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Whales, wood and kelp islands in the deep-sea: ecological succession and species overlap with other chemosynthetic habitats in the Californian continental slope (NE Pacific)

**Tese apresentada ao Instituto Oceanográfico da Universidade de São Paulo, como parte dos requisitos para obtenção do título de Doutor em Ciências, área de Oceanografia Biológica.**

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# Universidade de São Paulo

Instituto Oceanográfico

Whales, wood and kelp islands in the deep-sea: ecological succession and species overlap with other chemosynthetic habitats in the Californian continental slope (NE Pacific)

Angelo Fraga Bernardino

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## Abstract

Sunken parcels of macroalgae, wood and whale carcasses provide important oases of organic enrichment at the deep-sea floor, but sediment community structure and succession around these habitat islands are poorly evaluated. We experimentally implanted parcels of kelp and wood falls nearby a 30-ton deep-sea whale-fall at 1670 m in the Santa Cruz Basin (SCr; NE Pacific). At each organic island, we aimed to evaluate patterns of organic enrichment and spatial and temporal patterns of macrofaunal community structure and succession over time scales of 0.25 to 7y. Additionally, species overlap between kelp-, wood- and whale-falls with nearby cold-seep communities were investigated. In general, the abundance of infaunal macrobenthos was highly elevated at periods of intense organic enrichment at all organic falls, with decreased macrofaunal diversity and evenness within 0.5 meters of the falls. At kelp and wood falls opportunistic species and sulfide tolerant microbial grazers (dorvilleid polychaetes) abounded after the peak of sedimentary enrichment (0.25y and 1.8y, respectively), while the whale-fall macrofauna was highly abundant from 4.5 to 6.8 y, and was dominated by enrichment opportunist, chemoautotrophic-symbiont-hosting and heterotrophic species grazing sulfur-oxidizing bacterial mats. Sediments around kelp and wood parcels provided low-intensity reducing conditions, which sustain a limited chemoautotrophically-based fauna, with low levels of species overlap among other chemosynthetic habitats in the deep NE Pacific. Whale-fall sediments harbor many species and trophic types not present in background sediments, but there were low levels of species overlap between the whale-fall, cold seeps and hydrothermal vents, explained by differences in biogeochemistry and food webs among these habitats. We conclude that organically enriched sediments around kelp, wood and whale-falls may provide important habitat islands for the persistence and evolution of species dependent on organic- and sulfide-rich conditions at the deep-sea floor and contribute to regional and global diversity in deep-sea ecosystems.

## Resumo

Parcelas orgânicas de macroalgas, madeira e carcaças de baleia criam importantes oásis de enriquecimento orgânico no assoalho marinho de regiões profundas, mas a estrutura e sucessão ecológica da macrofauna sedimentar ao redor destes ambientes ainda é pouco conhecida. Parcelas de macroalgas e madeira foram artificialmente implantadas próximas a uma carcaça de baleia de 30-ton há uma profundidade de 1670 m na Bacia de Santa Cruz, Pacífico NE. Ao redor de cada ilha orgânica, foram estudados os padrões espaciais e temporais de enriquecimento sedimentar orgânico e a estrutura e sucessão temporal da macrofauna em escalas temporais que variam de 0.25 à 7 anos. Ainda, o nível de sobreposição entre espécies colonizadoras das parcelas orgânicas e na baleia, foram comparados com comunidades de exudações frias (uma localizada na bacia de São Clemente, Pacífico NE) e fontes hidrotermais. Em geral, a abundância da macrofauna sedimentar foi altamente elevada em períodos de intenso enriquecimento orgânico, com decréscimo da diversidade da macrofauna num raio de 0.5 metros das parcelas. Nas parcelas de macroalgas e madeira, espécies oportunistas e tolerantes à sulfetos atingiram altas densidades após o pico de enriquecimento orgânico sedimentar (0.25 e 1.8 anos, respectivamente), enquanto que ao redor da carcaça de baleia, a macrofauna foi também dominada por organismos quimiossintéticos com associações simbióticas bacterianas, e ainda espécies oportunistas que se alimentavam do abundante carpete bacteriano sobre a superfície sedimentar. Os sedimentos ao redor das parcelas de macroalgas e madeira sustentam baixas taxas de degradação microbiana e sulfeto intersticial, recrutando assim um limitado número de organismos quimioautotróficos e conseqüentemente com baixa sobreposição de espécies com outros ambientes redutores. Na carcaça de baleia, os sedimentos sustentam intensa degradação microbiana e altos níveis de sulfeto, mas diferenças marcantes nas biogeoquímica e nas cadeias tróficas presentes nestas carcaças resultam em baixa sobreposição de espécies com a fauna de exudações frias e fontes hidrotermais. Conclui-se que sedimentos enriquecidos organicamente ao redor de macroalgas, madeiras e carcaças de baleia criam importantes habitats para a persistência e evolução de espécies dependentes de condições sedimentares redutoras, e assim estas ilhas devem contribuir para a diversidade regional e global dos ecossistemas de mar profundo.

## Chapter 1. Introduction

Communities living at the deep-seafloor are directly affected the export of organic matter from the ocean's surface to depths, which significantly affects the structure and dynamics of deep-sea benthic ecosystems (Gage & Tyler 1991, Smith et al. 2008). Some special exceptions are found at cold seeps and hydrothermal vents, where chemosynthesis is a major process fueling local communities (Tunnicliffe 1988, Levin 2005). Therefore, the typical deep-sea benthos are dependent on a low flux rate of organic matter (e.g. phytoplankton detritus), which is partially decayed during its long and sluggish sink the deep-sea floor (Gage & Tyler 1991). However, the export of food to the sea bottom does not always occur at a slow pace and in some regions of the world's oceans, seasonal bursts in primary production give rise to massive exports of particulate organic material that settles rapidly to the sea bottom (Billett et al. 1983, Beaulieu 2002).

Although settling of pelagic organic matter may largely contribute to the bulk of energy that is available to deep-sea ecosystems, the massive episodic deliveries of labile organic carbon to these ecosystems are also important (Stockton & DeLaca 1982, Smith 1985, Rice & Lamshead 1994). Macroalgae, terrestrial plants debris, and carcasses of pelagic animals occur at variable temporal and spatial scales (Stockton & DeLaca 1982, Grassle & Morse-Porteous 1987). Woody and macroalgal debris are frequently deposited on continental slopes through physical or biological processes. Kelps can be released from the coastal zone during storms, high swell events, and from herbivory (Duggins et al. 1989, Harrold et al. 1998). Kelp and wood debris can then be concentrated and transported to depths in submarine canyons to form enormous accumulations of organic material in the deep ocean (Vetter 1994,

Vetter & Dayton 1998, 1999, McLeod & Wing 2007). Wood and kelp parcels also float out to sea, sinking as individual parcels spanning a broad range of sizes (1 - >200 kg) to the seafloor (Wolff 1979). Carcasses of nektonic animals (*e.g.* fishes, dolphins and whales) are additional sources of energy to the deep sea as they provide fresh organic material into a food-poor environment (Stockton & DeLaca 1982, Smith 1985, 1986, Smith & Demopoulos 2005). Although the flux of whale-detritus is not as quantitatively significant as the total particulate organic carbon (POC) flux to deep-sea oceans, dead whales sink as enormous organic-rich packages that disturb a very limited area of the sea-bottom (Smith 2006). For example, the seabed immediately underlying a typical whale fall (*i.e.* roughly 50 m<sup>2</sup>), can experience the equivalent of 2000 yr of background POC flux in a single pulse of organic enrichment (Smith & Baco 2003). Consequently, whale carcasses provide an immense and very rich source of labile organic matter to the food limited deep-sea ecosystems.

