks of pinewood was affixed (20 m off the seafloor) to a mooring that also had oceanographic instruments and a whale-bone deployment attached. The mooring was recovered during the 374th voyage of the *RRS Discovery* three hours after the release was triggered from on board the vessel. Fauna may have been lost from this wood deployment during the time taken for the mooring to travel to the surface and be recovered. Once on deck, the wood was transferred into buckets of chilled water and

photographed. One *Xylophaga* boring was located, which was sawed free from the rest of the deployment, photographed and preserved in 100% ethanol.

When samples arrived back at the Natural History Museum, London, the morphology of *Xylophaga* from each wood location was examined in detail to try and identify each species. Light microscopy was undertaken for every specimen using a Zeiss V.20 stereomicroscope with AxioCam camera.

5.2.2 Micro-computed tomography and the analyses of scans

Subsamples were sawn from three pieces of wood from each of the two SWIR locations as well as from the deployment from the MCSC. These were taken to allow the samples to fit into the scanner. Volume measures for each block can be seen in Table 5.2. Six whole blocks from the TOTO samples were chosen for scanning. The 13 samples (Tables 5.1 and 5.2) were scanned using the Nikon Metrology X-Tek HMX ST-225 Micro-CT scanner at the Imaging and Analysis Centre at the Natural History Museum in London. This system is equipped with a detector panel (20 x 20 cm) with a maximum resolution of 5 μm/pixel and a maximum energy of 225 kV. A tungsten target source and a scanning medium of air were used for each scan of the 13 samples. Individual scanning parameters can be found in Table 5.2. Images acquired during the scanning process were subsequently reconstructed using the software CT-Pro, which employs a modified version of the back-projection algorithm created by Feldkamp *et al.* (1984). This enabled the production of a volume image file that can be opened in VG Studio Max 2.0 (Volume Graphic GmbH, Heidelberg, Germany) for each sample. This program allowed the creation of a stack of greyscale bitmap images, which were then imported into the Drishti software suite (Ajay Limaye, *Drishti - Volume Exploration and Presentation Tool,* Poster presentation, Vis 2006, Baltimore).

All work in Drishti had to be completed on a computer with the following specifications: an Intel Xeon 2.3 GHz 16 core dual processor, 128 GB RAM and a Nvidia Quadro K5000 4 GB graphics card. Despite these specifications, the data sets for each scan were extremely large and this slowed processing time significantly. In Drishti, different transfer functions were created for *Xylophaga* shell, wood and air using the density of each. Manual defining of each transfer function using the 'mopcarve' function in Drishti was needed to remove artifacts created during the scanning process, and also all the air surrounding the wood during each scan. The specimens of *Xylophaga depalmai* were not imaged effectively; this may have been as a result of the shells degrading significantly in the IMS since sampling in 1993/4. *Xylophaga-depalmai* individual diameters in each piece of wood were supplied by Professor Paul Tyler from the study published as Tyler *et al*. (2007). Volume measurements of the wood, air in borings, as well as

diameters of every individual *Xylophaga* shell in each sample were measured using the 'get volume' and 'path length' function in Drishti (Tables 5.2, 5.3 and 5.4). Numbers of *Xylophaga* measured per region are also in Table 5.3. *Xylophaga* individual volumes were calculated manually from the diameters assuming that they were spherical. There were also some technical issues in estimating the volume of wood bored in the samples from Atlantis Bank, SWIR. This is discussed further in the Results section of this chapter. In total, the entire Micro-CT process (scanning and post-scan processing) for all samples took 320 hours approximately.

All statistical analyses were performed in SPSS Statistics v. 20. Non-parametric tests were used to measure significant differences between *Xylophaga* abundances, sizes and growth rates for each species. The percentage of total wood volume bored, *Xylophaga* population rates of wood degradation and individual *Xylophaga* rates of wood degradation for each species were also compared.

Table 5.1 Experimental localities and details of all CT-scanned wood. Abbreviations represent Southwest Indian Ocean Ridge (SWIR) and Mid-Cayman Spreading Centre (MCSC).

Location	Latitude	Longitude	Depth (m)	No. of samples	Type of wood	Deployed	Recovered	Days on seafloor	Height above seafloor	Temperature at the deployment site (°C)
Atlantis Bank, SWIR	32°42.71'S	57°16.31'E	750	3	Mango	18.11.09	14.12.11	756	<1 m	10.1
Coral Seamount, SWIR	41°22.38'S	42°54.64'E	732	3	Mango	04.12.09	20.11.11	716	<1 m	4.3
Beebe Vent Field, MCSC	18°22.57'N	81°40.34'W	4773	1	Pine	15.04.10	18.03.12	703	20 m	4.4
Tongue of the Ocean, Bahamas	25°52.88'N	77°32.24'W	480-520	2	Oak	06.02.93	07.08.93	182	<1 m	10.0-13.0
	26°52.88'N	78°32.24'W	480-520	2	Spruce	06.02.93	07.08.93	182	<1 m	10.0-13.0
	27°52.88'N	79°32.24'W	480-520	2	Spruce	04.11.93	11.04.94	158	<1 m	10.0-13.0

Table 5.2 Micro-CT scanning parameters for all wood samples.

Sample	Sample code	Voltage (kV)	Amps (µA)	Exposure time per image (ms)	Number of projection images	Resolution (µm per pixel)	Total volume of sample (mm^3)
Atlantis Bank, SWIR	SWR06	180	200	500	3142	0.0762	947145
	SWR24	180	200	500	3142	0.0860	637386
	SWR37	180	200	500	3142	0.0913	860650
Coral Seamount, SWIR	SWR55	180	200	500	3142	0.0898	130213
	SWR56	180	200	500	3142	0.1006	998641
	SWR64	180	200	500	3142	0.0742	387103
Beebe Vent Field, MCSC	CAYMAN1	180	200	500	3142	0.0456	3181
TOTO, Bahamas	1STOAKFEBAUG93_1	190	200	500	3142	0.1152	546046
	1STOAKFEBAUG93_2	190	200	500	3142	0.1152	484910
	2NDSPRUCEFEBAUG93_1	180	200	500	3142	0.1136	506524
	2NDSPRUCEFEBAUG93_2	180	200	500	3142	0.1136	421234
	2NDSPRUCENOV93APR94_1	180	200	500	3142	0.1136	337024
	2NDSPRUCENOV93APR94_2	180	200	500	3142	0.1136	373467

5.3 Results

5.3.1 Colonising *Xylophaga* species and morphology of borings

The wood packages from the two localities on the SWIR were colonised by two different species of *Xylophaga*: at Coral Seamount *Xylophaga murrayi* Knudsen 1967 was recorded (Figs. 5.2a and 5.2e, and Table 5.3), whilst at Atlantis Bank the species was *X.* cf. *indica* Smith 1904 (Figs. 5.2d, 5.2h and 5.4, and Table 5.3). The wood from the TOTO experiments was colonised by *X. depalmai* Turner, 2002 (Tyler *et al.*, 2007)(Figs. 5.2b and 5.2f, and Table 5.3), and the wood from the MCSC experiments was colonised by one unidentified specimen of *Xylophaga* sp. (Figs. 5.2c and 5.2g, and Table 5.3). It was not possible to gain a positive identification for this species, as there were no soft parts present. The *Xylophaga* sp. collected from the wood in the MCSC was not included in any of the comparisons that consider the entire population as only one specimen was collected.

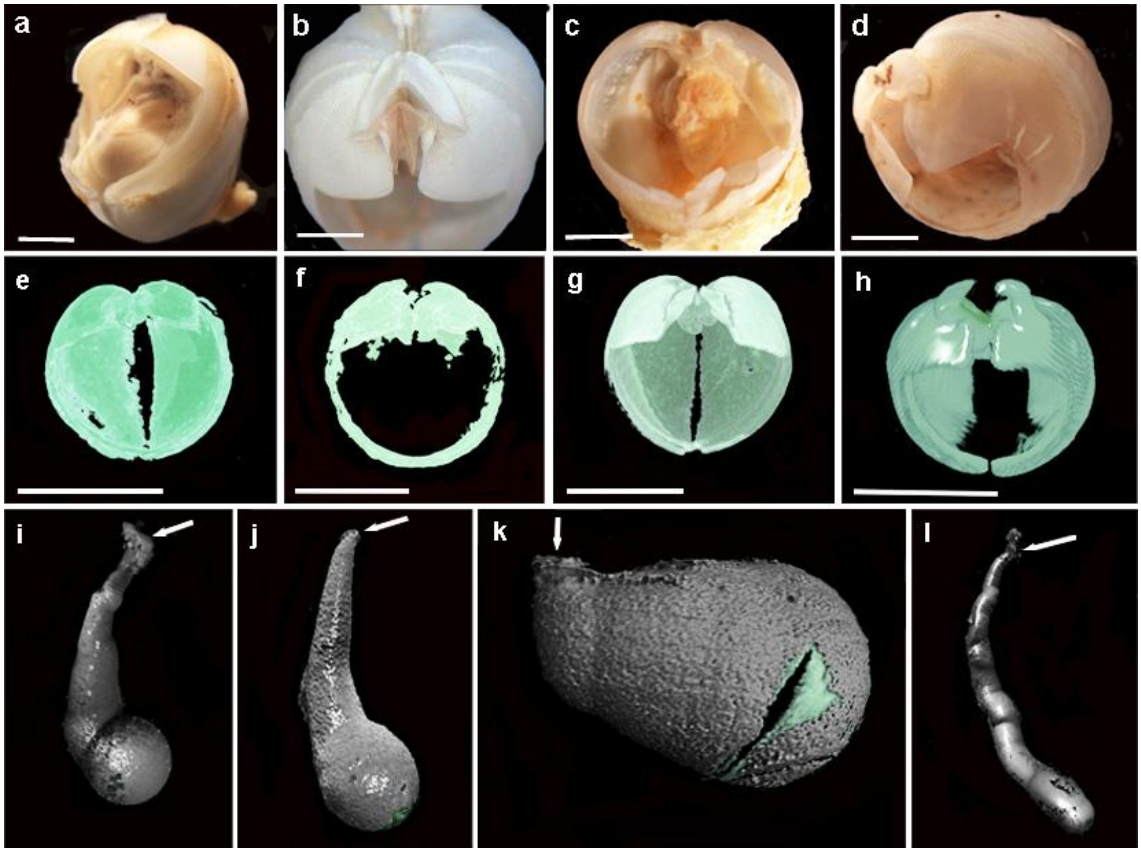

Figure 5.2 Colonising *Xylophaga* species and their typical boring morphology.(a) *Xylophaga murrayi* imaged using light microscopy; (b) *Xylophaga depalmai* imaged using light microscopy; (c) *Xylophaga* sp. imaged using light microscopy; (d) *Xylophaga* cf. *indica* imaged using light microscopy; (e) *Xylophaga murrayi* imaged using Micro-CT; (f) *Xylophaga depalmai* imaged using Micro-CT; (g) *Xylophaga* sp. imaged using Micro-CT; (h) *Xylophaga* cf. *indica* imaged using Micro-CT; (i) a *Xylophaga murrayi* boring imaged using Micro-CT; (j) *Xylophaga depalmai* boring imaged using Micro-CT; (k) *Xylophaga* sp. boring imaged using Micro-CT; (l) *Xylophaga* cf. *indica* boring imaged using Micro-CT. The transfer functions for *Xylophaga* shells and air can be seen in images (e-h) and (i-l)

respectively. The individual *Xylophaga* would be better resolved if smaller scans were done. The *Xylophaga depalmai* specimen in (f) has not been imaged properly (only the densest parts of the shell can be seen) and this may be as a result of shell degradation over time in IMS. All four boring openings are indicated by arrows. The shells of *Xylophaga* in each boring in (i-l) are indicated by a green colour. Scale bars for (a-d) are 2 mm (e-k) are 5 mm, (l) is 20 mm.

Micro-CT revealed a characteristic 3-D shape of *Xylophaga* borings in wood, which could be described as akin to a 'Prince Rupert's drop', or an elongated teardrop, so named for the formation created by liquid glass dropped into water (Figs. 5.2i-l and 5.3). We also observed some variation between species. *Xylophaga murrayi* and *X. depalmai* both have Prince-Rupert's-drop borings but *X. depalmai's* was larger (Figs. 5.2i and 5.2j). The unidentified *Xylophaga.* sp from the MCSC has a much shorter drop length (Fig. 5.2k) whilst the boring of *X.* cf. *indica* has a very elongated Prince-Rupert-drop shape quite distinguishable from the other three species (Fig. 5.2l) (Table 5.4). The borings of *Xylophaga* are very different to the borings created by terrestrial insects in wood; this is shown in Figure 5.8, where a reticulate network of thin tubes of generally equal width (not tapering to a point) is revealed by the Micro-CT, hypothesised to be created when the wood was on land prior to deployment.

Figure 5.3 A Prince Rupert's drop. This structure is made from heating glass and allowing it to fall into water where it cools rapidly. This image is taken from the website of the Physics Department at the University of Virginia, USA.

The boring shapes for *Xylophaga depalmai* did not differ between spruce and oak. A typical boring produced by *X. murrayi* had a volume of 392.6 mm^3 and a length of 15.4 mm. For *X.* cf. *indica*, a typical volume was 2686.2 mm^3 and a length of 114 mm. For *X. depalmai*, a typical volume was 617.2 mm^3 and a length of 27.6 mm. For *Xylophaga* sp., a typical volume of 231.0 mm^3 and a length of 12.1 mm (Figs. 5.2i-l and Table 5.4) was observed. All four of the boring shapes have boreholes less than 1.0 mm in diameter, which lead into a boring that is much wider (this width depending on the size of the individual within the boring) (Figs. 5.2i-l). The

borings in each piece of wood from the SWIR deployments were very similar in orientation; most originated from a similar area and extended in a similar direction (Figs. 5.8 and 5.9). This may have been as a result of the wood being colonised more heavily on the sides that were exposed to bottom water rather than the seabed. *Xylophaga* individuals made an active effort to keep their individual borings separate and prevent burrows joining, unlike the terrestrial wood infestation (Fig. 5.8). *Xylophaga* specimens within the borings did not appear to orientate themselves in similar ways as could be seen with *X. indica* in Figure 5.4.

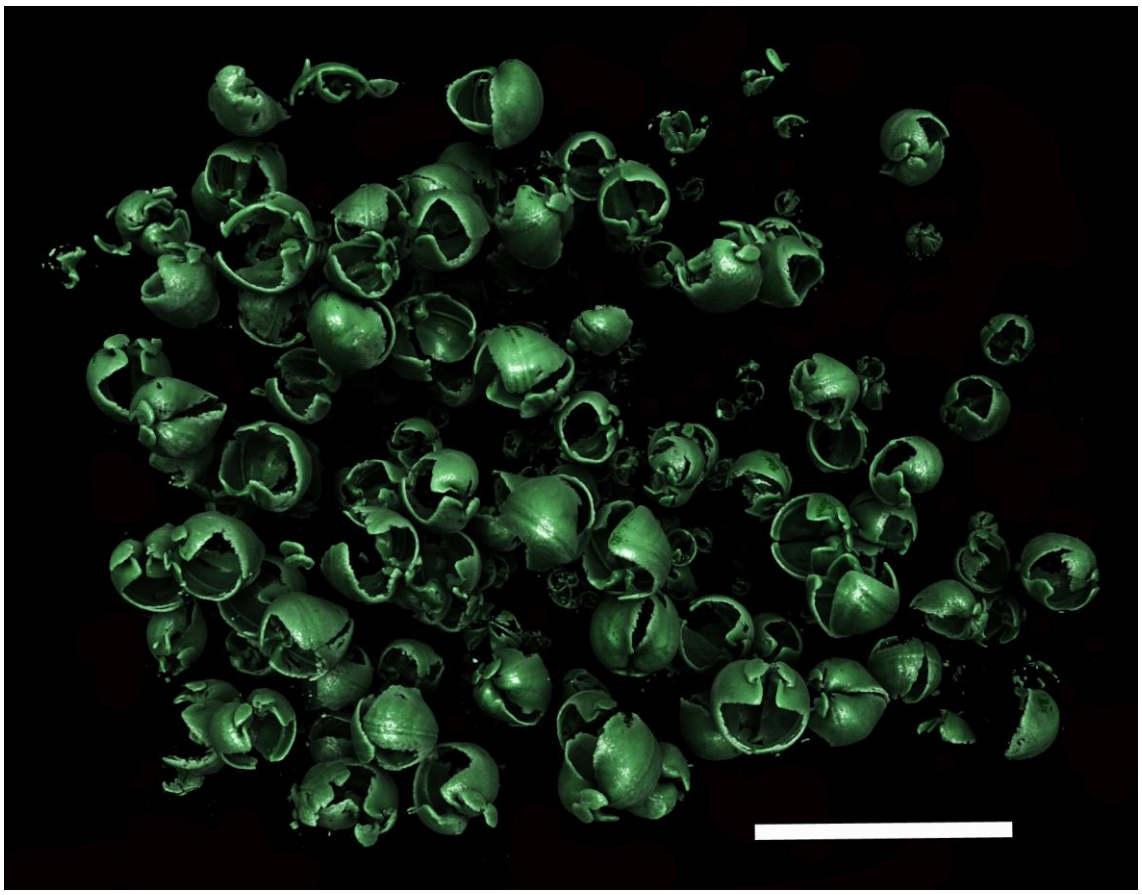

Figure 5.4 *Xylophaga* cf. *indica* population *in situ*. Only the transfer function for *Xylophaga* shells can be seen in this image. Scale bar is 30 mm.

5.3.2 Abundance and population size structure

Abundances of *Xylophaga* present in the 12 scanned pieces of wood from the Bahamas and SWIR were significantly different from one another (H=9.346, p=0.009) (Table 5.3). *Xylophaga murrayi* from the Coral Seamount (SWIR) had the lowest mean abundance with approximately 117±99.0 individuals dm^{-3} and *X.* cf. *indica* from the Atlantis Bank (SWIR) had 300±114.3 individuals dm^{-3} (Fig. 5.5b and Table 5.3). *X. depalmai* in oak from TOTO (Bahamas) had 1446±34.8 individuals dm^{-3}, while those in spruce had the highest mean abundance,

1484.5±259.8 individuals dm^{-3} (Fig. 5.5b and Table 5.3). There was no significant difference between the TOTO sample sets.

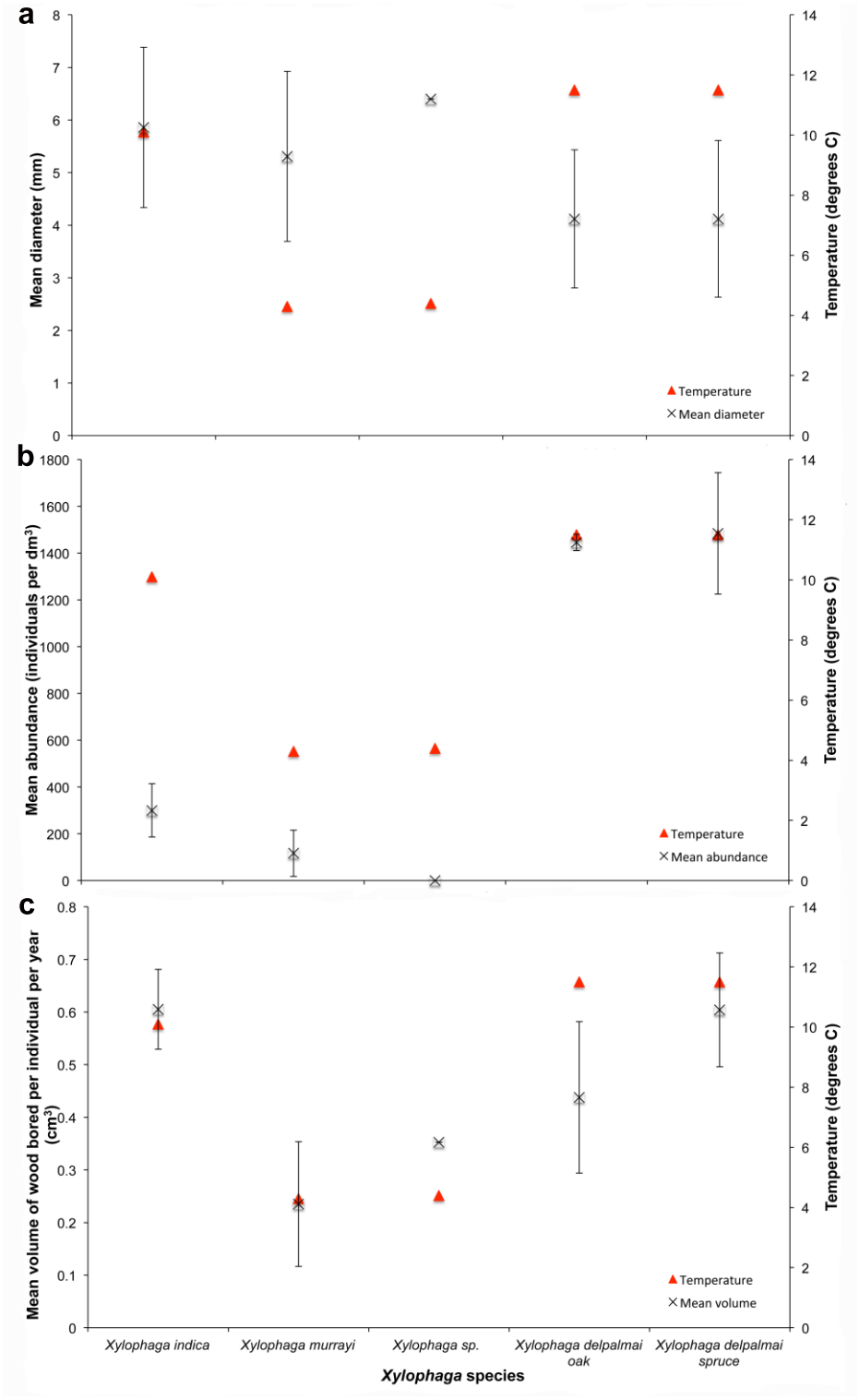

Figure 5.5 *Xylophaga* ecological statistics compared with the water temperature at each site. (a) Mean diameter of each *Xylophaga* species plotted with the water temperatures at the sites where each was collected. (b) Mean abundance of each *Xylophaga* species plotted with the water temperatures at the sites where each was collected. (c) Mean volume of wood bored by an individual of each *Xylophaga* species in a year, plotted with the water temperatures at the sites where each was collected.

There was a significant difference in the mean sizes of the four different species of *Xylophaga* from all 13 scanned pieces of wood (H=9.703, p=0.021) but not between the populations of *Xylophaga depalmai* in different types of wood (Fig. 5.5a and Table 5.3). The mean diameter of *X. murrayi* recorded was 5.31±1.6 mm, *X.* cf. *indica* was 5.86±1.5 mm and *X. depalmai* had a mean diameters of 4.12 mm in oak (±1.3 mm) and spruce (±1.5 mm) (Fig. 5.5 and Table 5.3). The only specimen of *Xylophaga* sp. found in the wood from the MCSC had a diameter of 6.40 mm (Table 5.3). The modal diameters differed slightly from the mean; 4.86 mm for *X. murrayi*, 5.13 mm for *X.* cf. *indica*, and *X. depalmai* individuals had modal diameters of 2.26 mm for oak deployed for 182 days, 4.78 mm for spruce deployed for 182 days and 3.97 mm for spruce deployed for 158 days (Fig. 5.6 and Table 5.3). The population structures of *X.* cf. *indica* and *X. depalmai* were unimodal suggesting that only one recruitment event had occurred and was not continuous (Fig. 5.6). However, for *X. murrayi*, some evidence of a bimodal distribution was observed in 2 of the 3 samples (Fig. 5.6a), but this cannot be considered strong evidence for more than one recruitment event.

5.3.3 Growth rates

The minimum growth rates of the four *Xylophaga* species were calculated based on the assumption that settlement occurred on the first day of deployment. Whilst this is unlikely to be the case, it does provide a minimum growth rate that can be compared across the samples. For *Xylophaga murrayi*, evidence of a bimodal size structure creates the possibility that more than one recruitment event has occurred, and as such it is unlikely that we can really consider these figures even a minimum growth rate for that species. Statistically, growth rates of each species were significantly different (H=9.791, p=0.020) (Table 5.3). *X. murrayi* had a mean minimum growth rate of 0.007±0.016 mm d^{-1}, *X.* cf. *indica* had a mean minimum growth rate of 0.008±0.008 mm d^{-1} and the *X. depalmai* populations had a much faster overall mean minimum growth rate of 0.024 mm d^{-1} (Table 5.3). The *X. depalmai* populations in oak for 182 days had a mean minimum growth rate of 0.023±0.007 mm d^{-1}, the *X. depalmai* in spruce for 182 days had a mean minimum growth rate of 0.022±0.009 mm d^{-1} and the *X. depalmai* populations in spruce for 158 days had a mean minimum growth rate of 0.026±0.007 mm d^{-1} (Table 5.3). There was no significant difference in minimum growth rates of *X. depalmai* populations with varying deployment times or wood type. *Xylophaga* sp. from the MCSC at 4773 m depth had an estimated minimum growth rate of 0.009 mm d^{-1} based on a single observation (Table 5.3).

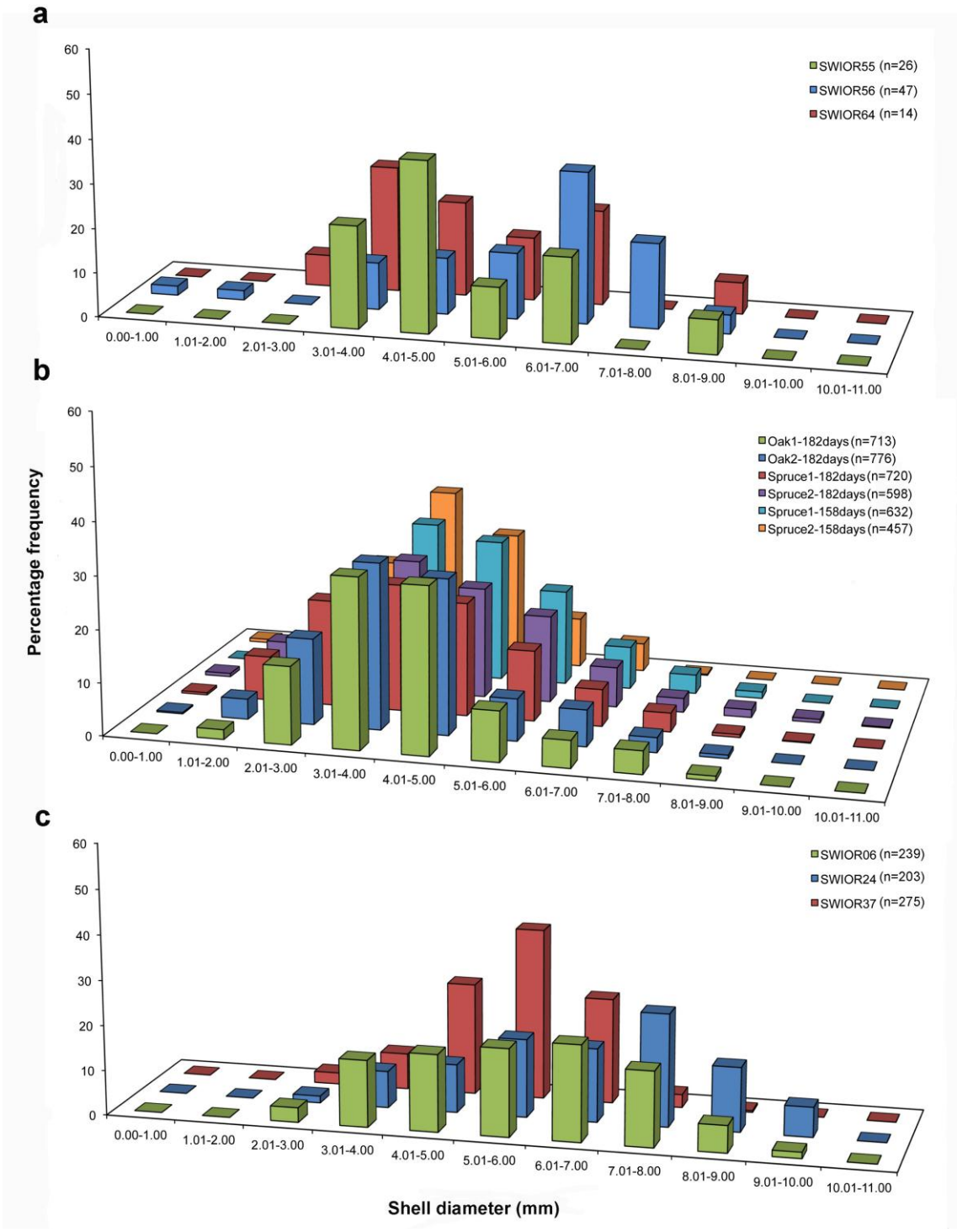

Figure 5.6 Population structure of three *Xylophaga* species. (a) Size class graph for *Xylophaga murrayi*; (b) *Xylophaga depalmai*; (c) *Xylophaga* cf. *indica*. The three samples displayed in (a) and (c) are replicates whereas the six samples in (b) have had variables altered; samples in (b) are labelled according to wood type (oak or spruce) and the deployment length (182 days or 158 days). The total population sizes in the scanned pieces of wood for each species are indicated as n.

5.3.4 Wood consumption rates

We used the Micro-CT data to measure the percentage volumes of the borings of an entire *Xylophaga* population in the blocks of wood. That is, the amount of wood consumed by the animals during the experimental period. The wood bored by *Xylophaga murrayi* at Coral Seamount, SWIR, was the least bored, ranging from 1.43% to 12.62% (Table 5.4). Atlantis Bank, SWIR, *X.* cf. *indica* consumed from 31.16% to 46.25% of the wood, and at TOTO, *X. depalmai* consumed from 24.65% to 50.03% (Table 5.4). The percentage volumes of each block that was bored were significantly different between the three different species, *X. murrayi, X.* cf. *indica* and *X. depalmai* (H=6.231, p=0.044). Examples of this can be seen in Figures 5.7, 5.8 and 5.9. After observing the wood visually and confirming with the scans, it was felt that the percentages of wood bored by *X.* cf. *indica* were underestimated. The wood bored by *X.* cf. *indica* was so heavily bored that the blocks were structurally compromised and could be crushed by hand whereas the wood bored by *X. depalmai* had many boreholes covering the surfaces but were still solid. The wood bored by *X. murrayi* and *Xylophaga* sp. (MSCS) were largely intact, yet *X.* cf. *indica* and *X. depalmai* recorded similar percentage volumes. This would indicate that the percentage volume bored by *X.* cf. *indica* should be much higher than both *X. depalmai* and *X. murrayi*. This inaccuracy may have been caused by the inability of the Micro-CT scanner to distinguish between wood pulp within burrows created by the boring activity of *X.* cf. *indica* and solid unbored wood, resulting in much higher volumes of unbored wood being recorded. The borings created by *X. murrayi* and *X. depalmai* (shown as air) were well defined and did not contain bored wood pulp (Figs. 5.7 and 5.8), whereas those air spaces created by *X.* cf. *indica* seemed to be much more 'rugged' indicating the presence of small particles of wood pulp instead of the smooth boring edges (Fig. 5.9).