Organic falls are important sources of disturbance to benthic communities nearby continental margins and in the deep-sea (Stockton & DeLaca 1982, Smith 1985, 1986, Smith *et al.* 1998). As the scavenging and decaying of the organic falls take place, organic detritus [*i.e.* particulate organic carbon (POC)] is released into the surrounding sediments providing food for the infauna (Turner 1977, Wolff 1979, Stockton & DeLaca 1982, Grassle & Morse-Porteous 1987). The spatial succession along organic-enrichment gradients exhibits very low diversity and extremely high densities of “opportunistic” species in disturbed areas, with moderate macrofaunal enhancement and species diversity at intermediate distances, and very low abundances and high diversity in the background community (Pearson & Rosenberg 1978, Smith *et al.* 2002, Smith & Baco 2003). Consequently, the type and the magnitude of the organic enrichment will create distinct responses by the benthic

fauna. The organic enrichment attracts organisms through both numerical (e.g., reproduction of opportunistic species) and functional responses (e.g., omnivores and scavengers), thus modifying the structure of natural deep-sea communities (Grassle & Grassle 1974, Pearson & Rosenberg 1978). High levels of organic disturbance may additionally promote intense microbial decomposition, depletion of pore-water oxygen and increase levels of sulfides in the sediments (Boetius & Lochte 1996, Smith & Baco 2003, Treude et al. 2003). Reducing sediments around organic islands attracts sulfophilic organisms that are capable of deriving carbon from chemosynthetic symbiosis and are adapted to survive in low oxygen sediments (Fisher 1999, Dubilier et al. 2008).

Whale skeletons generate and sustain organic-rich conditions for years in the deep-sea. The broad range of ecological niches present at whale carcasses attracts organisms not found on background areas, including whale-fall “specialists” and heterotrophic opportunists (Baco-Taylor & Smith 2003, Smith & Baco 2003). The creation of hard substrata for marine invertebrates also attracts dense assemblages of megafaunal animals, including fish and invertebrates (Vetter & Dayton 1998, 1999). Wood and kelp falls also attract their own set of specialists, including wood-boring bivalves which degrade the wood matrix as they grow (Turner 1973). Consequently, organic patches open new areas for colonization of rare, opportunistic and specialist species, and have the potential to increase species richness locally. The role of organic patches in increasing deep-sea diversity has been raised decades ago, but studies well designed to address this hypothesis are scarce (Snelgrove & Smith 2002). The comparison of community structure and succession at distinct food parcels can help elucidate the contribution of organic falls for beta diversity (i.e. regional scale) in the deep sea. The long-term comparisons of community structure also allow identifying

levels of species overlap among distinct types organic and sulfide enrichments, such as around whale-, wood- and kelp falls and cold seeps.

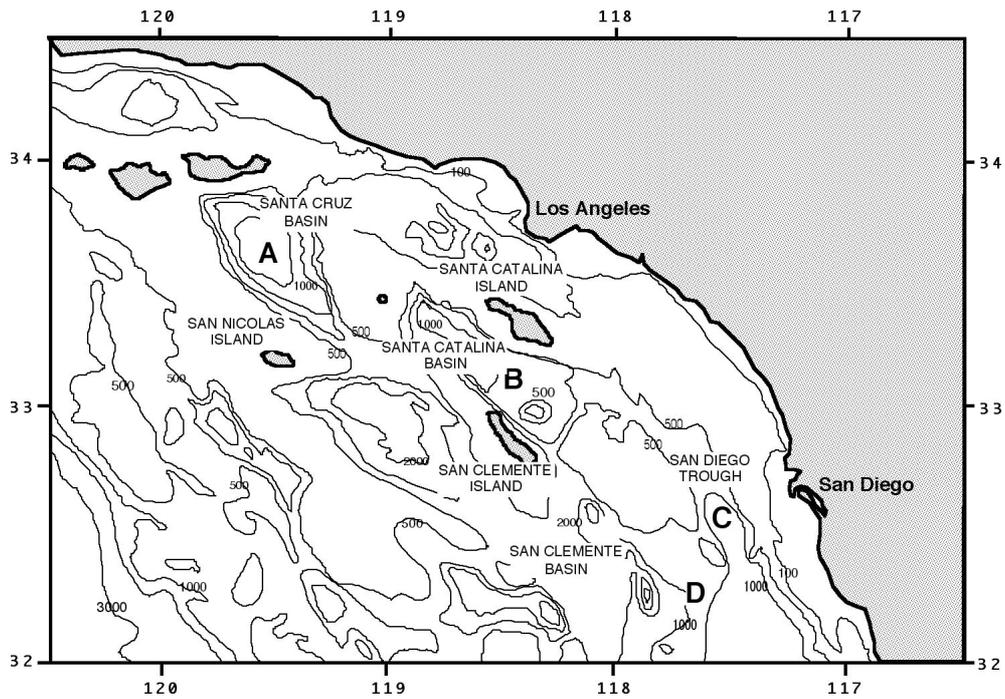
The present study will look at community structure and succession at artificially deployed parcels of wood, kelp (*Macrocystis pyrifera*) and a large whale-fall; and also investigate the San Clemente cold-seep macrofauna, which are all at similar depths on the California continental slope (Figure 1.1). This study will focus at the sediment macrofauna at each site and patterns of sediment organic and sulfide enrichment will be used to help explain ecological patterns at spatial and temporal scales. Through the comparison and characterization of community structure and succession at a variety of reducing habitats and organic islands on the southern California slope (cold seeps, whale-, kelp- and wood-falls) we expect to test the following general hypothesis:

*i) Kelp, wood, and whale falls produce highly distinct time courses of sediment organic loading, which create distinct patterns of community succession;*

*ii) Kelp- and wood-fall communities are less diverse than background sediments and host a smaller set of specialized fauna compared to whale-fall communities;*

*iii) Whale-fall sediment communities increase local species diversity but share a small proportion (<20%) of species with assemblages at southern California seeps, kelp falls and wood falls at similar ocean depths.*

*iv) The San Clemente seep macrofaunal sediment community contains few specialized species, and exhibits similar levels of diversity to background sediments;*



*Figure 1.1. Map from the Southern California coast (USA) showing the position of known whale carcasses and San Clemente cold seep (isobaths in meters) A: Deployment site of kelp and wood parcels, and of a whale carcass implanted in 1998; B: Natural whale carcass found at Santa Catalina Basin by C. Smith in 1987; C: Small (5.000-kg) whale carcass artificially implanted at San Diego Through in 1996; D: San Clemente cold seep.*

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## Chapter 2. Macrofaunal succession in sediments around kelp and wood falls in the deep NE Pacific and community overlap with other reducing habitats

### **Abstract**

Sunken parcels of macroalgae and wood provide important oases of organic enrichment at the deep-sea floor, yet sediment community structure and succession around these habitat islands are poorly evaluated. We experimentally implanted 100-kg kelp falls and 200 kg wood falls at 1670 m depth in the Santa Cruz Basin to investigate (1) macrofaunal succession, and (2) species overlap with nearby whale-fall and cold-seep communities over time scales of 0.25 to 5.5 y. The abundance of infaunal macrobenthos was highly elevated after 0.25 and 0.5 y near kelp parcels with decreased macrofaunal diversity and evenness within 0.5 meters of the falls.

Apparently opportunistic species (e.g., two new species of cumaceans) and sulfide tolerant microbial grazers (dorvilleid polychaetes) abounded after 0.25 -0.5 y. At wood falls, opportunistic cumaceans become abundant after 0.5 y, but sulfide tolerant species only became abundant after 1.8 - 5.5 y, in accordance with the much slower buildup of porewater sulfides at wood parcels compared to kelp falls. Species diversity decreased significantly over time in sediments adjacent to the wood parcels, most likely due to stress resulting from intense organic loading of nearby sediments (up to 20-30% organic carbon). Dorvilleid and ampharetid polychaetes were among the top-ranked fauna at wood parcels after 3.0 - 5.5 y. Sediments around kelp and wood parcels provided low-intensity reducing conditions, which sustain a limited chemoautotrophically-based fauna. As a result, macrobenthic species overlap among kelp, wood, and other chemosynthetic habitats in the deep NE Pacific are primarily restricted to apparently sulfide tolerant species such as dorvilleid polychaetes, opportunistic cumaceans, and juvenile stages of chemosymbiont containing vesicomid bivalves. We conclude that organically enriched sediments around wood falls may provide important habitat islands for the persistence and evolution of

species dependent on organic- and sulfide-rich conditions at the deep-sea floor and contribute to  $\beta$  and  $\gamma$  diversity in deep-sea ecosystems.