The maximum rate of wood degradation by a *Xylophaga murrayi* population was 6.57% per year of deployment (Table 5.4). This is equal to an approximate average value of 0.2351±0.1186 cm^3 being degraded per year per individual of *X. murrayi* (Fig. 5.5 and Table 5.4). The maximum rate of wood degradation by a *X.* cf. *indica* population was 22.26% per year of deployment (Table 5.4). This is equal to an approximate average value of 0.6052±0.0759 cm^3 being degraded per year per individual of *X.* cf. *indica* (Fig. 5.5 and Table 5.4), that is approximately 3x the rate of consumption by *X. murrayi.* These values for *X.* cf. *indica* are however, probably underestimates as per the limitation explained above. The mean rate of wood degradation per individual of *X. depalmai* per year was equal to an average value of 0.084% from oak deployed and 0.150% from spruce (Table 5.4). The maximum volume of wood that could be degraded in a year by the population of *X. depalmai* would be 103.66% or all the wood would be gone in a year (Table 5.4). This is equal to an approximate average value of 0.4380±0.1440 cm^3 being degraded per year per individual of *X. depalmai* in oak and

0.6040±0.108 cm^3 in spruce (Fig. 5.5 and Table 5.4). The rates of wood degradation for each block were significantly different between the three species populations (H=9.346, p=0.009) as well as the approximate rates of wood degradation for each individual *Xylophaga* for the three species (H=6.846, p=0.033).

Mean *Xylophaga* diameter, mean *Xylophaga* abundance and mean volume of wood bored per individual of *Xylophaga* per year for each species in this study were plotted with the temperatures at each sample site (Fig. 5.5). There did not appear to be any correlation between mean *Xylophaga* size and temperatures but mean *Xylophaga* abundance and mean volume of wood bored per individual of *Xylophaga* per year, those values correlated well (Fig. 5.5).

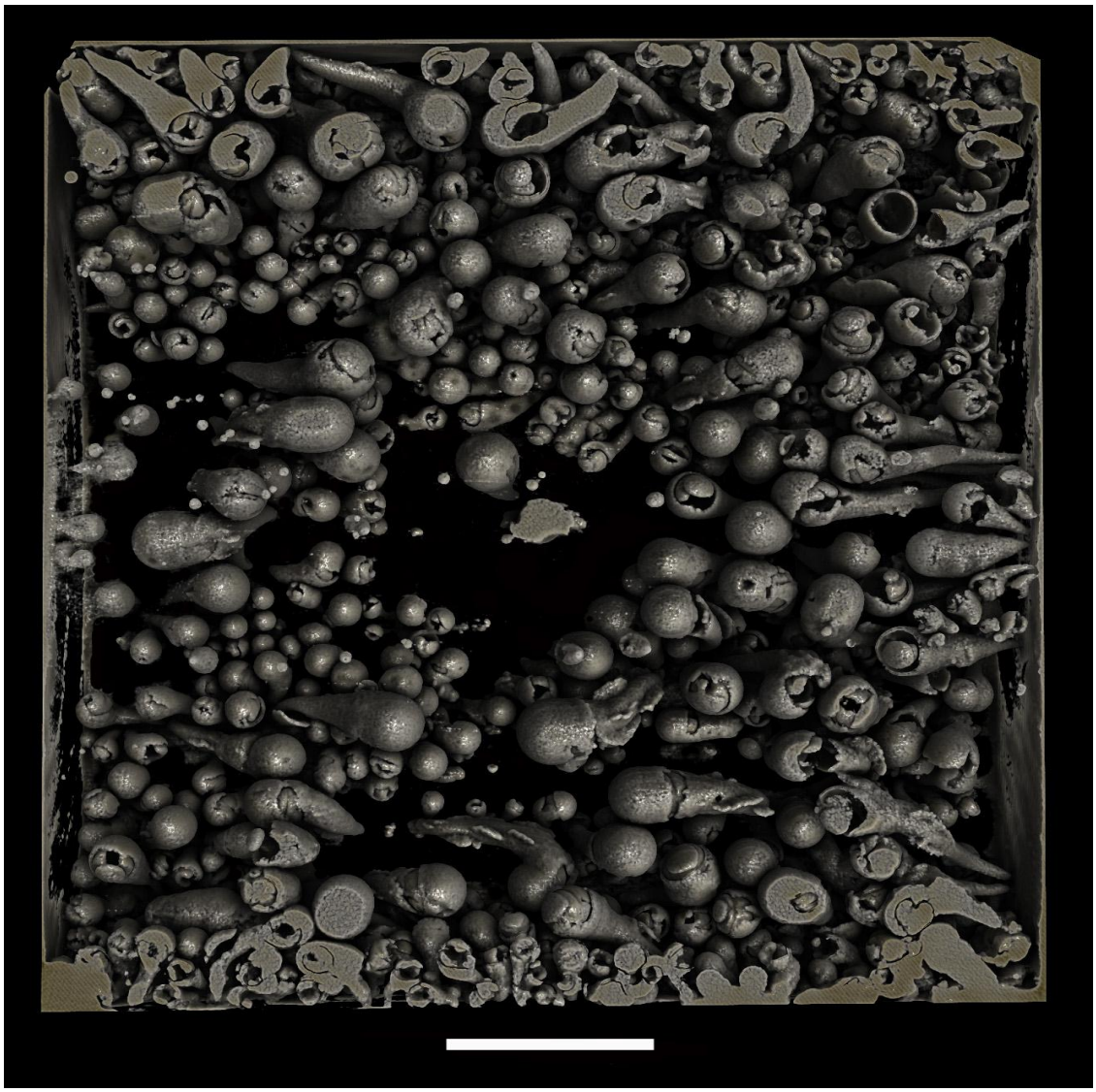

Figure 5.7 Borings of a population of *Xylophaga depalmai.* Only the transfer function for air can be seen in this image. Some of the air surrounding the piece of wood was purposefully left in to allow the reader to gauge the shape and size of the wood and the relative position of the borings within the wood. Scale bar is 30 mm.

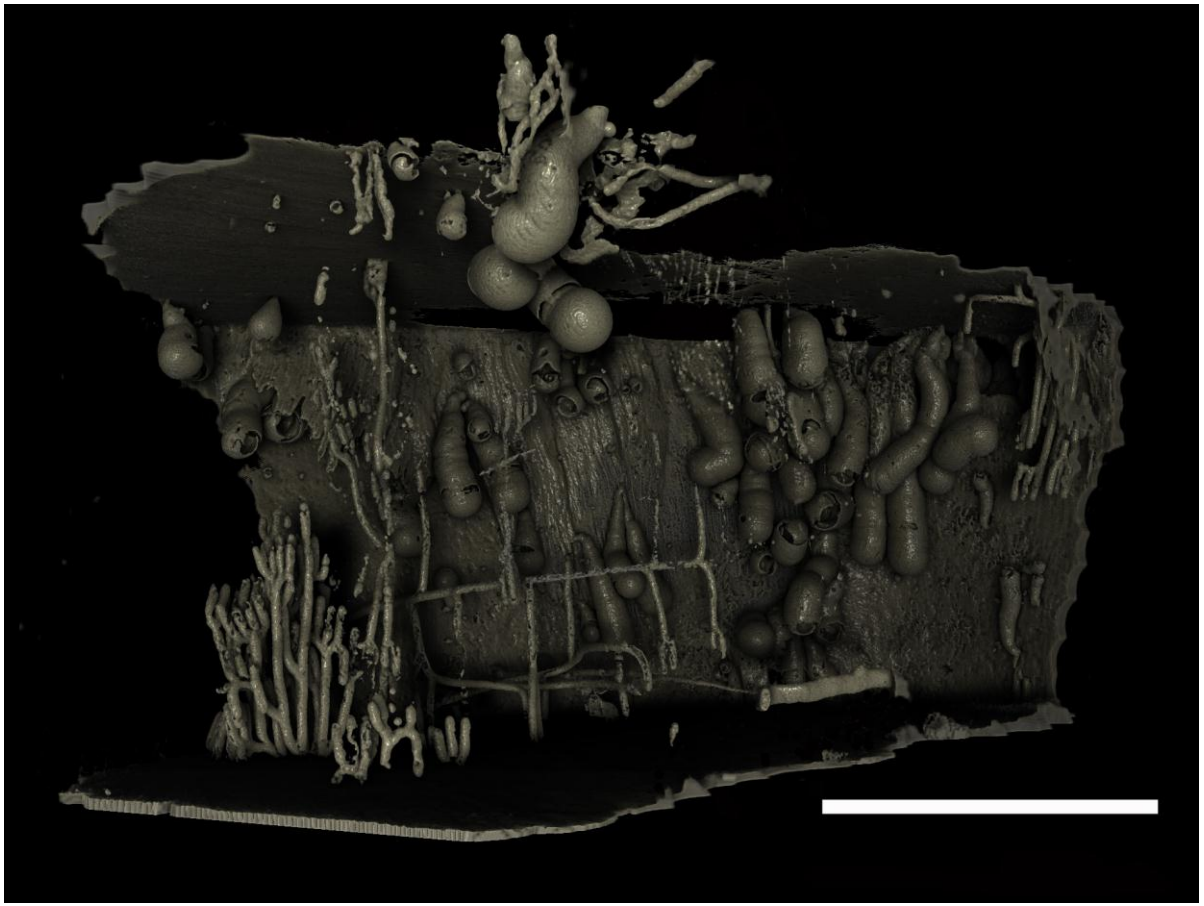

Figure 5.8 Borings of a population of *Xylophaga murrayi.* Only the transfer function for air can be seen. Some of the air surrounding the piece of wood was purposefully left in to allow the reader to gauge the shape and size of the wood and the relative position of the borings within the wood. The thin pipe-like boring network seen on the left was not created by *Xylophaga* and may belong to wood-boring terrestrial insects that attacked the wood when it was on land. Scale bar is 30 mm.

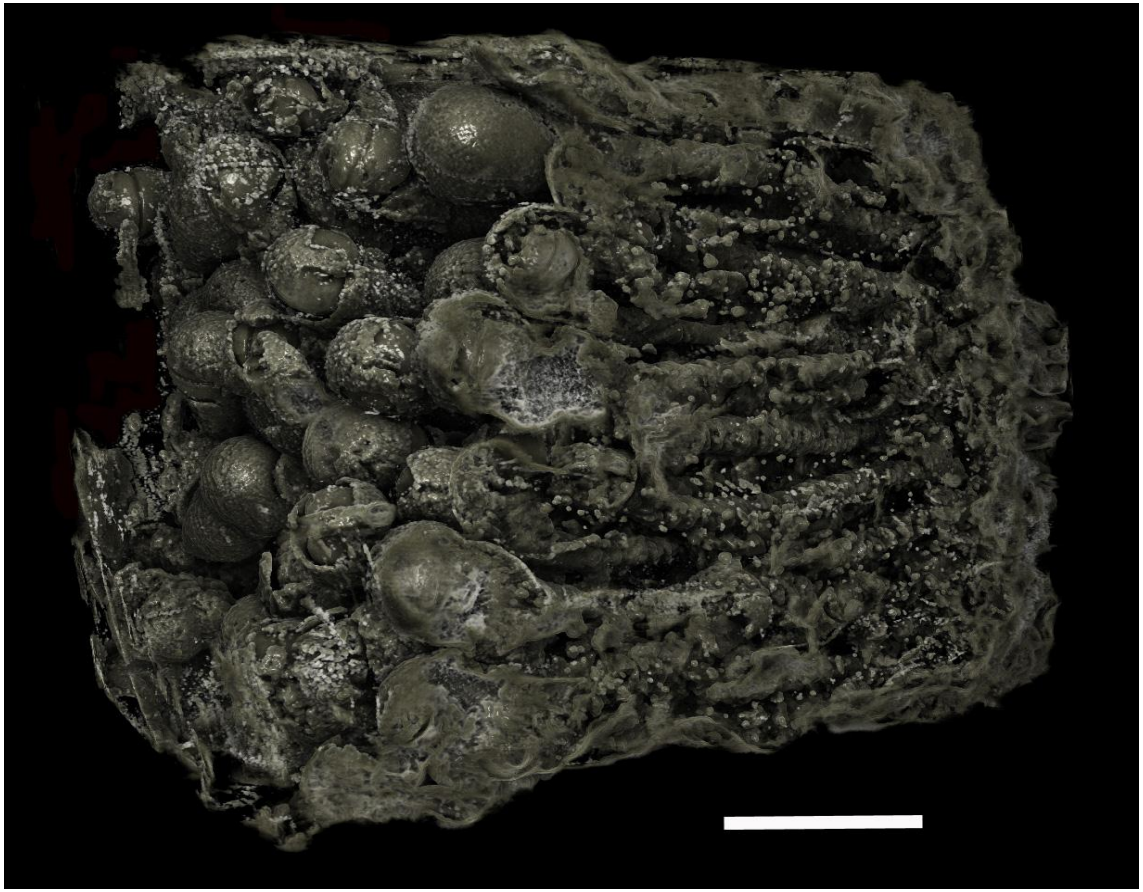

Figure 5.9 Borings of a population of *Xylophaga indica.* Only the transfer function for air can be seen in this image. These borings are not as easily distinguished, as the air in the borings was not well defined due to the wood being highly degraded to a pulp-like substance. Scale bar is 30 mm.

Table 5.3 Measurements of *Xylophaga* populations. Measurements with an asterisk (*) next to them denotes that those do not represent a mean measurement but rather are from the only specimen present in that sample. A dash (-) is placed where population measurements could not be made as only one individual was present.

Species	Wood	Days on seafloor	*n* (number of specimens measured)	Minimum diameter	Maximum diameter	Mean diameter	Modal diameter	Mean abundance (individuals dm^{-3})	Mean minimum growth rate
Xylophaga indica	Mango	756	697	2.04 mm	9.79 mm	5.86 mm	5.13 mm	300	0.008 mmd^{-1}
Xylophaga murrayi	Mango	716	87	0.94 mm	8.54 mm	5.31 mm	4.86 mm	117	0.007 mmd^{-1}
Xylophaga sp. (MSCS)	Pine	703	1	-	-	6.40 mm*	-	-	0.009 mmd^{-1}*
Xylophaga depalmai	Oak	182	1489	0.97 mm	8.80 mm	4.12 mm	2.26 mm	1446	0.023 mmd^{-1}
	Spruce	182	1318	0.28 mm	10.12 mm	4.11 mm	4.78 mm	1445	0.022 mmd^{-1}
	Spruce	158	1089	0.50 mm	8.56 mm	4.13 mm	3.97 mm	1524	0.026 mmd^{-1}

Table 5.4 Measurements of the boring sizes and *Xylophaga* boring rates. Measurements with an asterisk (*) next to them are underestimates.