## 2.1. Introduction

Plant remains such as wood and macroalgal debris have long been known from the deep-sea floor and the fossil record, with first reports dating from *The Challenger* expedition (Wolff 1979, Kiel & Goedert 2006). Deep-sea imaging and trawl studies suggest that wood and macroalgal falls occur widely on the ocean floor (Wolff 1979, Pailleret et al. 2007). Woody and macroalgal debris are frequently deposited on continental slopes through physical or biological processes. For example, kelp is released from the coastal zone during storms, high swell events, and from herbivory (Duggins et al. 1989). Kelp and wood debris can be concentrated and transported to depths in submarine canyons to form enormous accumulations of organic material in the deep ocean (Vetter 1994, Vetter & Dayton 1998, 1999, McLeod & Wing 2007). Wood and kelp parcels also float out to sea, sinking as individual parcels spanning a broad range of sizes (1 - 1000 kg) to the seafloor (Wolff 1979, Smith 1983, C. Smith personal observations during >100 submersible and remotely operated vehicle dives off California and Hawaii).

It is long recognized that food falls at the deep-sea floor can contribute to beta diversity in deep-sea by creating patches of organic enrichment and chemical or physical disturbance (Stockton & DeLaca 1982, Smith & Hamilton 1983, Smith 1985, 1986, Grassle & Morse-Porteous 1987, Snelgrove & Smith 2002). However, patterns of succession resulting from the arrival of kelp and wood falls, and the identity of their characteristic species remain essentially unstudied along the bathyal Californian slope. Such information is essential to understanding the recycling of different types

of organic parcels (e.g., sunken kelp paddies, logs) on the continental slope and their roles in maintaining biodiversity in the deep northeast Pacific (Snelgrove et al. 1992, Snelgrove et al. 1996, Snelgrove & Smith 2002).

The effects of small-scale, low intensity disturbances on deep-sea infaunal communities, resulting from algal enrichment and scavenger disturbance, have been evaluated in a number of deep-experiments; these disturbances produce modest increases on species diversity (Levin & Smith 1984, Snelgrove & Smith 2002). However, substantial organic enrichment of deep-sea sediments (e.g., from macroalgae, wood falls, diatom detritus, and whale falls) can cause dramatic population enhancement of opportunistic and sulfophilic species (e.g. capitellid and dorvilleid polychaetes, leptostracans and cumaceans; (Turner 1977, Smith 1986, Grassle & Morse-Porteous 1987, Snelgrove et al. 1994, Smith et al. 2002, Smith & Baco 2003). Community patterns in organic-rich sediments around whale falls resemble the classic spatial succession described along organic-enrichment gradients in shallow-water [e.g., (Pearson & Rosenberg 1978)] with very low diversity and extremely high densities of opportunistic polychaetes species adjacent to the whale fall, moderate macrofaunal enhancement and species diversity at intermediate distances, and low abundances and high diversity in the background community (Smith et al. 2002, Smith & Baco 2003). During later stages of succession, deep-sea whale falls also support substantial assemblages of animals with chemoautotrophic endosymbionts, producing reducing habitats ecologically similar to vents and seeps (Smith & Baco 2003, Treude et al. 2009).

Large wood and kelp parcels also have the potential to produce intense organic enrichment and reducing habitats in deep-sea sediments. Deep-sea wood falls are initially processed by wood-boring xylophaganae bivalves (Turner 1977, Wolff

1979, Distel & Roberts 1997, Turner 2002, Voight 2007b) which may broadcast organic-rich fecal material onto surrounding sediments (Turner 1977), potentially creating organic and sulfide-rich sediments. The breakdown of large kelp accumulations at the deep-sea floor also has the potential to create organic and sulfide-rich sediment patches against an oligotrophic background (Vetter 1994, 1996, Vetter & Dayton 1998).

While deep-sea wood and kelp falls are common on regional scales, temporal patterns of infaunal community succession around large wood and kelp parcels remain very poorly studied. Thus, the contribution of wood- and kelp-fall communities to beta diversity in the deep sea, and their relationship to deep-sea reducing assemblages (e.g., vent, seep and whale-fall communities) cannot be evaluated. In this study, we investigated macroinfaunal structure for over 5 years around experimentally implanted kelp and wood parcels in the bathyal NE Pacific to test the following hypotheses:

- 1) *Large wood and kelp falls exhibit distinct patterns of infaunal macrobenthic community succession, promoting beta diversity in the deep sea;*
- 2) *There is little species overlap between sediment communities around large wood and kelp parcels and other reducing habitats (whale falls, seeps and vents) at similar depths in the deep sea.*

## **2.2. Materials and Methods**

### *Study site, deployments and sampling*

Experimental implantations of kelp and wood parcels were conducted near well-studied whale falls off southern California (Smith & Baco 2003) (Fig. 1.1). In

particular, wood and kelp parcels were implanted on the flat sediment-covered floor of Santa Cruz Basin (SCrB), California ( $\sim 33^{\circ} 27' N$ ,  $119^{\circ} 22' W$ , 1670 m depth, bottom temperature  $\sim 4^{\circ}C$ , bottom-water oxygen concentration  $260 \mu M$  (Treude et al. 2009).

Four parcels of wood and kelp were experimentally deployed at a mean water depth of 1670 m in the SCrB (Fig. 1.1, point A; Table 2.1), approximately 100 m from an experimental 30-ton whale carcass (Smith & Baco 2003, Treude et al. 2009). Wood parcels were deployed in October 1999 and May 2002 (Table 2.1). Kelp parcels were deployed in May 2002 and July 2002 (Table 2.1). Each kelp parcel consisted of  $\sim 100$  kg (4-5 whole plants) of fresh *Macrocystis pyrifera* contained in a 5-cm stretch mesh nylon net bag with one kelp holdfast protruding from the bag to facilitate sampling of kelp associated fauna; Fig. 2.1). The wood parcels consisted of  $\sim 200$  kg of untreated Douglas fir (*Pseudotsuga menziesii*) with four small, 2-kg wood packages inside a 2.5-cm stretch mesh nylon net bags attached to the upper surface (Fig. 2.1).

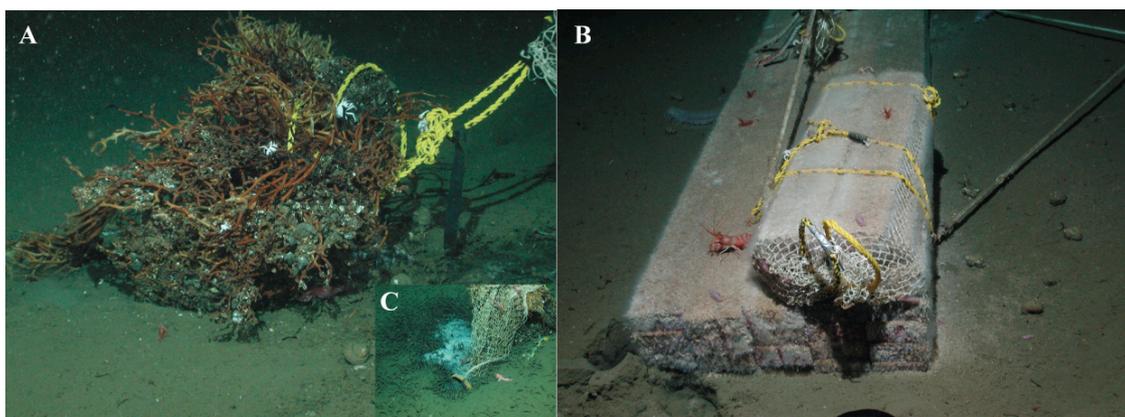


Figure 2.1 A. Kelp parcel deployed at Santa Cruz Basin after 0.25 y on the sea floor. Notice the presence of a whitish bacterial mat over the sediment nearby the parcel. Dark sediments around it represent sulfide rich sediments. B. 200Kg wood parcel that has been deployed at SCrB for two years. Small wood packs inside 2.5cm mesh net nylon bags are attached on the top of the parcel. C. Detail showing the presence of bacterial mats nearby the kelp falls at 0.25 y.

Kelp and wood parcels were sampled using the Remotely Operated Vehicle (ROV) *Tiburón* during three cruises on board of the RV *Western Flyer* (Table 2.1). Kelp parcels were sampled at 0.25 and 0.5 years after deployment, while wood parcels were sampled at 0.5, 1.8, 3 and 5.5 y after deployment (Table 2.1). Sampling occurred around distinct parcels for each time interval; because parcels were located within several hundreds meters of one another on the homogenous basin floor, we assumed that all fall types were bathed in the same larval pool, i.e., location effects were modest compared to treatment effects. At all sites, three randomly located transects radiating outward from the treatment were sampled, collecting tube cores (7 cm diameter, 10 cm deep) at 0 m, 0.5 m, 1.0 m and 2.0 m from the parcel. Background samples (17 replicate tube cores) were additionally collected in 2002, 2004 and 2005 at random locations of 9-100 meters away from any parcel, allowing averaging of temporal patterns in the background Santa Cruz Basin. Tube cores were immediately sectioned into 0-1, 1-5 and 5-10 cm horizons and fixed in 4% buffered formaldehyde-seawater solution until laboratory analysis, or frozen at -20°C for organic carbon analysis in laboratory.

*Table 2.1. Drop and sampling date from organic parcels artificially deployed at SCrB. Note that each wood and kelp parcels were sampled on distinct cruises, thus representing community succession on several time scales. Background cores were collected at three different cruises (see text).*

Parcel type / deployment number	Drop date	Depth (m)	Bottom position	Sampling date/ROV dive	Age at sampling
Wood (CRS 397)	Oct. 15, 1999	1670	33°29.604'N	Oct. 25, 2002/TD 494	3 years
			119°21.967'W	Mar. 1, 2005/TD 827	5.5 years
Wood (CRS 800)	May 1, 2002	1670	33°29.643'N	Oct. 24, 2002/TD 492	0.5 years
			119°22.107'W	Mar. 1, 2004/TD 652	1.8 years
Kelp (CRS 799)	May 1, 2002	1670	33°29.600'N 119°22.088'W	Oct. 25, 2002/TD 493	0.5 years

Kelp (CRS 806)	July 16, 2002	1670	33°29.691'N 119°22.078'W	Oct. 22, 2002/TD 489	0.25 years
				2002/ TD 490, 502, 505	
SCr Background		1670		2004/ TD 655 2005/ TD822, 823, 824	

### *Laboratory and data analysis*

In the laboratory, macrobenthic samples were sieved on a 300- $\mu$ m sieve, stained with Rose Bengal, sorted and identified to the lowest attainable taxonomic level. Metazoan trophic group classification was made according to (Kukert & Smith 1992). Sediment samples for organic carbon and nitrogen analysis were acidified to remove carbonates by repeated additions of sulfurous acid (8 % v/v) until no effervescence was observed (Verardo et al. 1990) and then analyzed using a Perkin-Elmer 2400 CHN Elemental Analyzer, with a limit of detection of 1 mg and 1.2 mg for C and N, respectively, while the precision was 0.3 % and 0.4 % for C and N, respectively. CHN standards were made with acetanilide and blanks were made of both non-acidified and acidified cups, both giving minor signals of TOC and TN.

A few top-ranked macrofaunal organisms at representative stages of community succession on kelp and wood parcels were selected for stable isotope analysis. Individuals were sorted using methanol-cleaned forceps and specimens were rinsed and cleaned in DI water. Calcareous shelled organisms were decalcified with phosphoric acid and placed in pre-weighed tin cups for overnight drying (35-40 °C). Multiple individuals of single species were combined in one sample to make up the necessary dry weight of 0.5-2 mg for analysis. Samples were combusted in a Eurovector elemental analyzer and resulting N<sub>2</sub> and CO<sub>2</sub> gases were separated by gas chromatography and admitted into an IRMS mass spectrometer for determination of

$^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios (reproducibility:  $\pm 0.5$  ‰ for  $\delta^{15}\text{N}$  and  $\pm 0.2$  ‰ for  $\delta^{13}\text{C}$ ).

Macrofaunal C-isotopic ratios were measured against a Pee Dee Belemnite (PDB) standard for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ . Results are expressed as delta ( $\delta$ ) notation representing the relative difference between sample and standard, where  $\delta X$  (‰) =  $[(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$ , where  $R = ^{15}\text{N}/^{14}\text{N}$  or  $R = ^{14}\text{C}/^{13}\text{C}$ .

Macroinfaunal organisms were fixed in 4% formaldehyde solution, potentially introducing artifacts in stable isotope values, although shifts in carbon isotope ratio are usually small compared to the wide natural C variability in marine food sources (Fry & Sherr 1989, Edwards et al. 2002, Sarakinos et al. 2002). In this study, we corrected for preservation artifacts by adding 1‰ to  $\delta^{13}\text{C}$  (Baco-Taylor 2002, Sarakinos et al. 2002, Demopoulos et al. 2007). Trophic shift boxes from potential organic matter sources at kelp and wood parcels helped to determine potential food sources (Fry 2006). Trophic changes of 3 ‰ were added to the range of  $\delta^{15}\text{N}$  values obtained for each organic matter source (DeNiro & Epstein 1978, Minagawa & Wada 1984, Fisher et al. 1994). Species that exclusively use a particular source for nutrition are expected to fall within the appropriate trophic-shift box. Isotope values from kelp plants were obtained from the published literature (Page et al. 2008). A multi-source mixing model was used to calculate proportional contributions of each primary organic matter source (i.e.: kelp, wood, sedimentary organic carbon and bacterial mats growing on sulphidic sediments around treatments) to the benthic food web [IsoSource software; (Phillips & Gregg 2003)]. This model examines all possible combinations of each source contribution (0-100%) in small increments (e.g., 1%), where the combinations that best fit to the observed species isotope signature are considered feasible (Phillips & Gregg 2003). A mixing polygon was drawn around fauna samples, with polygon *apices* representing end members for each organic

matter source (Demopoulos et al. 2007). Mixing contributions were calculated for consumers that fit within the mixing polygon, using *a priori* faunal corrected (i.e. trophic fractionation) isotope measurements for each trophic group (Kukert & Smith 1992, Phillips & Gregg 2003, Demopoulos et al. 2007). We assumed a  $\delta^{15}\text{N}$  fractionation of 3‰ for omnivores, deposit feeders and “other” trophic levels (Fry 1988, 2006, Demopoulos et al. 2007). Statistical tests to detect differences on the contribution of organic sources to the fauna were performed using the IsoError software (Phillips & Gregg 2001).

Statistical analyzes on sediment organic content were examined with Student t test of means or Kruskal-Wallis, when the number of replicates allowed. Patterns of faunal abundance, faunal similarity and diversity were compared among distances and time for each treatment type. Total densities around treatments and in ambient sediments were examined with One-Way ANOVA if normality of variances was present. If homogeneity of variances was not attained, a non-parametric Kruskal-Wallis test was performed. For significant ANOVA and Kruskal-Wallis results, *post-hoc* tests were used to examine difference in means [statistical package BioEstat© (Zar 1996)]. Species diversity was evaluated for pooled replicate cores at each distance sampled (n=1-4) due to the low density of metazoans. Hulbert’s rarefaction curves ( $ES_n$ ) were used to compare species diversity between treatments.  $ES_n$  gives an estimative of the number of species that would be found in a given number of individuals, interpolated from the number of species collected in each sample assuming a random distribution of individuals within samples (Hulbert 1971). Although this index tends to overestimate within-sample diversity, it is widely use to compare deep-sea samples of different sizes (Levin et al. 2001). Values of  $ES_n$  were compared at n=10, n=25 and n=50, where possible. Near treatment samples were

compared at higher  $n$  values, because these samples contained higher abundances and were particularly interesting as they were directly influenced by the treatments. Background replicate cores ( $n=17$ ) from 2002, 2004 and 2005 were combined to calculate a composite diversity from the background community; with confidence limits calculated from pooled cores. From rarefaction “knots” obtained in each pooled sample, a one-tailed 95% confidence interval was calculated using the  $T$  distribution. Diversity curves were then compared to the background confidence curve in order to test for statistical differences on diversity (Smith 1986). Additionally, Pielou’s evenness values ( $J'$ ) is given to provide further information on community structure (Clarke & Warwick 2001). The relative abundance of trophic guilds at each period sampled was integrated by distance in order to increase the power of statistical analyzes. Samples from 0 m and 0.5 m were compared against the background in order to evaluate changes in trophic structure nearby our treatments; and examined with ANOVA or Kruskal-Wallis.