Species	Wood	Days on seafloor	Shape of boring	*n* (number of measured blocks)	Individual boring volume	Individual boring length	Range of % wood bored by *Xylophaga* population during study	Maximum boring rate of *Xylophaga* populations (% of wood bored per year)	Individual mean boring rate (cm^3 of wood bored per year)
Xylophaga indica	Mango	756	'Prince Rupert's drop'	3	2686.2 mm^3	114.0 mm	31.16-46.25*	22*	0.605*
Xylophaga murrayi	Mango	716	'Prince Rupert's drop'	3	392.6 mm^3	15.4 mm	1.43-12.62	7	0.235
Xylophaga sp. (MCSC)	Pine	703	'Prince Rupert's drop'	1	231.0 mm^3	12.1 mm	-	-	-
Xylophaga depalmai	Oak	182	'Prince Rupert's drop'	2	617.2 mm^3	27.6 mm	24.64-38.25	77	0.438
	Spruce	182	'Prince Rupert's drop'	2	-	-	36.77-50.03	100	0.606
	Spruce	158	'Prince Rupert's drop'	2	-	-	27.40-44.94	104	0.602

5.4 Discussion

5.4.1 Distributions, body sizes, settlement and growth rates of *Xylophaga*

Xylophaga species colonising the deployments in the SWIR have both been found once previously in the Indian Ocean. *Xylophaga murrayi* was described from material collected during the 'John Murray' expedition from depths of 347-384 m off Zanzibar (Knudsen, 1967). The second record for *X. murrayi* from Coral Seamount (SWIR) has extended the geographic range of this species down the eastern coast of Africa, the depth range by approximately 400 m and the temperature range (water temperatures of approximately 12.9°C off Zanzibar and 4.3°C at Coral Seamount). At Atlantis Bank (SWIR), we recorded the species *X. indica,* previously described from 339 m depth off the Andaman Islands by Smith (1904) during the 'Investigator' Expedition (Knudsen, 1961). The second locality from this study represents a large increase in geographic range across the Indian Ocean and also an increase in the depth range of this species by approximately 400 m. From TOTO, Bahamas, we recorded *X. depalmai*, which was originally described from off Florida but is now known to inhabit depths of 30 – 520 m depth from the Bahamas to Massachusetts (Turner, 2002; Tyler *et al.*, 2007). The species of *Xylophaga* found in the MCSC could not be definitively identified due to lack of soft morphology. There have been several species of *Xylophaga* found in the Caribbean Sea: *X. gerda* (283-2072 m) (Turner, 2002) *X. clenchi* (35-4862 m) (Turner, 2002), *X. whoi* (338-914 m) (Turner, 2002), *X. profunda* (1722-2066 m) (Turner, 2002), *X. abyssorum* (252-3950 m) (Turner, 1955, 2002; Voight, 2009), *X. tipperi* (152 m) (Turner, 2002), *X. bayeri* (152-365 m) (Turner, 2002) and *X. depalmai* (as above). Only one of these species, *X. clenchi,* has been found as deep as 4773 m however; this is thus a likely candidate for our specimen, but further collection is required to confirm this.

In terms of body size as measured by shell diameter, the previous dimensions of the one specimen of *Xylophaga murrayi* recorded by Knudsen (1967) (5.0 mm x 5.6 mm x 5.7 mm) were similar to mean and modal diameters found in the specimens from this paper. The one specimen of *X. indica* observed by Knudsen (1961) was almost twice as large as any of the specimens observed during our study (a diameter of 12 mm compared to our mean 5.8 mm). The type specimen of *X. depalmai* described by Turner (2002) noted a diameter of 9.8 mm, but Tyler *et al*. (2007) and this study recorded modal diameters between 2.26-4.78 mm with a maximum diameter of 10.12 mm. The *X. depalmai* population had the smallest diameters of the three species, which may be as a result of this experiment having the shortest deployment time (six months compared with two years). The *X. depalmai* diameters did not vary with deployment length or wood type. Deployment length did only vary by 24 days at the TOTO

site; possibly not enough for significant differences to develop in *Xylophaga* size. Turner (2002) and Turner (1955) reported malformed individuals and stenomorphism (individuals with stunted growth) in harder substrates but this can also be caused by crowding. Increasing substrate hardness has been observed to result in shorter and thicker shells in pholadids, *Penitella penita* (Evans, 1968c, b).

Previous studies have shown members of the genus *Xylophaga* to have seasonal rather than continuous recruitment (Turner, 1973; Berg Jr. *et al.*, 1987). Our results are suggestive of this with regard to the unimodal population structures of *Xylophaga* cf. *indica* and *X. depalmai*. For *X. murrayi*, the evidence is less certain as there are two distinct peaks in the size distribution for two of the three blocks examined. It is noteworthy that the SWIR deployments had been on the seafloor for nearly two years, compared with the six months for the Bahamas deployments. The study by Tyler *et al*. (2007) from which the Bahamas samples came, showed that there was only one recruitment event despite the population reproducing, suggesting "recruits did not come directly from local populations, and that zygotes produced were advected away from their parents". Settlement and recruitment that did occur during summer rather than winter may have been higher but may have been a product of experimental design (Tyler *et al.*, 2007). This was not seen in the particular sample blocks chosen for this study. Conversely, other studies have shown continuous recruitment in other *Xylophaga* species (Haderlie, 1983) and 'pulsing' recruitment in others (Romano *et al.*, In press).

In terms of abundance, *Xylophaga murrayi* from Coral Seamount, SWIR, had the lowest mean abundance (117 individuals dm^{-3}) of all three *Xylophaga* populations (excluding the MCSC population) whereas *X.* cf. *indica* from the more northerly Atlantis Bank, SWIR had more than double the abundance (300 individuals dm^{-3}) of *X. murrayi*. This may have been as a result of the environmental setting; in terms of the general species composition of the entire experiments, there was a significantly lower abundance of molluscs at Coral Seamount, compared to Atlantis Bank (see Chapter 4). Environmental settings on the two seamounts differed in terms of substratum type at the deployment site and physical, chemical and biological oceanographic parameters, especially temperature (see Chapter 4). Warmer temperatures seen at Atlantis Bank may have been more conducive to recruitment than the colder temperatures at Coral Seamount. Predation may also have been a factor contributing to the differences as there were more predatory polychaetes and amphipods at Coral Seamount (Chapter 4) (Turner, 1973; Berg Jr. *et al.*, 1987; Ockelmann and Dinesen, 2011; Romano *et al.*, In press). Berg Jr. *et al*. (1987) also noted that competition and succession could have effects on abundances of *Xylophaga*.

At TOTO, *X. depalmai* had the highest abundance of all the localities (~1500 individuals dm^{-3}), approximately five times higher than *X.* cf. *indica* and 15 times higher than *X. murrayi.* There was no difference in *X. depalmai* abundances due to varying the type of wood or deployment

length, as was seen by Haderlie (1983) when it was observed that there was no difference between fir, oak and redwood. There was scarcely any information in the literature about the abundances of these species prior to this study and the study by Tyler *et al*. (2007), but when compared with abundance data of other *Xylophaga* species, they appear to be in a similar range. *X. dorsalis* was observed in abundances of 100-500 individuals dm^{-3} (Bienhold *et al.*, 2013) and 525 individuals dm^{-3} (Gaudron *et al.*, 2010) while *Xylophaga* spp. were found in abundances of 290 individuals dm^{-3} (Romano *et al.*, In press). Another member of the family Xylophaginae, *Xyloredo ingolfa*, has been reported with extremely high abundances of 14412 individuals dm^{-3} (Gaudron *et al.*, 2010). Our results are strongly suggestive that water temperature plays a key role in controls on the relative abundance of *Xylophaga*.

There was only one specimen of *Xylophaga* sp. recovered from the deployments in the MCSC. This was initially thought to be as a result of the mooring design which was dictated by the requirements for suspended oceanographic instruments: the wood was suspended 20 m above the seafloor and hence potentially into a region of the water column with fewer *Xylophaga* larvae (Haderlie, 1983; Turner, 2002). Supporting this, Haderlie (1983) found that the intensity of settlement and damage to wood decreased with distance above the bottom as shallow as 2 m above seabed. However, since then, a recent study has showed that wood suspended 20 m above the seafloor can be colonised in large numbers by *Xylophaga* (Romano *et al.*, In press). The height that *Xylophaga* larvae are able to swim up into the water column may differ according to species present and the depths of deployments though (Romano *et al.*, In press). The study by Romano *et al.* (2013) did show however, that wood was more heavily recruited and bored on the seafloor rather than suspended 20 m above the seafloor. It is not likely that this lack of prolific settlement is as a result of the extreme depth (4773m) at our MCSC locality as several species of *Xylophaga* have been found at similar depths in the Caribbean Sea: *X. gerda* (2072 m) (Turner, 2002) *X. clenchi* (4862 m) (Turner, 2002), *X. profunda* (2066 m) (Turner, 2002) and *X. abyssorum* (3950 m) (Turner, 1955, 2002; Voight, 2009).

Growth rates can vary due to genetics, length of growing season, temperature, substrate quality and crowding (Romey *et al.*, 1994). *Xylophaga* are known to have very rapid growth rates when compared with other animals in the deep sea but excluding animals at hydrothermal vents and whale falls (Gage and Tyler, 1991; Lutz *et al.*, 1994). The species in this study are no exception to that rule; *Xylophaga murrayi, X.* cf. *indica* and *Xylophaga* sp. had similar minimum growth rates but *X. depalmai* had a minimum mean growth rate that was approximately three times faster but did not differ with substrate type or deployment length (Tyler *et al.*, 2007). Although it is important to point out that our estimates of growth rate are based on an assumption of the settlement time, we are the first to report any sort of growth rates in these particular species. Some other *Xylophaga* species have had variable growth rates recorded. *X. alexisi* and *X. ricei*

had growth rates of 0.011 mm day^{-1} (Harvey, 1996; Voight and Segonzac, 2012). *X. atlantica* has growth rates that differ with crowding: 0.085 mm day^{-1} in individuals that settled at the deployment beginning and 0.031 mm day^{-1} in individuals that settled one year after deployment (Romey *et al.*, 1994). The difference in growth rates between the *X. depalmai* and the other species of *Xylophaga* in this study may be explained by the S-shaped growth curve (Romey *et al.*, 1994). As the *X. depalmai* were growing for a maximum of only 158-182 days, whereas the other species could have been growing for a maximum of 756 days, the Bahamian species may still have been in the rapid growth phase rather than the plateau phase like the other species. This was seen in a study by Romano *et al*. (2013) where the mean growth rate was 0.070 mm d^{-1} in the first three months of deployment but then slowed in the next nine months to 0.021-0.016 mm d^{-1}. That study also saw faster growth rates in pine than in oak and it was hypothesised that this was because oak was a harder wood and so may have been more difficult to bore. Alternatively, *Xylophaga* may have recruited earlier to the pine. Stenomorphism cannot be blamed for the slower growth rate in *X. murrayi* but perhaps in *X.* cf. *indica* and other studies where wood was disintegrating with *Xylophaga* growth rates around 0.01mm day^{-1} (Harvey, 1996; Voight and Segonzac, 2012).

5.4.2 The effects of *Xylophaga* on wood

Until now, *Xylophaga* borings have never been visualised in 3-D. In general, the shape of the borings is very characteristic, and closest to the shape formed by molten glass when cooled as a droplet in cold water – the 'Prince Rupert's drop' – which became famous in the 17th century for its unique biomechanical properties. However, our study has also shown that the borings of different species vary in morphology and size. In fact, boring size and shape is much more variable between species than between type of wood (no difference was observed between *Xylophaga depalmai* in oak and spruce) or the age of the wood block (*X. murrayi* and *X.* cf. *indica* colonised mango wood and after two years, the populations and boring networks differed hugely). Knudsen (1961) remarked that *Xylophaga* borings were ‘oblong-pearshaped’. From this study however, it can be seen that there is much more to the boring morphology. *Xylophaga* make the borehole as larvae, hence the very small size of the visible surface hole. *Xylophaga* then bore into the wood for nutrition making the burrows larger and simultaneously resulting in their growth. Whether the increase in boring size occurs in width or length mostly depends on the species and possibly other factors such as wood hardness (see below). The age of the *Xylophaga* individual may also be influential as has been noted in other pholadids (Savazzi, 1999). Savazzi (1999) observed “Pholadid bivalves are characterised by an active boring stage, in which the shell grows and the organisms bores, and a subsequent adult or static phase, in which neither shell growth (with the exception of secondary thickening) nor boring takes

place." Whether this is true for *Xylophaga* remains to be seen based on evidence from higher-resolution temporal studies.

In terms of *Xylophaga* boring sizes, volumes have never been reported previously but lengths have: Turner (1973) reported 20 mm burrows in an undescribed *Xylophaga* sp., which is comparable with the lengths we observed in *Xylophaga murrayi* and *X. depalmai*. Turner (1973) however, cited overcrowding to explain these burrow sizes; that may be the case in *X. depalmai* but not *X. murrayi* in this study. Turner (2003) noted that *X. profunda* had borings of 45-50 mm length or approximately twice the shell depth, fitting in the middle of the range of boring sizes observed in this study. Dons (1940) observed similar size borings (50 mm) in *X. dorsalis*. Even though no difference was observed in boring morphology between different wood types in *X. depalmai,* in *Penitella penita*, increasing hardness of the substrate has resulted in proportionally shorter and stouter boreholes (Evans, 1968a). Our results show that the borings of more than one *Xylophaga* never joined and when borings were in close proximity, they grew in similar directions to avoid joining with each other. This suggests that *Xylophaga* individuals never link borings to facilitate reproduction within the wood. Savazzi (1999) observed that the morphology of pholadids combined with the positioning of the animal in the "roughly hemispherical anterior region of the borehole" results in the substrate being evenly abraded and allows the bivalve to change the direction of the borehole within a very small radius of curvature thus allowing it to avoid adjacent boreholes.

The miniscule size of the borehole in comparison to the rest of the boring has interesting implications for *Xylophaga* life history in terms of reproduction and predation. *Xylophaga,* once within the borings, are protected from most predators allowing only very small animals (e.g. polychaetes) to enter (Ockelmann and Dinesen, 2011). With regard to reproduction, the inability of *Xylophaga* to leave the boring to find a mate could have led to the development of several types of reproduction depending on the species. Hermaphroditism has been reported in *Xylophaga depalmai,* wood-boring teredinids and the whale-fall bivalve *Idas washingtonia*, erasing the need of finding a mate (Eckelbarger and Reish, 1972; Tyler *et al.*, 2007; Tyler *et al.*, 2009). Alternatively siphons may play a role in the reproduction of *Xylophaga* as has been seen in teredinids in which several methods of fertilisation are used. External fertilisation may occur in the water column after gametes are expelled out of the borehole, sperm may be released by the male into the water column which the female then draws in using her siphon, or sperm from the male is inserted into the female siphon using his siphon so fertilisation occurs internally (Eckelbarger and Reish, 1972). Siphons can only play a role in reproduction if they are long enough to extend from the animal (which is in the anterior region of the boring due to the borehole narrowing closer to the posterior end by the borehole) out of the borehole and into the water column. The last possible reproductive mode to circumvent the small borehole size is

sexual dimorphism in the form of dwarf males. First reported in the teredinids and then *Xylophaga* (Yakovlev and Malakhov, 1985; Ockelmann and Dinesen, 2011; Haga and Kase, 2013), these studies have shown that previous reports of abundant 'brooding' larvae are probably erroneous (Knudsen, 1961; Harvey, 1996; Turner, 2002; Voight, 2008).

How much wood can *Xylophaga* eat? Our Micro-CT data has allowed us for the first time to estimate wood consumption rates for this enigmatic deep-sea genus. The percentage of bored wood by the population of each *Xylophaga* species was related to the abundance of the animals, but this was not the entire story. *Xylophaga murrayi* from Coral Seamount had between 14 and 26 individuals present in each sample and this corresponded to 1 to 13% of wood being bored. *X.* cf. *indica* from Atlantis Bank had 203-239 borings in each sample and these corresponded to 31 to 46% of wood being bored. However, *X. depalmai* from TOTO had the highest frequency of borings (457-776 borings) but the amount of wood consumed (25 to 50%) was similar to that of *X.* cf. *indica*. We normalised this by calculating the mean individual boring rate per day for each species, and it was then apparent that per individual, *X.* cf. *indica* was the most efficient borer and *X. depalmai* only had the highest frequency of wood bored because of the large population size. Even though our calculations show individual boring efficiency was only marginally higher in *X.* cf. *indica* than *X. depalmai,* the figure may in fact be much higher due to methodological limitations after visual inspection of the wood.

An individual of *Xylophaga murrayi* was able to bore 0.2351 cm^3 of wood in a year. *X. depalmai* individual could bore 0.4380 cm^3 in oak and 0.6040 cm^3 in spruce and *X.* cf. *indica* individual bored 0.6052 cm^3 in a year (though this is probably an underestimate). This study showed the variability in the rates of degradation by *Xylophaga*, however we are unsure whether this is as a result of intrinsic variables relating to the species themselves (e.g. metabolic rates) or extrinsic variables that the species have reacted to (wood hardness, water temperature etc.). Turner (1973) noted experimental wood blocks disintegrating while being picked up with Alvin after 104 days off Massachusetts. The blocks bored by *X.* cf. *indica* were very close to disintegrating even though only a maximum of 46% of wood was calculated to be bored (proposed to be an underestimate). A maximum of 50% of the wood was bored by *X. depalmai* but this wood was not crumbling. Thus, we can say that the wood observed by Turner (1973) was probably significantly more than 50% (perhaps 70-85%) bored after nearly four months. Haderlie (1983) noted panels were disintegrating after 4-6 months of deployment (also 70-85%), a degradation rate higher than those seen during this study.