Cluster analyses and non-metric multi-dimensional scaling (MDS) based on species-abundance data from standardized quantitative samples (PRIMER v6, (Clarke & Gorley 2006); were used to compare community structure across distance and time. Square-root transformations were used prior to multivariate analysis in order to balance the importance of common and rare species (Clarke & Warwick 2001). Clustering and ordination analyses were often combined to verify mutual consistency. Analysis of similarities (ANOSIM) was performed on standardized quantitative samples to determine significant differences between groups (distance and time) and dissimilarity percentages identified species contributions to these patterns (SIMPER analysis, (Clarke & Warwick 2001).

In order to determine the level of species overlap between kelp, wood and other reducing habitats, we restricted our direct comparisons to relatively abundant species (i.e., over 1% rel. ab. at any treatment type) collected nearby our treatments (i.e. 0 to 0.5 meters) and that were not sampled in ambient sediments (i.e. total of 17 background cores from 2002, 2004 and 2004). We excluded rare species (less than 1% rel. abundance) from our comparisons in order to account for difficulties in taxonomic identification and undersampling biases. Comparisons of species overlap with other published studies were carried out at the genus and species level in order to account for differences in taxonomic identification between studies.

### **2.3. Results**

#### *Sedimentary organic carbon and nitrogen at kelp and wood parcels*

After 0.25 years, percent organic carbon (TOC) in surface sediments near kelp falls was only 0.5% above background levels, with a spatial decrease in sedimentary TOC from 0 m to 1m (Fig. 2.2). After 0.5 years, the TOC levels at 0 meters was significantly higher than 0.5 meters away ( $t=-11.045$ , d.f.=2,  $p=0.008$ ), although no significant temporal increase in TOC was observed at 0 meters (Fig. 2.2). The combined TOC content in nearest 0-0.5 meters from the kelp falls at 0.5 y was significantly higher than 0.25 y and background sediments (Kruskal-Wallis,  $H=7.833$ , d.f.=2,  $p=0.02$ ). Sedimentary percent organic nitrogen (TON) was also slightly elevated near kelp parcels at 0.25 y, with significant differences from 0.5 meters and background sediments ( $t=6.05$ , d.f.=5,  $p=0.0018$ ; Fig. 2.2). Sedimentary C/N ratios confirm the presence of relatively labile (i.e., nitrogen-rich) material at 0 m after 0.25

y. C/N ratios were significantly lower at 0.25 y at 0 meters than all other distances and background sediments ( $t=-15.94$ , d.f.=4,  $p<0.01$ ; Fig. 2.2).

Sedimentary TOC and TON around wood parcels resembled those in background levels at 0.5 and 1.8 y after deployment (Fig. 2.2). However, by the 3 y, surface sediments adjacent to wood parcels had become massively enriched in organic enrichment, with TOC 3-6 times higher than background values ( $t=17.37$ , d.f.=1,  $p<0.001$ ; Fig. 2.2). In addition, C/N ratios were extremely high (up to 82.9 C:N;  $t=52.49$ , d.f.=1,  $p<0.001$ ) compared to all other distances sampled, suggesting enrichment from relatively refractory woody material. Mean TOC and C/N ratios in surface sediments near wood falls were still well above ambient levels after 5.5 y (Fig. 2.2). In addition, percent organic nitrogen was depressed in surface sediments near the wood parcel to distances of 2 m after 5.5 y (ANOVA,  $F=61.64$ , d.f.=1,  $p=0.0005$ ), suggesting that low C/N ratio woody organic material had spread some distance from the wood parcel (Fig. 2.2).

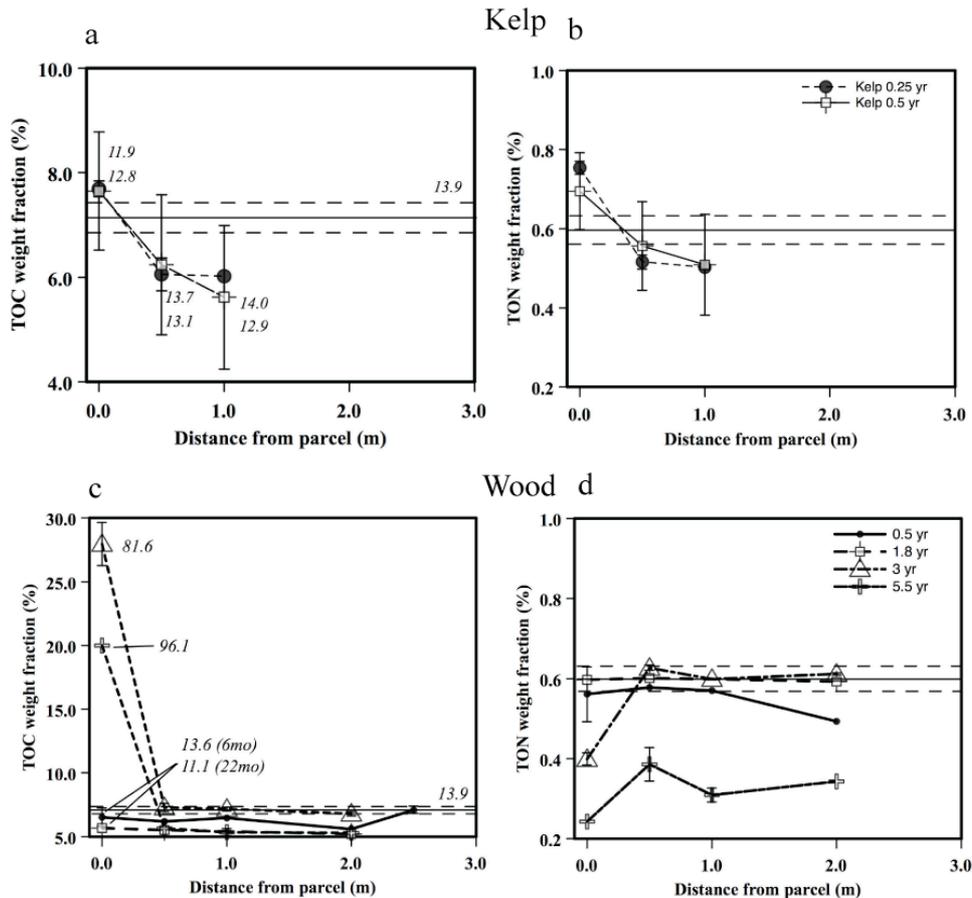


Figure 2.2. Upper panels: Mean ( $\pm 1$  SE) TOC and TON in surface sediments (0-2 cm) around kelp parcels. C/N ratios are shown in text format, with upper and lower values representing 0.25- and 0.5 y averages, respectively. Horizontal parallel lines demark average %TOC/TON ( $\pm 1$  SE) of background sediments and C/N ratio (in *italics*). Lower panels: Temporal variability on TOC and TON in surface sediments (0-2 cm) around wood parcels ( $\pm 1$  SE when  $n > 1$ ). C/N ratios are shown for 0 m and background samples only (in text format).

### Macrofaunal density, composition and patterns of succession

#### Kelp parcels

Spatial and temporal patterns of species composition and trophic structure indicate that kelp falls dramatically influenced infaunal communities over short spatial scales ( $< 1$  m) for at least 0.5 y. At 0.25 y, macroinfaunal abundance within 0.5 m of kelp parcel was significantly enhanced by  $\sim 5$ -fold relative to background

sediments (Fig. 2.3A). This pattern persisted relative unchanged after 0.5 y (Kruskal-Wallis,  $H = 24.05$ ;  $d.f. = 8$ ,  $p = 0.002$ ; Fig. 2.3A - *Inset*). At distances of over 0.5 meters from the kelp, macroinfaunal abundance remained near ambient levels at both 0.25 and 0.5 y (Fig. 2.3A).

Dorvilleid polychaetes and cumaceans dominated sediments within 0.5 m of kelp falls after 0.25 y and 0.5 y, but were rare in ambient sediments (Fig. 2.3B). The four top-ranked species at 0.25 y constituted over 88% of all individuals at 0 m, and dorvilleids and cumaceans represented over 36 and 34 % of the total macrofauna, respectively (Table 2.2, Fig. 2.4). At 0.5m, cumaceans still responded for over 55% of all macrofaunal individuals at 0.25 y (Fig. 2.3B, Fig. 2.4). At distances of  $\geq 1$  m from the kelp parcels at 0.25 y, background species dominated the infaunal community. At 0.5 y, cumaceans and dorvilleids still dominated the macrofaunal community at a distance of 0 m, with background species becoming abundant at distances  $\geq 0.5$  m from the kelp (Fig. 2.4, Table 2.2). The rapid increase in abundance of the two species of cumaceans (*Cumella* sp. A and Cumacean sp. K) and the dorvilleid *Ophryotrocha* sp. A within 0.5 m from the kelp treatments suggests this species are opportunistically responding to enrichment and disturbance conditions (Grassle & Grassle 1974, Pearson & Rosenberg 1978). The cumacean species that responded to the kelp enrichment in this study were not typically encountered in our background communities, but they colonize enrichment experiments in deep-sea regions, including fish and whale-falls in the NE Pacific (Smith 1986, Snelgrove et al. 1996, Smith et al. in prep).

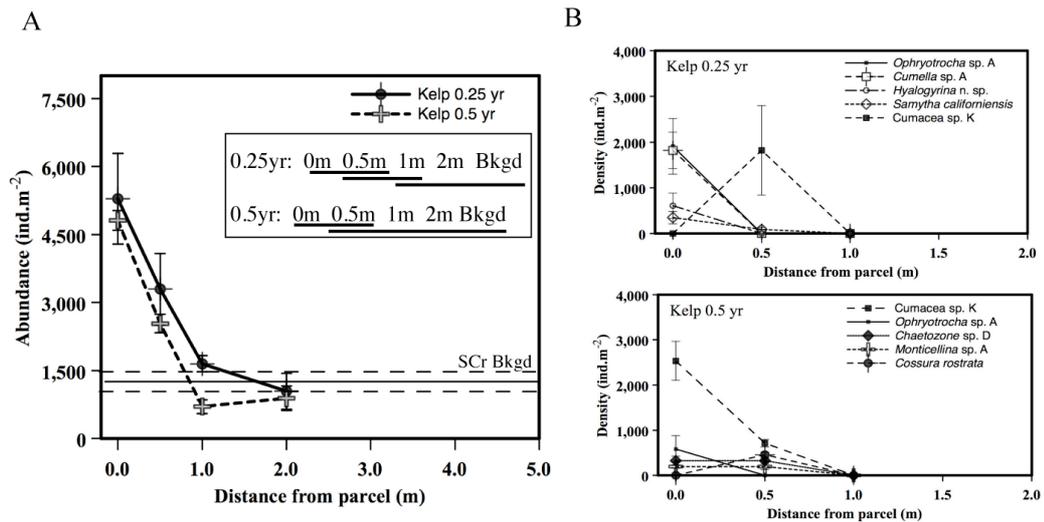


Figure 2.3. A. Mean macrofauna abundance at 0.25- and 0.5-y kelp parcels deployed in SCr Basin. The solid black line denotes the average background density ( $\pm$  1 SE, dotted line). Inset: A posteriori multiple comparisons based on Kruskal-Wallis test ( $H=24.05$ ,  $p<0.001$ ). Groups not underscored by a common line are statistically different at  $p<0.05$ . No significant difference was found between 0.25- and 0.5-y at the same distance. B. Abundance of top-ranked species at 0.25 y (top panel) and 0.5 y (bottom panel) kelp parcels.

The dominance of a *Ophryotrocha* sp. A and the two cumacean species, lowered species evenness ( $J'$ ) and diversity nearby kelp treatments, at both times sampled (Table 2.3). At 0 m, species dominance was significantly higher than background sediments during both periods sampled (Kruskal-Wallis,  $H= 12.96$ , d.f.= 4,  $p=0.01$ ). Species diversity ( $ES_n$ ) increased from 0.25 y to 0.5 y within 0.5 meters of kelp parcels (Table 2.3, Fig. 2.5). At 0.25 y, rarefaction diversity was significantly lower within 0.5 m of the kelp treatments, if compared to background samples (Fig. 2.5). At 0.5 y, diversity increased towards background sediments at distance of over 0.5 m, but diversity was still significantly depressed at the 0 m macroinfaunal community (Fig. 2.5). The diversity changes around kelp treatments suggest that although areas nearby the parcels were being negatively affected by the enrichment and disturbance conditions after 0.5 y, the macrofauna in sediments over 0.5 m had returned to background conditions.

Table 2.2. Mean density per core ( $38.5 \text{ cm}^{-2}$ ) and relative abundances of top 5 ranked macrofaunal species at 0 m and 0.5 m from kelp parcels, and background sediments in SCr Basin. (P) Polychaeta, (Cr) Crustacea, (M) Mollusca.

Kelp 3 months									
Rank	Species	0 m		0.5 m		1 m		2 m	
		Mean density	Rel %						
1	<i>Ophryotrocha</i> sp. A (P)	7.3 (2.3)	36.1%						
2	<i>Cumella</i> sp. A (Cr)	7.0 (1.5)	34.4%						
3	<i>Hyalogyrina</i> n. sp. (M)	2.3 (1.0)	11.5%						
4	<i>Samytha</i> cf. <i>californiensis</i> (P)	1.3 (0.5)	6.6%	0.3 (0.2)	2.6%				
5	Cumacea sp. K (Cr)			7.0 (3.8)	55.3%	0.3 (0.2)	5.3%		
<b>Total percent</b>			88.6%		57.9%		5.3%		
Kelp 6 months									
1	Cumacea sp. K (Cr)	9.8 (1.7)	52.7%	2.8 (0.6)	28.2%	0.3 (0.3)	10.0%		
2	<i>Ophryotrocha</i> sp. A (P)	2.3 (1.1)	12.2%						
3	<i>Chaetozone</i> sp. D (P)	1.3 (0.4)	6.8%	1.3 (0.3)	12.8%	0.7 (0.3)	20.0%	1.7 (1.2)	38.5%
4	<i>Monticellina</i> sp. A (P)	0.8 (0.1)	4.1%	0.8 (0.5)	7.7%	0.7 (0.7)	20.0%	0.3 (0.3)	7.7%
5	<i>Cossura</i> cf. <i>rostrata</i> (P)			1.8 (0.5)	17.9%	0.3 (0.3)	10.0%	0.3 (0.3)	7.7%
<b>Total percent</b>			75.7%		66.7%		60.0%		53.8%
Background (100m)									
		Mean density		Rel %					
1	<i>Cossura</i> cf. <i>rostrata</i> (P)	0.8 (0.4)		18.4%					
2	<i>Chaetozone</i> sp. D (P)	0.7 (0.3)		15.8%					
3	<i>Monticellina</i> sp. A (P)	0.4 (0.2)		10.5%					
4	<i>Tharyx</i> sp. A (P)	0.3 (0.2)		7.9%					
<b>Total percent</b>				52.6%					

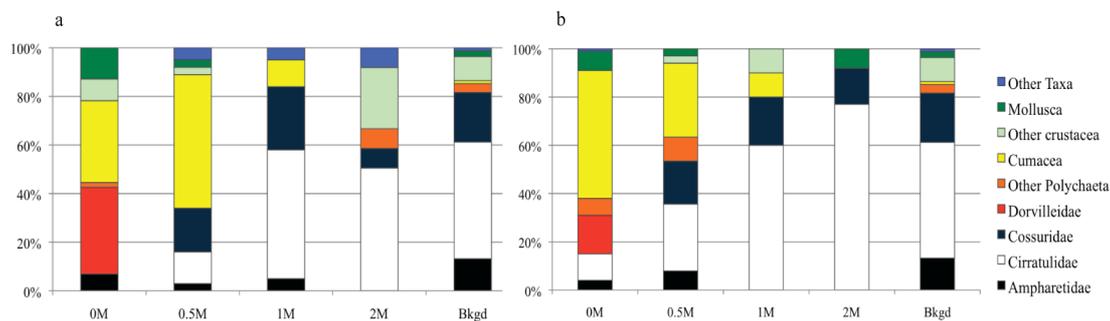


Figure 2.4. Macro-infauna composition around kelp parcels at 0.25 y (a) and 0.5 y (b) in SCr Basin. Bkgd - Background fauna from SCr Basin in 2002, 2004 and 2005 ( $n=17$  cores).