The strong relationship between temperature and wood consumption should be noted. At the coldest sites (Coral Seamount and MCSC), the wood was the least heavily colonised and bored. Warmer water temperatures may perhaps be more conducive to survival and recruitment of *Xylophaga* larvae accounting for the high degradation rates in *Xylophaga* cf. *indica* and *X.*

depalmai. Metabolic rates of animals are also known to be higher in warmer temperatures and perhaps this is also a reason for the high degradation rates in those two species. Recent observations of the absence of typical wood-eating fauna such as *Xylophaga* from Antarctic waters (Glover *et al.*, 2013) are also supportive of this, although the authors hypothesised that this was a result of lack of larval input rather than the cold temperatures. Antarctic bathyal depths are the coldest deep-sea regions in the world, with temperatures of about 0°C at 1000 m water depth (compared to 4°C at Coral Seamount) and as such *Xylophaga* may grow very slowly there even if the larvae are able to reach the region.

It has only recently become possible due to technological advances for these types of measurements to be made of the borings, thus there are very few other studies to compare with. The only other study of a boring invertebrate from an organic fall has been done on the bone-boring polychaete, *Osedax*. Degradation rates of approximately 6% per year were recorded for this worm, similar to those recorded during this study for *Xylophaga murrayi* (7%) but much less than *X. indica* (22%) or *X. depalmai* (104%). This may be as a result of the differences in boring mechanisms (chemical in *Osedax* but mechanical in *Xylophaga*) and also the nature of the substrate they bore into (bone for *Osedax* and wood for *Xylophaga*). It should also be noted that even though *Xylophaga* borings were the only ones studied here, it is likely that other wood-eroding organisms such as cocculiniform limpets and limpets from the family Pectinodontidae, also play a significant role in wood degradation (Pailleret *et al.*, 2007a).

5.5 Conclusions

This study has provided new insights into *Xylophaga* ecology and their use of wood as a resource including the first quantitative measurements of the degradation rates of wood by *Xylophaga*. This study has also reiterated that *Xylophaga* are keystone species in wood-fall ecosystems and are vitally important in the remineralisation of wood in the deep sea. If future studies can be directed into estimating how much wood enters deep sea and assessing its importance as a food resource, our data can be used to estimate the overall remineralisation rates for terrestrial wood inputs in deep-sea areas with known presence of *Xylophaga*.

6. The ecology of organic falls

6.1 Introduction

Following more than 30 years of research on organic falls, our knowledge of these unique ecosystems has increased enormously. Whale falls and wood falls are now known to occur widely in the deep sea (Dell, 1987; Gibbs, 1987; Marshall, 1987; McLean, 1992; Fujioka *et al.*, 1993; Bennett *et al.*, 1994; Marshall, 1994; Wada *et al.*, 1994; Berrow *et al.*, 1995; Dell, 1995; Smith and Baco, 2003; Pailleret *et al.*, 2007a; Lundsten *et al.*, 2010a; Samadi *et al.*, 2010; Fagervold *et al.*, 2012; Amon *et al.*, 2013). Many of these nutrient-rich habitats support diverse, trophically-complex faunal assemblages and, in most cases, are colonised by specialist fauna such as *Osedax* and *Xylophaga*, which are adapted to exploiting the organic matter from in the substrate and are effectively 'endemic' to organic falls (Turner, 1955, 1973, 1977, 2002; Smith and Baco, 2003; Rouse *et al.*, 2004; Glover *et al.*, 2005b; Pailleret *et al.*, 2007a; Tyler *et al.*, 2007; Voight, 2007, 2008, 2009; Lundsten *et al.*, 2010a; Samadi *et al.*, 2010; Voight and Segonzac, 2012). There are however, still many gaps in our knowledge.

The aims of this thesis were to try and fill some of these gaps by further investigating the taxonomic compositions of organic falls, especially in areas where no studies had previously been conducted. It was hoped that both specialist organic-fall fauna such as *Osedax* and *Xylophaga*, as well as opportunistic background fauna would be found in the new experimental localities, as has been seen in past studies at other locations. Only by first making the key taxonomic assessments, will a better understanding of the degree of connectivity and overlap in faunal assemblages between organic falls themselves and other chemosynthetic habitats, such as vents and seeps, be possible. Lastly, an important goal was to make an improvement in our knowledge of the functional ecology of these systems, in particular how quickly the organic matter is used by the fauna attending them.

In the preceding chapters, I have presented data on the fauna attending organic falls from several ocean basins, as well as the ecology and function of the communities. Throughout these chapters, there have been several recurring themes that have been investigated; the taxonomic composition of organic falls, the presence of novel organisms and the study of the length of time which resources can be provided by organic falls. I will now discuss these themes while also synthesising my findings during this project. I will also suggest how this has advanced our previous understandings, discuss the frequency with which these habitats occur and propose potential directions for organic-fall research to proceed in the future.

6.2 Taxonomic compositions of organic falls

Biogeographic provinces exist for fauna at hydrothermal vents (Van Dover, 2000), but it is not clear whether organic falls are also geographically structured in the same way. One aim of this thesis was to further our understanding of the taxonomic compositions of faunal assemblages at organic falls from multiple ocean basins. Whale and bone falls have previously been observed in the north and northeast Atlantic Ocean, Southern Ocean, and the northeast, northwest, south and west Pacific Ocean (Marshall, 1987, 1994; Wada *et al.*, 1994; Naganuma *et al.*, 1996; Jones *et al.*, 1998; Smith and Baco, 2003; Goffredi *et al.*, 2004; Dahlgren *et al.*, 2006; Fujiwara *et al.*, 2007; Vrijenhoek *et al.*, 2008a; Amon *et al.*, 2013; Glover *et al.*, 2013). Data presented in this thesis has described the several novelties: bone and wood falls from the Indian Ocean (Chapter 4), a natural whale fall from the Southern Ocean (Chapter 2) and a study within this thesis also expanded on the findings from implanted bones in the Southern Ocean (Chapter 3). Here I attempt a broad global comparison of the dominant taxa composition of sulfophilic-stage whale falls.

The natural whale fall discovered in the Southern Ocean (Chapter 2) had nine species encrusting and another 21 in the immediate vicinity. The most abundant were the malacostracans (amphipods and isopods), then polychaetes (*Osedax*, dorvilleids and capitellids) and finally limpets (Fig. 6.1). Glover *et al*. (2013) and Chapter 3 of this thesis studied some implanted whale bones in this region also. Observations suggested that polychaetes were the most abundant fauna present, namely *Osedax*, dorvilleids and cirratulids, although the sampling method may have resulted in loss of motile fauna (Fig. 6.1).

Whale bones implanted on two seamounts in the Southwest Indian Ridge discussed in Chapter 3 had very similar taxonomic compositions but abundances varied. The bones on Coral Seamount had malacostracans (amphipods and isopods) as the most abundant fauna, followed by polychaetes (dorvilleids and capitellids) and then mytilid bivalves (Fig. 6.1). Bivalves were the most abundant fauna on the bones at Atlantis Bank, followed by the malacostracans and then polychaetes (Fig. 6.1).

In the Atlantic Ocean, there has been one study done in the northeast region (Dahlgren *et al.*, 2006). Polychaetes were the dominant fauna noted in the sulfophilic stage and so can be assumed to be the most abundant (Fig. 6.1). The main polychaete families were dorvilleids, chrysopetalids and siboglinids (*Osedax mucofloris*) (Dahlgren *et al.*, 2006; Wiklund *et al.*, 2009a; Wiklund *et al.*, 2009b). A more recent study of the molluscan fauna, has noted the presence of chemosynthetic bivalve molluscs (*Thyasira sarsi* Philippi, 1845) associated with the whale carcass several years after deployment (Danise *et al.*, 2013). A whale bone trawled up off

Iceland was noted to have limpets living on them (Waren, 1989) (Fig. 6.1). Many of the other fauna may have been lost during the collection process (trawling) however.

There have been six studies on two main whale-fall events off Japan; one carcass located on the Torishima seamount (Fujioka *et al.*, 1993; Wada *et al.*, 1994; Naganuma *et al.*, 1996) and then 12 carcasses in Kagoshima Bay (Fujikura *et al.*, 2006; Fujiwara *et al.*, 2007; Fujiwara *et al.*, 2009). Similar fauna was noted in both areas. The most abundant fauna on both whale falls were mytilid bivalves (Fig. 6.1). Other gastropods (buccinids and cocculinids) were noted, as well as polychaetes (cirratulids, dorvilleids, spionids and *Osedax*) and galatheids (Naganuma *et al.*, 1996; Fujiwara *et al.*, 2007) (Fig. 6.1).

There have been 12 whale carcasses observed in the northeast Pacific (Smith and Baco, 2003; Lundsten *et al.*, 2010a; Lundsten *et al.*, 2010b). Three whale carcasses discussed by Smith and Baco (2003), Bennett *et al.* (1994) and Baco and Smith (1999) found off southern California all had mytilid bivalves as the most abundant fauna present (over 80% of the total fauna) (Fig. 6.1). Gastropods were also very abundant (cocculinids, pyropeltids, and provannids) (Fig. 6.1). Isopods (*Illyarachna profunda*) were also very abundant), as well as polychaetes (Fig. 6.1). *Osedax* worms were present (A. Glover, personal communication) but in low abundances. The other whale carcasses in Monterey Bay were studied by Goffredi *et al.* (2004), Braby *et al.* (2007) and Lundsten *et al*. (2010b). These whale falls seemed to have a different faunal assemblage; *Osedax* worms and other polychaetes were the most abundant fauna on most of the carcasses, followed by actinarians (Fig. 6.1). Decapods, gastropods as well as echinoids, were also in high abundance. The last carcass was the most northerly on the western coast of Canada and was studied by Lundsten *et al.* (2010a). This carcass again had a very different faunal assemblage to the other two main areas of whale carcasses off California. Although there were no observations of abundances of fauna (not included in Fig. 6.1 as a result), the most speciose phyla were noted; cnidarians had the highest number of species, then molluscs and arthropods, followed by teleosts, annelids and echinoderms. Evidence that *Osedax* once occupied this carcass was present however.

Quadruped bones were noted off Papua New Guinea with *Osedax* worms flourishing on them but no other faunal observations were made (Vrijenhoek *et al.*, 2008a) (Fig. 6.1). Whale bones trawled off New Zealand were said to have large amounts of mytilid bivalves and sipunculids encrusting them (Gibbs, 1987; Marshall, 1987, 1994) (Fig. 6.1). Osteopeltid limpets, other gastropods, harpacticoid copepods and polychaetes were also noted (Marshall, 1987, 1994). This may not be a complete picture of the bone-encrusting fauna of this region as many motile animals such as amphipods and isopods may have been lost during collection by trawling.

A broad-scale analysis of these data has many caveats associated with it; the collection methods varied greatly, and in many cases species dominance is based on informal observations and notes rather than quantitative comparisons. Also, there are very few known whale falls that have been studied. However, some general patterns are apparent. On looking at the global picture, the most abundant classes at whale falls are the Bivalvia, Gastropoda, Malacostraca and Polychaeta. The differences between the sites lie in the relative abundances of each of these classes (Fig. 6.1). There appears to be three distinct regions in the eastern Pacific with the northerly two regions being very different from most others in the world, in that cnidarians are present in high abundance (Fig. 6.1). The Southern Ocean, Southwest Indian Ocean, Northeast Pacific, Northeast Atlantic, North Atlantic and the remainder of the Pacific are all different in their most abundant faunal assemblages.

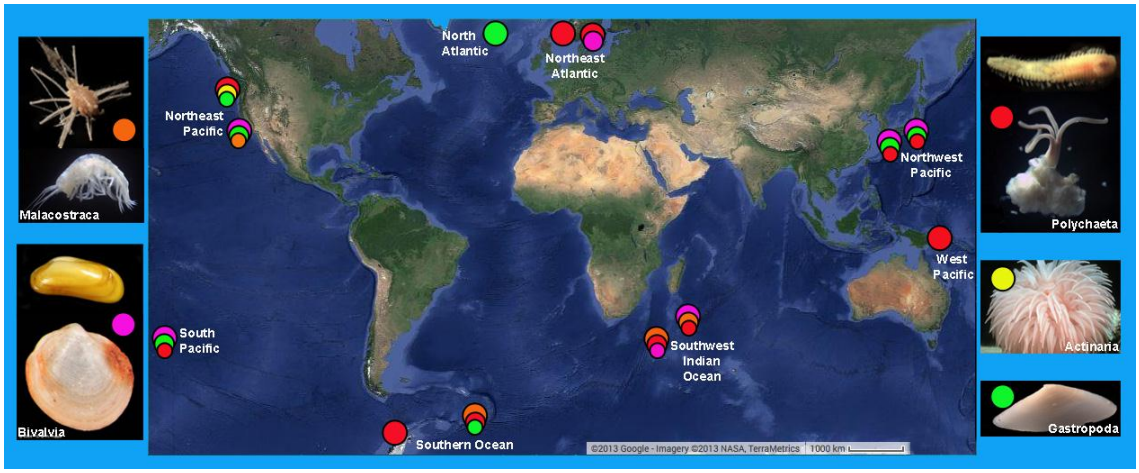

Figure 6.1 The most abundant classes of fauna found at whale or bone falls in different regions of the world. The abundance of different classes of fauna present are indicated by circle size; the largest circle corresponds with the most abundant class at each site and so forth. Those regions that only have one class represented are because that was the only observation made. The photograph of the Actinarian was taken from the Monterey Bay Aquarium website and the photograph of the thyasirid bivalve was taken from website of Conchology, Inc.

There have been few papers discussing the ecology of wood falls thus far; most tend to focus on one group of animals from the wood falls (*Xylophaga* or mytilids) (Fig. 1.4). Only sites where wood-fall ecology (ecology of lignified vegetative material) has been studied were included in this analysis; those that only mention one genus such as *Idas* or *Xylophaga* were not included. A map showing all the locations where *Xylophaga* have been recorded can be seen in Fig. 1.4. Wood falls however have been observed nearly worldwide. In the West Pacific, bivalves (*Xylophaga* and mytilids) have been noted as the most abundant class at wood falls, then limpets (Gros *et al.*, 2007; Pailleret *et al.*, 2007a; Lorion *et al.*, 2009; Samadi *et al.*, 2010) (Fig. 6.2). Echinoderms and polychaetes were also noted as very abundant (Becker *et al.*, 2009). The wood-fall fauna from two seamounts I investigated on the Southwest Indian Ridge showed very

similar abundance trends (Chapter 4). The most abundant class were the bivalves by a large percentage (mytilids and xylophagids), followed by malacostracans and polychaetes (Fig. 6.2).

Gaudron *et al.* (2010) looked at the ecology of wood deployments on the Mid-Atlantic Ridge, in the Arctic and in the eastern Mediterranean. All three sites showed similar faunal trends; bivalves were the most abundant phyla, followed by polychaetes and then arthropods (Fig. 6.2). Nemerteans, sipunculids, actinarians and echinoderms were also observed. In the Northwest Atlantic Ocean, bivalves (*Xylophaga* and mytilids) have been noted as well as the presence of polychaetes (capitellids, chrysopetalids, polynoids), echinoderms (ophiuroids, echinoids) and galatheids (Turner, 1955, 1973, 1977; Dean, 1993) (Fig. 6.2). Studies in the Tongue of the Ocean, Bahamas, have observed very high numbers of *Xylophaga* present though (Turner, 1977; Tyler *et al.*, 2007) (Fig. 6.2). Turner (1977) also noted *Xyloredo* sp. and galatheids in high numbers, but the most species-rich were the polychaetes (chrysopetalids, hesionids, polynoids and capitellids) and gastropods. Ostracods have also been noted by Maddocks and Steineck (1987). Bienhold *et al*. (2013) made similar observations in the eastern Mediterannean; *Xylophaga* and *Idas* were the most abundant fauna, followed by polychaetes, echinoids, decapods and lastly sipunculids.