Table 2.3. Diversity indices from kelp parcels and background sediments at SCrB. Mean number of individuals ( $M$ ) per core ( $38.5 \text{ cm}^{-2} \pm \text{ISE}$ ).  $J'$ -Pielou evenness index,  $ES_n$ - Expected number of species at 10, 25 and 50 individuals (inside brackets) per sample.

	M		J'		ES 10		ES 25 (50)	
	0.25 y	0.5 y	0.25 y	0.5 y	0.25 y	0.5 y	0.25 y	0.5 y
<b>0m</b>	19.7(6.1)	18.5(1.7)	0.71	0.64	4.1	4.6	5.7 (6.8)	7.8 (11.1)
<b>0.5m</b>	12(5.7)	8.8(1.2)	0.76	0.93	3.5	6.0	5.2	10.4
<b>1m</b>	3.7(0.9)	2.3(0.3)	0.97	1.00	4.9	-	-	-

<b>2m</b>	3.7(2.7)	3.3(1.5)	0.90	1.00	6.0	5.0	-	-
<b>Bkgd</b>	3.3 (0.1)		0.94		6.2		9.8(13.0)	

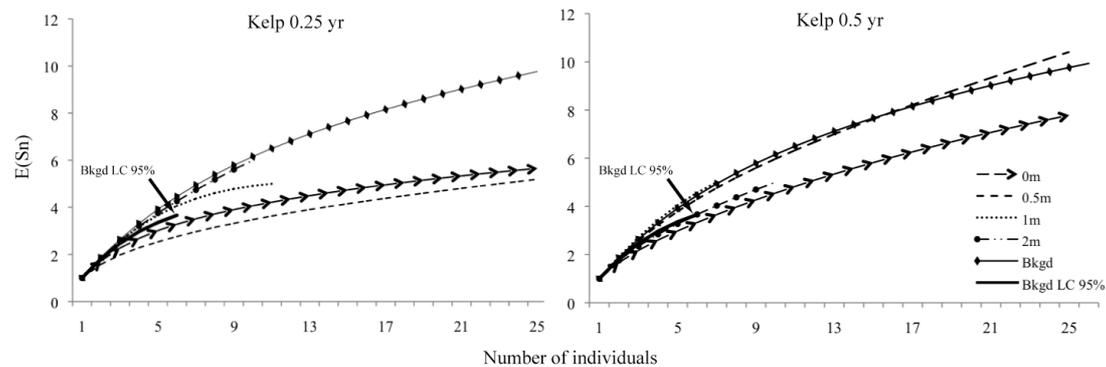


Figure 2.5. Rarefaction diversity for Kelp macrofauna 0.25 y and 0.5 y.

Trophic-group analysis revealed large changes adjacent to kelp parcels (i.e.,  $\leq 0.5$  m) after 0.25 – 0.5 y. At 0.25 y, the high abundance of dorvilleids and cumaceans dramatically increased the proportion of omnivores (OMNI, Kruskal-Wallis,  $H=6.836$ ,  $d.f.=2$ ,  $p=0.02$ ) and the “other” trophic group (Kruskal-Wallis,  $H=16.624$ ,  $d.f.=2$ ,  $p<0.01$ ) within 0.5 meters from the treatments (Fig. 2.6). The augmented number of “other” trophic group at 0 m was still significant after 0.5 y ( $p<0.01$ ; Fig. 2.6). Surface deposit feeders (SDF) were slightly depressed nearby kelp parcels compared to background sediments, but no significant trend was observed (Fig. 2.6). In addition, sub-surface deposit feeders (SSDF) were wholly absent adjacent to kelp parcels (0 m) after 0.25 -0.5 yr (Kruskal-Wallis,  $H=5.70$ ,  $d.f.=1$ ,  $p=0.018$ ; Fig. 2.6). This suggests that sub-surface deposit feeders were particularly affected by the physical and organic disturbances in sediments nearby the kelp parcels. Trophic differences between 0.25 and 0.5 y at 0.5 m suggests recovery towards background conditions at 0.5 y, with an increase in the importance of SDF accompanied by a

strong decrease in the “other” trophic group (Fig. 2.6). The elevated number of omnivores and “other” trophic guilds are consistent with the opportunist species that colonized sediments and possibly with the augmented amount of detritus within 0.5 m from the treatments. Complete abundance and relative contribution of species at kelp parcels are presented in Appendix 2A.

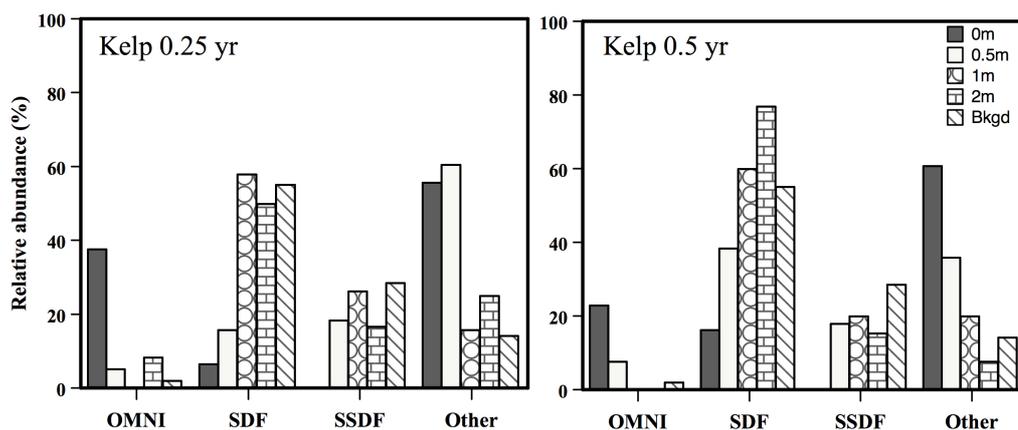


Figure 2.6. Percentage of total abundance in trophic groups at 0.25 y and 0.5 y kelp parcels at SCr Basin. OMNI-Omnivores, Carnivores and Scavengers; SDF-Surface deposit feeders; SSDF-Sub-surface deposit feeders, Other- Trophic groups not determined (see methods).

### Wood parcels

Wood parcels also exhibited dramatic effects on macrofaunal community structure in space and time. The sediment near the wood parcels accumulated a massive number of *Xylophaga washingtonia* recruits during the first 0.5 – 3 years (Fig. 2.7A), presumably resulting from settling response to available wood substrate nearby. Brooder species of *Xylophaga* present on the wood parcels may have been the source of juveniles that rained down to the sediments after 0.5 y (Voight 2007a). The *Xylophaga* bivalves never attained adult size within the sediments, although adults were present on the wood parcels from 0.5 y onward. Thus, *Xylophaga* represents a sink population resulting from mass effects of the wood parcel (Leibold et al. 2004).

Because *Xylophaga* appear to represent sink populations in the sediment community derived from mass effects of the wood parcels, we also evaluated macrofaunal community patterns with *Xylophaga* removed from the analyses. At 0.5 y, mean macrofaunal abundance, without *Xylophaga*, was significantly higher at 0 m than in background sediments; at further distances macrofaunal abundances resembled background levels (Fig. 8B; Kruskal-Wallis test,  $H=27.98$ , d.f.=13,  $p=0.03$ ).