Wolff (1979) made observations of wood-fall fauna in the Caribbean. He noted that the most speciose groups were the polychaetes, isopods, limpets and decapods. During a research expedition (JC083) in early 2013 to investigate the effects of the eruptions of the Soufriere Hills volcano on Montserrat on the surrounding deep sea, wood was frequently noted on the seafloor (Fig. 6.4) (Amon & Glover, unpublished data). After sampling some of this wood, a large abundance of gastropod limpets were noted (Fig. 6.2). *Idas* and *Xylophaga* were present but in low numbers. There were also many different species of polychaetes (Fig. 6.2).

Many species of *Xylophaga* and *Xylopholas* have been observed in the Northeast and East Pacific Ocean (Voight, 2007, 2008, 2009). Bernardino *et al.* (2010) did a study of fauna in the sediments surrounding wood falls and found that the assemblage changes with distance from the wood fall and time since on the bottom. That study noted that *Xylophaga* were very abundant but polychaetes were in fact the most abundant group. Cumaceans were also very prevalent.

It can be said with confidence that the wood-boring genus *Xylophaga* is found at wood falls in nearly all of the world's oceans but in differing abundances. The one ocean where it has not been found thus far is the Southern Ocean (Glover *et al.*, 2013). Wood deployed there by Glover *et al*. (2013) was recovered almost intact, prompting them to hypothesise that due to the Antarctic Circumpolar Current and absence of wood entering the Southern Ocean from Antarctica, *Xylophaga* larvae are not present.

On looking at the global picture, there appear to be seven biogeographic provinces of wood-fall fauna. The four most abundant classes at wood falls are the Bivalvia, Gastropoda, Malacostraca and Polychaeta but in varying orders in different regions around the world (Fig. 6.2). Bivalves are nearly always the most abundant fauna at wood falls, no matter the region (Fig. 6.2). Fauna characteristic of wood-fall habitats no matter the region include bivalves and polychaetes. The Northeast Pacific is one province where polychaetes dominate, followed by *Xylophaga* (Fig. 6.2). There appears to be another distinct region in the West Pacific where mytilid bivalves are the most abundant fauna present, followed by gastropods and then polychaetes (Fig. 6.2). The Southwest Indian Ocean may be another province where *Xylophaga* and mytilid bivalves dominate, followed by malacostracans and polychaetes. The northeastern, northwestern and central Atlantic, and the Bahamas region is another province dominated by *Xylophaga*, polychaetes and then malacostracans (Fig. 6.2). The Mediterannean is very similar to the Atlantic province but echinoids are the third most abundant faunal class (Fig. 6.2). The lower Caribbean forms another province where limpets are the most abundant fauna. Lastly, the Southern Ocean is distinct from all other provinces in that no wood-fall fauna have been observed (Fig. 6.2).

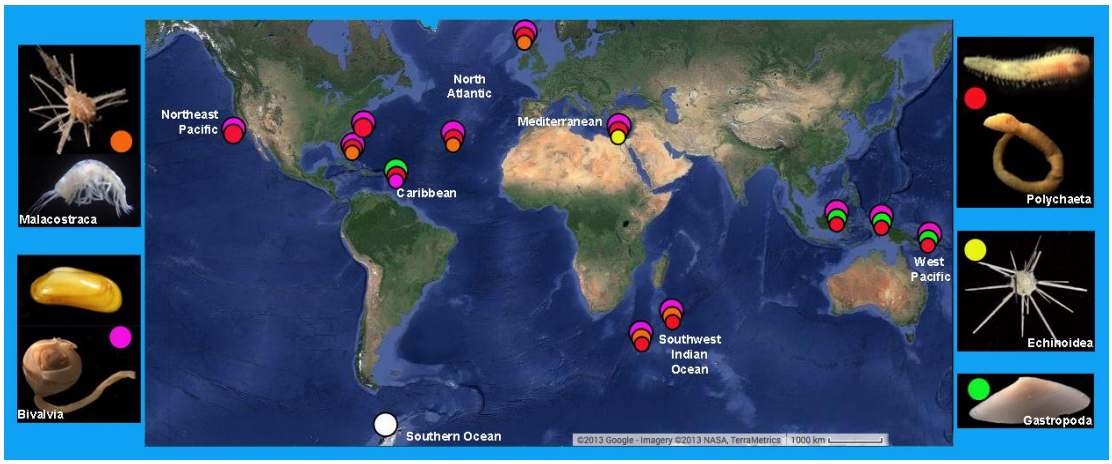

Figure 6.2 The most abundant classes of fauna found at wood falls in different regions of the world. The abundance of different classes of fauna present are indicated by circle size; the largest circle corresponds with the most abundant class at each site and so forth. Those regions that only have one class represented are because that was the only observation made. Records where only *Xylophaga* were reported were not included on this map; that can be seen in Figure 1.4.

What can be said for certain is that organic falls are not like hydrothermal vents; distinct biogeographic provinces do not exist in different regions where the dominant fauna differs in terms of higher taxonomic levels (for example, the dominance of crustaceans at hydrothermal vents on the Mid-Atlantic Ridge compared to polychaetes at the East Pacific Rise). The trends observed at hydrothermal vents are thought to be as a result of the combination of dispersal limitations and vicariance. Instead there seem to be a set of fauna widely distributed amongst

organic-fall habitats (organic-enrichment specialists), found in varying abundances regardless of the region e.g. mytilids, limpets, siboglinids (on whale falls), xylophagids (wood falls), dorvilleids, amphipods and isopods. From this, we can suggest that there are fewer barriers to dispersal over evolutionary timescales for organic-fall fauna.

More similar sampling methodologies with more frequent sampling events within varying regions will help to resolve this broad picture. The accumulation of all raw data from organic-fall studies would also be useful by allowing a meta-data analysis to be undertaken. This analysis for wood falls and whale falls has hinted at interesting potential patterns in faunal assemblages at these nutrient-rich islands, which could perhaps be used as hypotheses when designing future organic-fall studies.

Not only was it confirmed that taxonomic compositions of faunal assemblages differ between whale falls but the community composition was also observed to vary within whale falls for the first time (Chapter 2). This chapter was able to prove that fauna followed the 'oil gradient' hypothesis and that there was also some truth in the 'oil protection' theory (Higgs *et al.*, 2011b). The second assessment of functional ecology at wood falls and the first at whale falls (Chapter 4) showed that not only does the taxonomic composition of fauna vary at organic falls but also the functional composition. This may be the key to how these organic falls are able to support such a large number and high diversity of fauna.

6.3 New species described from organic falls

Since the study of organic-fall habitats began, novel species have been recorded from them. Three out of four chapters in this thesis have emphasised this point. The study of the caldera whale fall yielded ten putative new species encrusting the bones. This was initially reported as nine in Chapter 2 but it was discovered in Chapter 3 that the *Osedax* species was actually three species (*Osedax antarcticus, Osedax rogersi* and *Osedax crouchi*) (Fig. 6.3). Also in Chapter 3, *Osedax nordenskioeldi* was described from implanted whale bones in the Southern Ocean. In Chapter 4, there have been three species, *Eualus oreois, Lebbeus ketophilus* and *Munidopsis mandelai*, described from these experiments already, and there are thought to be at least twenty more putative new species (Fig. 6.3).

Organic falls support specialised communities in the deep sea and play fundamental roles in maintaining diversity, in turn facilitating evolutionary novelty. This wealth of new species reported is to some extent as a result of the deep sea being poorly explored due its inaccessibility. Cumulative summary tables of the number of new species described and putative new species recorded for the first time at whale or bone falls, and wood falls (including

those from this thesis) are seen below (Tables 6.1 and 6.2 respectively). In less than thirty years of research, there have been 116 species discovered from whale or bone falls; 51 of these have been formally described and 65 are putative. During over 60 years of the study of wood falls, there have been at least 93 species formally described and 62 more putative new species discovered at wood falls (155 in total) (Table 6.2). Organic falls have thus not only yielded a cumulative 271 putative new species, but within these findings, there have been numerous new genera (*Osedax, Osteopelta, Pectinodonta, Xylophaga, Xyloplax* etc.*)*, several new families of fauna (Osteopeltidae and Xylophaginae etc.), as well as a new class discovered (Concentricycloidea) (Fig. 6.3).

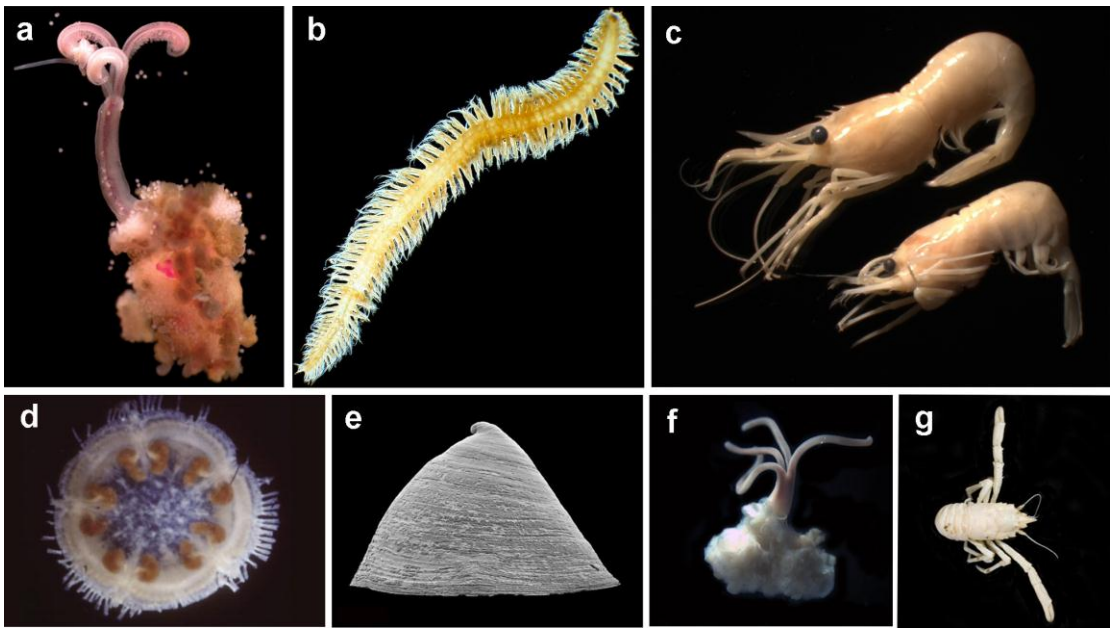

Figure 6.3 Species described from organic falls. *(a) Osedax mucofloris* described from whale bones off Sweden in 2005 by Glover *et al*. (2005). Photo courtesy Adrian Glover. (b) *Vigtorniella flokati* described from whale bones off California in 2004 by Dahlgren *et al*. (2004). Photo courtesy Adrian Glover. (c) *Lebbeus ketophilus* and *Eualus oreois* described from whale bones deployed during research for this thesis (Chapter 4) by Nye (2013). (d) *Xyloplax tunerae* (Concentricycloidea) described from a wood fall found off the Bahamas by Rowe *et al.* (1986). (e) *Osteopelta praeceps* described from a whale vertebrae off New Zealand by Marshall (1994). Photo courtesy Te Papa Tongarewa, Museum of New Zealand. (f) *Osedax antarcticus* described from implanted whale bones in the Bransfield Strait by Glover *et al*. (2013) but also discussed in Chapter 3 of this thesis. (g) *Munidopsis mandelai* described from wood experiments from the Southwest Indian Ridge by Macpherson *et al*. (In press) but discussed in Chapter 4 of this thesis.

Looking at the tables, it is apparent that the rate of discovery is steadily increasing, due to the increase in technology allowing exploration to push deeper into our world's oceans (Tables 6.1 and 6.2). However, the rate of description has not also increased, leaving many species recorded but undescribed (Tables 6.1 and 6.2). This is probably due to over-saturation of limited taxonomists. This is unfortunate because taxonomy is necessary and vital, as it provides a basic understanding of the components of biodiversity and without that fundamental information of what lives where, biogeography and other areas of ecological science cannot be done

effectively. As humans push ever further into the deep sea with advancing technology, more discoveries should be forthcoming; whether these species will be described is another matter.

Table 6.1 Cumulative records of species described from whale or bone falls thus far. [a] These records came from several whale-fall sites off California. [b] These species were also recorded at wood falls in nearby locations. All rows without authorities correspond with the last authority in the rows above.

Locality	Depth (m)	Species	Year described	Reference/Authority
South Africa		*Adipicola pelagica*	1854	Woodward, 1854
South Africa	400-1800	*Adipicola pacifica*	1897	(Dell, 1987)
Scotland & Ireland		*Idas simpsoni*	1900	(Tebble, 1966)
South Africa	400-1800	*Adipicola osseocola*	1987	(Dell, 1987)
New Zealand	880	*Phascolosoma saprophagicum*	1987	(Gibbs, 1987)
New Zealand	880	*Osteopelta mirabilis*	1987	(Marshall, 1987)
Iceland		*Osteopelta ceticola*	1989	(Waren, 1989)
Santa Catalina Basin, USA	1240	*Cocculina craigsmithi*	1992	(McLean, 1992)
Point Sur, California, USA	940	*Pyropelta wakefieldi*	1992	(McLean, 1992)
Santa Catalina Basin, USA	1240	*Harmothoe craigsmithi*	1993	(Pettibone, 1993)
Santa Catalina Basin, USA	1240	*Peinaleopolynoe santacatalina*	1993	
N. Atlantic		*Idas pelagica*	1993	(Waren, 1993)
N. Atlantic		*Idas ghisottii*	1993	(Waren & Carrozza, 1990)
New Zealand	372-1242	*Osteopelta praeceps*	1994	(Marshall, 1994)
New Zealand	372-1242	*Bruceiella laevigata*	1994	
New Zealand	372-1242	*Bruceiella pruinosa*	1994	
New Zealand	372-1242	*Xylodiscula osteophila*	1994	
New Zealand	880	*Adipicola arcuatilis*	1995	(Dell, 1995)
Iceland		*Protolira thorvaldssoni*	1996	(Waren, 1996)
San Clemente Basin, USA	1960	*Paralomis manningi*	2000	(Williams *et al.*, 2000)
Santa Cruz Basin, USA	1674	*Vigtorniella flokati*	2004	(Dahlgren *et al.*, 2004)
Kyushu, Japan	200-300	*Asymmetron inferum*	2004	(Nishikawa, 2004)
Monterey Bay, USA	2891	*Osedax rubiplumus*	2004	(Rouse *et al.*, 2004)
Monterey Bay, USA	2891	*Osedax frankpressi*	2004	
Tjarno, Sweden	125	*Osedax mucofloris*	2005	(Glover *et al.*, 2005b)
Kyushu, Japan	224-250	*Osedax japonicus*	2006	(Fujikura *et al.*, 2006)
California, USA[a]	385-2893	*Anthosactis pearseae*	2007	(Daly and Gusmao, 2007)
California, USA[a]	385-2893	*Munidopsis bracteosa*	2007	(Jones and Macpherson, 2007)
Monterey Bay, USA	1018	*Osedax roseus*	2008	(Rouse *et al.*, 2008)
Tjarno, Sweden	125	*Ophryotrocha eutrophila*	2009	(Wiklund *et al.*, 2009a)
Tjarno, Sweden	125	*Ophryotrocha scutellus*	2009	
Tjarno, Sweden	125	*Ophryotrocha craigsmithi*	2009	
Tjarno, Sweden	125	*Vigtorniella ardabilia*	2009	(Wiklund *et al.*, 2009b)
Monterey Bay, USA	2893	*Rubyspira osteovora*	2010	(Johnson *et al.*, 2010)
Monterey Bay, USA	2893	*Rubyspira goffrediae*	2010	(Johnson *et al.*, 2010)
California, USA[a]	960-1960	*Ophryotrocha batillus*[b]	2012	(Wiklund *et al.*, 2012)
California, USA[a]	960-1960	*Ophryotrocha flabella*	2012	
California, USA[a]	960-1960	*Ophryotrocha langstrumpae*[b]	2012	
California, USA[a]	960-1960	*Ophryotrocha longicollaris*	2012	
California, USA[a]	960-1960	*Ophryotrocha magnadentata*[b]	2012	
California, USA[a]	960-1960	*Ophryotrocha nauarchus*	2012	
Southwest Indian Ridge	732-750	*Eualus oreois*	2013	(Nye, 2013)
Southwest Indian Ridge	732-750	*Lebbeus ketophilus*	2013	
Kemp Caldera, Southern Ocean	1446	*Osedax rogersi*	2013	(Amon *et al.*, In review)
Kemp Caldera, Southern Ocean	1446	*Osedax crouchi*	2013	
Bransfield Strait, Southern Ocean	568	*Osedax nordenskioeldi*	2013	
Bransfield Strait, Southern Ocean	568	*Osedax antarcticus*	2013	(Glover *et al.*, 2013)
Deception Island, Southern Ocean	21	*Osedax deceptionensis*	2013	
Deception Island, Southern Ocean	21	*Ophryotrocha clava*	2013	(Taboada *et al.*, 2013b)
Deception Island, Southern Ocean	21	*Ophryotrocha orensanzi*	2013	
Deception Island, Southern Ocean	21	*Cirratulus balaenophilus*	2013	(Taboada *et al.*, 2013a)
Kemp Caldera, Southern Ocean	1446	*Pyropelta* sp.	-	(Amon *et al.*, 2013)

Kemp Caldera, Southern Ocean	1446	Osteopeltidae sp.	-	
Kemp Caldera, Southern Ocean	1446	*Lepetodrilus* sp.	-	
Kemp Caldera, Southern Ocean	1446	*Jaera* sp.	-	
Kemp Caldera, Southern Ocean	1446	Lysianassidae sp.	-	
Kemp Caldera, Southern Ocean	1446	*Ophryotrocha* spp. (2)	-	
Kemp Caldera, Southern Ocean	1446	Capitellidae sp.	-	
Southwest Indian Ridge	732-750	*Ophryotrocha* spp.[b] (3)	-	Amon (Chapter 4)
Southwest Indian Ridge	732-750	*Austrodecus* sp. B[b]	-	
Southwest Indian Ridge	732-750	*Hedgpethia* sp. A[b]	-	
Southwest Indian Ridge	732-750	*Nymphon* sp. A	-	
Southwest Indian Ridge	732-750	*Rhynchothorax* sp. A[b]	-	
Southwest Indian Ridge	732-750	*Idas* spp.[b] (3)	-	
Southwest Indian Ridge	732-750	*Austrolaenilla* spp.[b] (2)	-	
Southwest Indian Ridge	732-750	*Heliometra* sp.	-	
California, USA[a]	960-1240	*Vesicomya* sp.	-	(Baco *et al.*, 1999)
California, USA[a]	385-2893	*Osedax* spp. (3)	-	(Braby *et al.*, 2007)
California, USA[a]	385-2893	*Phyllochaetopterus* sp.	-	
California, USA[a]	385-2893	Spionidae spp. (2)	-	
California, USA[a]	385-2893	Polynoidae spp. (2)	-	
California, USA[a]	385-2893	*Protodrilus* sp.	-	
California, USA[a]	385-2893	*Calyptogena* sp.	-	
California, USA[a]	385-2893	*Amphiplica* sp.	-	
California, USA[a]	385-2893	Hesionidae sp.	-	
Kyushu, Japan	200-300	*Cestopagurus* sp.	-	(Fujiwara *et al.*, 2007)
Santa Catalina Basin, USA	1244	Polynoid sp.	-	Glover *et al*. (in prep)
California, USA[a]	1820-2893	*Osedax* spp. (2)	-	(Jones *et al.*, 2008)
Japan	113	*Osedax* spp. (4)	-	Pradillon *et al*. (unpub.)
Sagami Bay, Japan	925	Chrysopetalidae spp. (3)	-	
Setubal Canyon, Portugal	1000	*Ophryotrocha* spp. (3)	-	Ravara *et al.* (in prep.)
Monterey Bay, USA	1018	*Osedax* sp. 'nude palp C'	-	(Rouse *et al.*, 2009)
California, USA[a]	960-1960	*Axinodon* sp.	-	(Smith and Baco, 2003)
California, USA[a]	960-1960	Aplacophora sp.	-	
California, USA[a]	960-1960	Ampharetid sp.	-	
California, USA[a]	960-1960	*Asabellides* sp.	-	
California, USA[a]	960-1960	*Anobothrus* sp.	-	
California, USA[a]	960-1960	*Palpiphtime* sp.	-	
California, USA[a]	113-1820	*Osedax* spp. (6)	-	(Vrijenhoek *et al.*, 2009)
Bransfield Strait, Southern Ocean	568	*Ophryotrocha* spp. (2)	-	Wiklund (in prep.)
Nuuk, Greenland	123	*Ophryotrocha* spp. (2)	-	Worsaae (pers. comm.)

Table 6.2 Cumulative records of species described from wood falls thus far. [a] These three species were also recorded at whale/bone falls in nearby locations. All rows without authorities correspond with the last authority in the rows above.

Locality	Depth (m)	Species	Year described	Reference/Authority
Norway	0-2500	*Xylophaga dorsalis*	1819	(Turton, 1819)
Chile	3-246	*Xylophaga globosa*	1835	(Sowerby, 1835)
Pacific Ocean	343-1861	*Ophiambix aculeatus*	1880	(Lyman, 1880)
Caribbean Sea	170-311	*Xylopagarus rectus*	1880	(Milne Edwards, 1880)
Caribbean Sea	252-3950	*Xylophaga abyssorum*	1886	(Dall, 1886)
England, UK	30-82	*Xylophaga praestans*	1903	(Smith 1903)
Andaman Islands	339	*Xylophaga indica*	1904	(Smith 1904)
East Pacific	35	*Xylophaga mexicana*	1908	(Dall, 1908)
West coast, USA	61-2066	*Xylophaga washingtonia*	1921	(Bartsch, 1921)
Japan	457	*Pectinodonta rhyssa*	1925	(Dall, 1925)
Sulawesi	1301	*Xylophaga tomlini*	1932	(Prashad 1932)
East coast, USA	15-3172	*Xylophaga atlantica*	1942	(Richards, 1942)
Japan	183-1281	*Xylophaga supplicata*	1950	(Taki and Habe, 1950)
Japan	183-1281	*Xylophaga rikusenica*	1950	
Japan	183-1281	*Xylophaga japonica*	1950	

Japan	183-1281	*Xylophaga teramachii*	1950	
Phillippines	5050	*Xylophaga erecta*	1961	(Knudsen, 1961)
Phillippines	5050	*Xylophaga lobata*	1961	
Gulf of Panama	3670-3720	*Xylophaga concava*	1961	
Gulf of Panama	915-975	*Xylophaga panamensis*	1961	
Gulf of Panama	915	*Xylophaga duplicata*	1961	
Gulf of Panama	915	*Xylophaga aurita*	1961	
Gulf of Panama	915	*Xylophaga turnerae*	1961	
Gulf of Panama	915	*Xylophaga obtusata*	1961	
Mindanao & Bali Sea	1500	*Xylophaga grevei*	1961	
Mindanao & Bali Sea	1500	*Xylophaga bruuni*	1961	
Kermadec Trench	6660-6770	*Xylophaga hadalis*	1961	
Tasman Sea, New Zealand	4530	*Xylophaga galatheae*	1961	
Zanzibar	347-384	*Xylophaga murrayi*	1961	
West Africa	2550	*Xylophaga africana*	1961	
West Africa	2550	*Xylophaga guineensis*	1961	
Borneo	2000	*Xylophaga foliata*	1961	
Borneo	2000	*Xylophaga tubulata*	1961	
Indonesia	5050	*Xylophaga wolffi*	1961	
Gulf of Panama	3193-3207	*Pectinodonta gilbertvossi*	1971	(Olsson, 1971)
West Atlantic	35-2223	*Xylophaga clenchi*	1971	(Turner and Culliney, 1971)
Scotia Sea	1660-1664	*Xylophaga rhjabtshikovi*	1975	(Kudinova-Pasternak, 1975)
West Pacific	3100	*Xylophaga knudseni*	1975	(Okutani, 1975)
Norway	10	*Xylophaga nidarosiensis*	1980	(Santhakumaran, 1980)
Norway	10	*Xylophaga noradi*	1980	
Indo Pacific	220-780	*Ophiambix meteoris*	1983	(Bartsch, 1983)
Caribbean Sea	366-1184	*Phascolosoma turnerae*	1985	(Rice, 1985)
New Zealand and NSW	366-958	*Pectinodonta kapalae*	1985	(Marshall, 1985)
New Zealand and NSW	366-958	*Pectinodonta morioria*	1985	
New Zealand	366-1100	*Pectinodonta aupouria*	1985	
New Zealand	1057-1208	*Xyloplax medusiformis*	1986	(Baker *et al.*, 1986)
Caribbean Sea & W Atlantic	2066-3560	*Xylocythere turnerae*	1987	(Maddocks and Steineck, 1987)
West Atlantic	3506	*Xylocythere tridentis*	1987	
Caribbean Sea	3506-4000	*Xylocythere pointillissima*	1987	
Gulf of Panama	3900	*Xylocythere rimosa*	1987	
West Atlantic	1830	*Paradoxostoma turnerae*	1987	
Caribbean Sea	1830-4000	*Paradoxostoma* sp. 1	1987	
Caribbean Sea	4000	*Cytherois lignincola*	1987	
West Atlantic	3506	*Cytherois paralignincola*	1987	
West Atlantic	1830-4000	*Propontocypris excussa*	1987	
West Atlantic	3506	*Propontocypris sectilis*	1987	
Caribbean Sea	2066	*Propontocypris repanda*	1987	
Gulf of Panama	3900	*Parapontoparta spicacarens*	1987	
Bahama Islands	2066	*Xyloplax turnerae*	1988	(Rowe *et al.*, 1988)
West coast, USA	2030-2875	*Munidopsis lignaria*	1989	(Williams and Baba, 1989)
Caribbean Sea	91-229	*Xylopagarus tayrona*	1993	(Lemaitre & Campos, 1993)
Caribbean Sea	219-362	*Xylopagarus anthonii*	1995	(Lemaitre, 1995)
Caribbean Sea	139-366	*Xylopagarus tenuis*	1995	(Lemaitre, 1995)
Rockall Trough, NE Atlantic	1370-2195	*Xylophaga ricei*	1996	(Harvey, 1996)
Madeira abyssal plain	5000	*Xylophaga anselli*	1996	
New Zealand	736-1364	*Pectinodonta marinovichi*	1998	(Marshall, 1998)
Caribbean Sea	283-2072	*Xylophaga gerda*	2002	(Turner, 2002)
Caribbean Sea	338-914	*Xylophaga whoi*	2002	
Bahama Islands	1722-2066	*Xylophaga profunda*	2002	
California USA	1615-1828	*Xylophaga muraokai*	2002	
East coast, USA	45-520	*Xylophaga depalmai*	2002	
West coast, USA	35-257	*Xylophaga bayeri*	2002	
Florida, USA	152	*Xylophaga tipperi*	2002	
Florida, USA	152-365	*Xylophaga bayeri*	2002	
Northeast Pacific Ocean	2675	*Xyloplax janetae*	2006	(Mah, 2006)
Juan de Fuca Ridge	1520-2211	*Xylophaga oregana*	2007	(Voight, 2007)
Juan de Fuca Ridge	1520-2211	*Xylophaga corona*	2007	
Juan de Fuca Ridge	1520-2211	*Xylophaga heterosiphon*	2007	

Juan de Fuca Ridge	1520-2211	*Xylophaga microchira*	2007	
Juan de Fuca Ridge	1520-2211	*Xylophaga zierenbergi*	2007	
Guaymas Basin	1792-1875	*Xylophaga crooki*	2007	
Guatemala	106-113	*Xylophaga multichela*	2008	(Voight, 2008)
Indo Pacific	220-780	*Asterechinus elegans*	2009	(Becker *et al.*, 2009)
California USA	1225	*Xylophaga siebenalleri*	2009	(Voight, 2009)
West coast, USA	1099-2850	*Xylophaga pacifica*	2009	
West coast, USA	1099-2850	*Xylophaga scrippsorum*	2009	
Nile Deep-Sea Fan	1694	*Glycera noelae*	2012	(Boggemann *et al.*, 2012)
Nile Deep-Sea Fan	1694	*Cryptonome conclava*	2012	(Borda *et al.*, 2012)
Cape Verde abyssal plain	4626	*Xylophaga alexisi*	2012	(Voight and Segonzac, 2012)
Santa Cruz Basin, USA	1672	*Ophryotrocha batillus*[a]	2012	(Wiklund *et al.*, 2012)
Santa Cruz Basin, USA	1672	*Ophryotrocha langstrumpae*[a]	2012	
Santa Cruz Basin, USA	1672	*Ophryotrocha magnadentata*[a]	2012	
Southwest Indian Ridge	732-750	*Munidopsis mandelai*	2013	(Macpherson *et al.*, In press)
Phillippines	219-1775	Mytilidae spp. (6)	-	(Duperron *et al.*, 2008)
Southwest Indian Ridge	732-750	*Ophryotrocha* sp.[a] (2)	-	Amon (Chapter 4)
Southwest Indian Ridge	732-750	*Austrodecus* sp. B[a]	-	
Southwest Indian Ridge	732-750	*Hedgpethia* sp. A[a]	-	
Southwest Indian Ridge	732-750	*Rhynchothorax* sp. A[a]	-	
Southwest Indian Ridge	732-750	*Idas* spp.[a] (2)	-	
Southwest Indian Ridge	732-750	*Austrolaenilla* spp.[a] (3)	-	
Southwest Indian Ridge	732-750	*Thysanometra* sp.	-	
Southwest Indian Ridge	732-750	Amphinomidae spp. (2)	-	
Southwest Indian Ridge	732-750	Nymphon spp. (3)	-	
Nile Deep-Sea Fan	1694	*Coccopigya* sp.	-	(Gaudron *et al.*, 2010)
Nile Deep-Sea Fan	1694	*Prionospio* spp. (2)	-	
Mid-Atlantic Ridge	2300	*Prionospio* sp.	-	
Norwegian Sea	1257	*Alvania* sp.	-	
Phillipines and Vanuatu	290-1764	Mytilidae spp. (2)	-	(Lorion *et al.*, 2009)
Vanuatu	560-580	*Pectinodonta* sp.	-	(Pailleret *et al.*, 2007a)
Gulf of Cadiz		*Ophryotrocha* spp. (4)	-	Ravara et al (in prep.)
Koster, Sweden	125	*Ophryotrocha* spp. (2)	-	Wiklund (pers. comm.)
Caribbean Sea	1656-7471	*Nothria* sp.	-	(Wolff, 1979)
Caribbean Sea	1650	*Marginaster* sp.	-	
Caribbean Sea	5800-6850	*Echinothambema* sp.	-	
Caribbean Sea	4417-8330	*Heteromesus* sp. (3)	-	
Caribbean Sea	7965	*Macrostylis* sp.	-	
Caribbean Sea	6800-8330	*Nannoniscidae*sp. (2)	-	
Caribbean Sea	4580	*Katianira* sp.	-	
Caribbean Sea	1650-6780	*Lepidopleurus* sp. (2)	-	
Caribbean Sea	1650	Cocculinidae sp.	-	
Caribbean Sea	2288	*Onesimoides* sp.	-	
Caribbean Sea	2970-8100	*Fedikovella* spp. (2)	-	
Caribbean Sea	3700-6800	*Pseudococculina* spp. (2)	-	
Caribbean Sea	2450-6780	Gastropoda spp. (2)	-	
Caribbean Sea	1517	Janiridae sp.	-	
Caribbean Sea	6840-6850	*Austroniseus* sp.	-	
Caribbean Sea	4580	Lysianassidae sp.	-	
New Zealand	1270-1280	*Caymanostella* sp.	-	
Vanuatu	271-445	*Pectinodonta* sp.	-	(Zbinden *et al.*, 2010)

Many of the novel species found at organic falls have intriguing unique adaptations that allow them to exploit the energy in these habitats and are rarely seen elsewhere. The genus, *Osedax* is able to produce and secrete acids to breakdown and bore into the calcified bone (Rouse *et al.*, 2004; Tresguerres *et al.*, 2013). New symbiotic bacteria have also been found in *Xylophaga,* which are probably able to produce cellulases and other enzymes that are capable of digesting woody substrates in a similar way to in the teredinids (Distel and Roberts, 1997; Yang *et al.*,

2009). Similar symbionts may also be found in other animals that digest wood such as *Munidopsis andamanica, Xyloplax* spp. and Pectinodonta spp. (Baker *et al.*, 1986; Rowe *et al.*, 1988; Mah, 2006; Hoyoux *et al.*, 2009; Zbinden *et al.*, 2010). Other organic-fall fauna that have chemosynthetic capabilities include mytilids and vesicomyids found on both wood and bone (Baco *et al.*, 1999; Lorion *et al.*, 2009).

6.4 How common are organic falls?

How many gigatons of wood or whale carcasses are sitting on the world's seafloor at any given moment? This question can only be answered by knowing 1) how much of these substrates enters the oceans and 2) how quickly are those substrates being utilised and remineralised by fauna? The research in this thesis has provided useful data to aid in eventually providing those answers, which is important for understanding ecosystem processes in the deep sea. A natural whale fall was located in the Southern Ocean (Chapter 2), as well as large quantities of wood off Montserrat were observed during a research expedition I took part in in early 2013 (JC083). Rates of use of wood by several species of the specialist *Xylophaga* were also calculated (Chapter 5), and can be used to calculate how long wood can provide resources for if these species are present. Estimates of the time on the seabed were also possible following the experimental deployments of wood and bone in the Southwest Indian Ridge (Chapter 4) and bone deployments in Chapter 3.

The natural Antarctic whale-fall discovery adds evidence to the hypothesis that whale and bone falls are more abundant in areas below migratory routes and feeding grounds e.g. the western coast of the USA and the Southern Ocean (Smith and Baco, 2003). The caldera whale fall studied in Chapter 2 was estimated using the bone appearance, to have been on the seafloor for between four and 64 years old. The whale bones deployed in the Bransfield Strait showed limited erosion after two years. The large quantities of wood off Montserrat were probably carried into the sea as a result of increased deforestation during the pyroclastic-flow event of the Soufriere Hills volcano in 2009 (Fig. 6.4). Some of this wood was collected and was definitely of anthropogenic origin as there was paint present on some but others were obviously trees. This would mean that the wood (which was somewhat degraded and heavily colonised) had been on the seafloor for around five years. Wood falls are thought to be more abundant in offshore areas from forests and the mouths of rivers and observations of wood in the Caribbean Sea have been numerous (Wolff, 1979; Tyler *et al.*, 2007). The bone deployed for two years on the Southwest Indian Ridge (Chapter 4) looked almost intact when it was recovered indicating that they could have probably provided resources for much longer but wood from one location could be crushed

by hand and the other was still in relatively solid. The wood and bone deployed in the Mid-Cayman Spreading Centre for two years (Chapter 5) was in pristine condition when recovered.

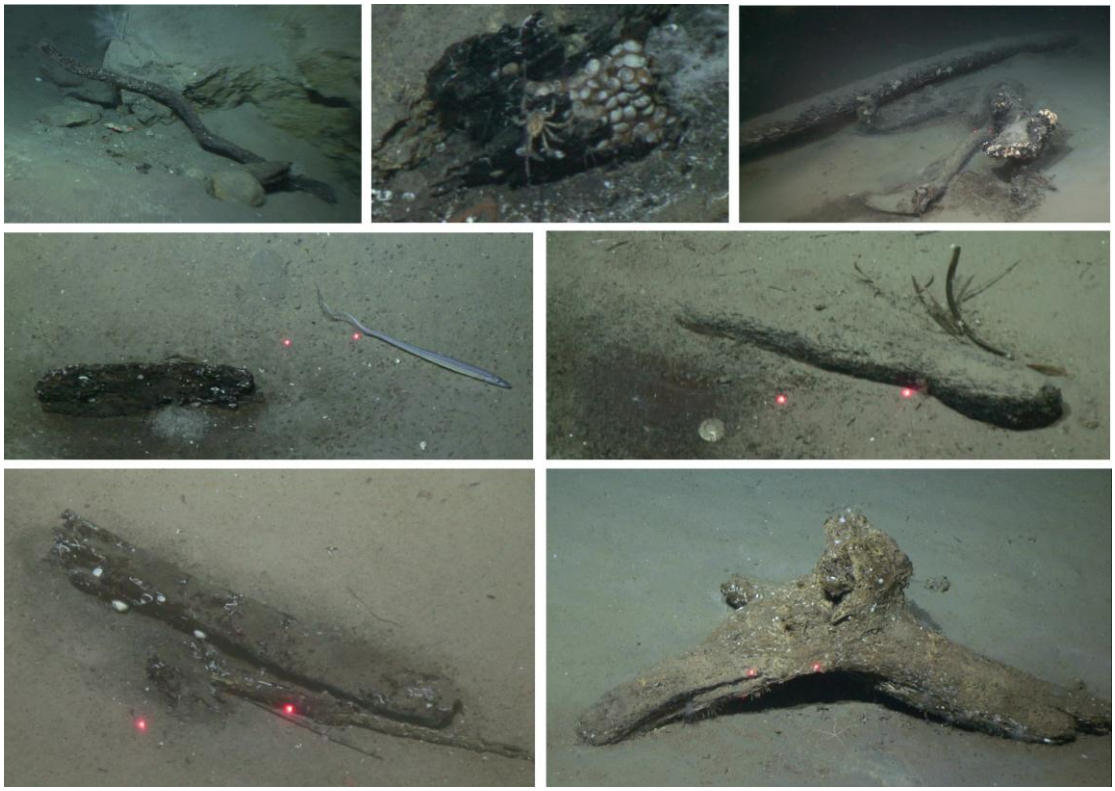

Figure 6.4 Wood observed on the deep seafloor off Montserrat. The red dots visible in the images represent a laser scale equal to 10 cm.

Despite several decades of research on organic falls, all one is able to say on the frequency of these habitats with any certainty, is that they occur widely. There has been no study thus far that has attempted to estimate the amounts of bone substrate or wood substrate entering the world's deep ocean. In recent times however, the inputs of whale bones and wood to the deep sea is likely to have been substantially altered by human activities such as whaling and deforestation (Butman *et al.*, 1995; Butman *et al.*, 1996; Smith, 2006; Voight, 2009). It has been calculated that there may be late-stage Gray whale skeletons as close as 5-16 km apart on the west coast of the USA and global estimates for the nine largest species of whales were calculated at 12-30 km apart (Smith *et al.*, 1989; Smith and Baco, 2003). Whale falls are thought to be able to provide resources from a few months to up to approximately 100 years depending on the size (Naganuma *et al.*, 1996; Smith and Baco, 2003; Schuller *et al.*, 2004; Fujiwara *et al.*, 2007; Lundsten *et al.*, 2010a; Lundsten *et al.*, 2010b), however whale falls have only been studied for about thirty years and so this cannot be said with certainty. Wood falls provide resources for much shorter periods probably, from a few months to several years (Turner, 1973, 1977; Romey *et al.*, 1994; Tyler *et al.*, 2007; Voight, 2007; Bernardino *et al.*, 2010; Voight and Segonzac,

2012; Bienhold *et al.*, 2013; Romano *et al.*, In press). No natural wood falls have been dated unfortunately so the only estimates we have are from implanted wood experiments and even in many of these studies, the observed state of the wood is not given in detail (Harvey, 1996; Gaudron *et al.*, 2010).

The length of time that organic falls are able to provide resources to the community depends on whether ecosystem-engineering species like *Osedax* and *Xylophaga* are present and in what numbers as they can rapidly breakdown the substrate. Higgs *et al*. (2011) found that *Osedax mucofloris* can degrade 6% of bone per year. Glover *et al*. (2013) found that wood deployed in the Southern Ocean for less than two years was almost intact when recovered due to absence of *Xylophaga* in the Southern Ocean. The study in Chapter 4 is a prime example that the presence of *Xylophaga* results in wood lasting shorter times as the wood on Atlantis Bank was heavily colonised and could be crushed by hand but the wood on Coral Seamount was the opposite. The wood type and the deployment times were the same for these two experiments.

6.5 Future areas of research

As the study of organic falls is fairly new to science, there are still many questions that need answering. Are there biogeographic provinces for organic-fall fauna and if so, are these provinces related to the distribution of whale-feeding grounds and migration routes or the mouths of rivers? Quantitative research into organic falls in previously unstudied areas needs to be done, simultaneously investigating the effects of bathymetric and regional variations on faunal assemblages attending these nutrient-rich islands. This will also unearth species as yet undiscovered, including specialists, and will answer questions about the distribution and frequency of these habitats on the deep ocean floor.

With regard to organic-fall specialists like *Osedax* and *Xylophaga,* are they present in all ocean basins? Are they restricted by temperature, bathymetry or location? By gaining a better understanding of which specialists attend organic falls in different areas, the reproductive and dispersal modes, and the rates of gene flow between organic falls and between other types of reducing habitats will also become clear. Also, as *Osedax* and *Xylophaga* are keystone species that engineer the organic substrate, how does their presence or absence affect the diversity of fauna attending organic falls? The settling cues and symbiont acquisition of these specialist genera are also areas needing further research.

We, deep-sea biologists, are lucky enough in our profession to be modern-day explorers who witness many discoveries. These can be anything from a new species of tiny polychaete, to an entire community of animals living on a decomposing whale carcass. The deep sea is a remote

and unexplored habitat, which makes it an extremely exciting habitat to work in but this seclusion is also obstructive. We want to ask all these scientific questions above and many more, but are hampered by the basic lack of knowledge of what actually lives down in the deep ocean. This needs to be the primary goal of our research in the future.

Appendix

Table Appendix 1. Location of data and samples used in this thesis.

Source of data	Location
Chapter 1	
'Osedax records' spreadsheet	Personal computer
'Xylophaga records' spreadsheet	Personal computer
Chapter 2	
Master video tapes of whale-fall footage from JC42	Office of Dr. Jon Copley (UoS)
Shortened JC42 video files x3	Personal computer
Master video tapes of whale-fall footage from JC55	Office of Dr. Jon Copley (UoS)
Shortened JC55 video files x3	Personal computer
Minke whale molecular sequences	Personal computer
Minke whale molecular samples	3 vials in freezer in 6th floor molecular lab (NHM)
Whale-species phylogenetic tree	Personal computer
Maps	Personal computer
Whale-bone samples collected on JC42 x3	Chest freezer of Dr. Adrian Glover, 1st floor wet lab (NHM)
Whale-bone samples collected on JC42 x1	1 jar ETOH, Polychaete lab, 6th floor (NHM)
Faunal subsamples from Dr. Katrin Linse (BAS) - *Pyropelta* sp.	1 vial in fridge of polychaete lab, 6th floor (NHM)
Faunal subsamples from Dr. Katrin Linse (BAS) - *Lepetodrilus* sp.	1 vial in fridge of polychaete lab, 6th floor (NHM)
Faunal subsamples from Dr. Katrin Linse (BAS) - Osteopeltidae sp.	1 vial in fridge of polychaete lab, 6th floor (NHM)
Faunal subsamples from Dr. Katrin Linse (BAS) - Lysianassidae sp.	1 vial in fridge of polychaete lab, 6th floor (NHM)
Faunal subsamples from Dr. Katrin Linse (BAS) - *Jaera* sp.	1 vial in fridge of polychaete lab, 6th floor (NHM)
Faunal subsamples from Dr. Katrin Linse (BAS) - *Ophryotrocha* spp. (x2)	2 vials in care of Dr. Helena Wiklund (NHM)
Whale-fall fauna-specimens photographs	Personal computer
Whale-fall photograph mosaic	Personal computer
Spreadsheets from JC42/JC55	Personal computer and office of Dr. Jon Copley (UoS)
'Quantitative faunal counts' spreadsheet	Personal computer
Figures from Amon *et al*. (2013	Personal computer
Chapter 3	
Osedax samples from Kemp Caldera (JC42)	Box of vials in fridge of polychaete lab, 6th floor (NHM)

Osedax samples from Smith Island	Vials in fridge of polychaete lab, 6th floor (NHM)
Osedax molecular sequences	Personal computer
Osedax molecular samples	Box of vials in freezer in 6th floor molecular lab (NHM)
Osedax phylogenetic tree	Personal computer
Osedax haploytype networks	Personal computer
Maps	Personal computer
Osedax sample spreadsheet	Personal computer
Osedax-specimens from Kemp Caldera photographs	Personal computer
Osedax-specimens from Smith Island photographs	Office of Dr. Adrian Glover (NHM)
Osedax SEM photographs	Personal computer
Chapter 4	
Master video tapes of bone and wood deployment footage from JC66	Office of Professor Alex Rogers (Oxford)
Shortened JC66 video files x2	Personal harddrive
Xylophaga and *Idas* molecular sequences	Personal computer
Xylophaga and *Idas* molecular samples	Vials in freezer in 6th floor molecular lab (NHM)
Maps	Personal computer
Wood samples collected on JC66	In ETOH bags by cupboard of Adrian Glover on 7th floor of the Collections (NHM)
Faunal samples	In cardboard box by cupboard of Adrian Glover on 7th floor of the Collections (NHM)
Munidopsis mandelai samples	In care of Dr. Enrique Macpherson (U. Barcelona)
Polynoidae spp. samples	In care of Lenka Nealova (NHM)
Pycongonidae spp. samples	In care of Dr. David Staples (U. Vic)
Amphipoda spp. and Isopoda spp. samples	In care of Dr. Tammy Horton (NOC)
Venustatrochus georgianus sample	In care of Dr. Suzanne Williams (NHM)
Dorhynchus sp. samples	In care of Dr. Paul Clark (NHM)
Spreadsheets from JC66	Personal computer and office of Professor Alex Rogers (Oxford)
Quantitative faunal spreadsheets	Personal computer
Fauna photographs	Personal computer
Chapter 5	
Raw Micro-CT data	Accessed at by Micro-CT lab (NHM)
Raw and processed Micro-CT data	Personal harddrive in Micro-CT lab (NHM)
Subsamples of woood from SWIR	Chest freezer of Dr. Adrian Glover, 1st floor wet lab (NHM)
Subsamples of wood from Cayman	Chest freezer of Dr. Adrian Glover, 1st floor wet lab (NHM)
Samples of wood from Bahamas	In 3 IMS buckets by cupboard of Adrian Glover on 7th floor of the Collections (NHM)
Scan images	Personal computer and harddrive

Micro-CT data spreadsheets	Personal computer
Chapter 6	
Fauna described from whale/bone falls' spreadsheet	Personal computer
Fauna described from wood falls' spreadsheet	Personal computer

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