Macrofaunal densities around the wood parcels were significantly elevated at 0 m after 1.8 y (ANOVA,  $F=12.906$ , d.f.=3,  $p=0.003$ ), dropping to background levels by 0.5 m from the parcels (Fig. 2.7B). At subsequent sampling times (3.0 and 5.5 y), macrofaunal abundance progressively increased adjacent to the parcels, reaching very high numbers ( $15,100 \pm 2400$  ind.m<sup>-2</sup>) at 0 m after 5.5 y ( $p<0.001$ ; Fig. 2.7B). However, macrofaunal enhancement was limited in a spatial scale, with slight increases after 1.8 – 5.5 y at 0.5 m, at all time points, and background levels of abundance at  $\geq 1$  m from parcels even at 5.5 y, when abundances at 0 m were 15 times background levels (Fig. 2.7B).

At the species level, Cumacean sp. K dominated macrofaunal assemblages adjacent to wood parcels after 0.5 y, although three background species (*Monticellina* sp. A, *Chaetozone* sp. D and *Cossura rostrata*) remained among the 5 dominant species (Fig. 2.8, Table 2.4). By 1.8 y, *Ophryotrocha* sp. A (a dorvilleid) and ampharetid polychaetes dominated assemblages at 0 - 0.5 m, with cumaceans also abundant at 0.5 m (Table 2.4); at further distances, background polychaetes continued to dominate (Fig. 2.8). By 3.0 y, dorvilleids (two species of *Ophryotrocha*) had achieved very high densities at 0 m, representing over 40% of the macrofauna (Fig. 2.8, Table 2.4) and the chemoautotrophic-symbiont-containing mytilid *Idas washingtonia* was first encountered at the wood parcels (Table 2.4). At 5.5 y,

dorvilleids (*Ophryotrocha* and *Parougia*) and two species of ampharetids dominated the macrofauna at 0 m, with very high abundances (Table 2.4, Fig. 2.8); Cumacean sp. K, was also abundant. None of the dorvilleids, ampharetids and cumaceans were collected in the background community, or even off distances of 2 m from the wood falls. This, plus their larger response to kelp parcels suggest that they are opportunistic species, or may be wood associated species enhanced by mass effects (Leibold et al. 2004) from the adjacent wood parcels.

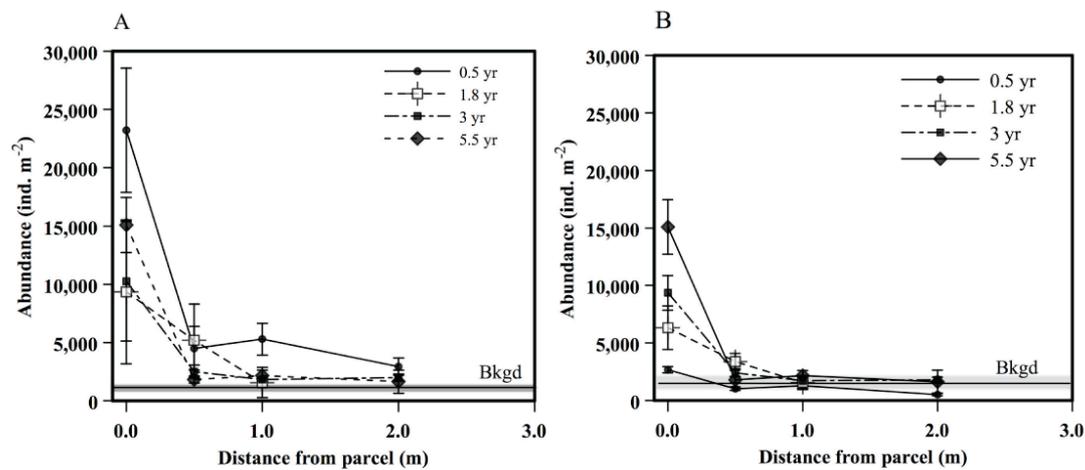


Figure 2.7. Temporal variability on macroinfaunal abundance at the wood parcels (Average  $\pm$  1SE). A. *Xylophaga* recruits included; B. *Xylophaga* not included. The solid black line and shading represent the average background (100m) abundances ( $\pm$  1SE).

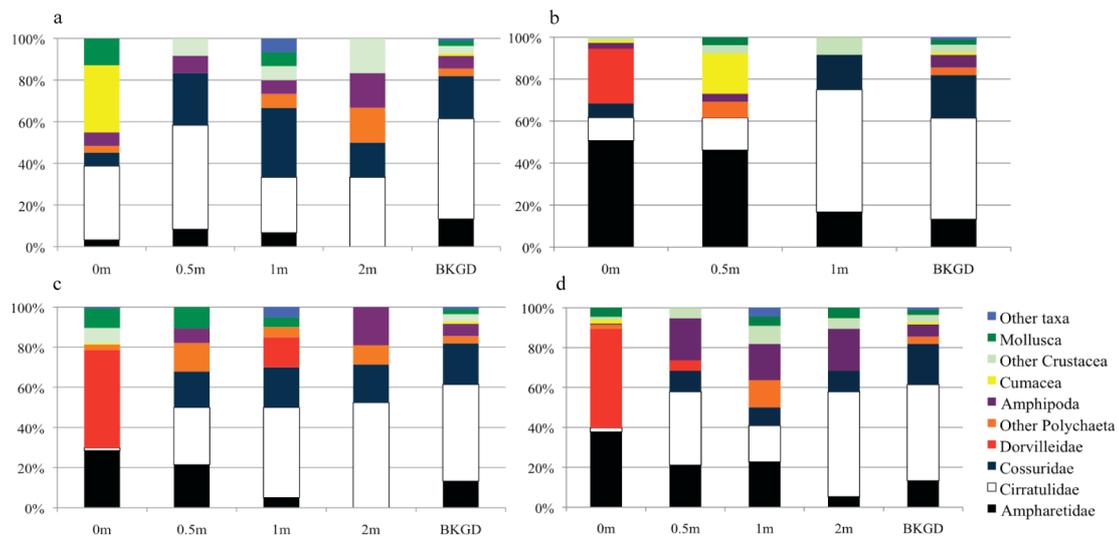


Figure 2.8. Sediment macrofaunal composition at the wood parcels deployed at SCr Basin. a. 0.25 y, b. 1.8 y, c. 3 y, d. 5.5 y.

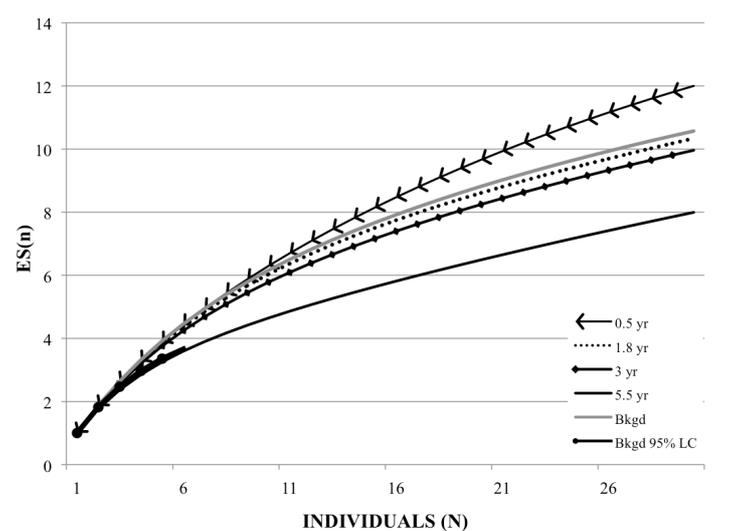
Table 2.4. Mean density per core ( $38.5 \text{ cm}^{-2}$ ) and relative abundances of top 5 ranked macrofaunal species at 0m and 0.5m at wood parcels and background fauna at SCr Basin. The total percentage of top ranked species is given below each list. (P) Polychaeta, (Cr) Crustacea, (M) Mollusca.

Wood 0.5 year									
		0 m		0.5 m		1 m		2 m	
Rank	Species	Mean density	Rel %						
1	Cumacea sp. K (Cr)	3.3 (1.3)	32.3%						
2	Monticellina sp. A (P)	1.7 (0.9)	16.1%	1.3 (1.3)	33.3%				
3	Bivalve sp. Q (M)	1.0 (0.6)	9.7%						
4	Chaetozone sp. D (P)	0.7 (0.7)	6.5%	0.3 (0.3)	8.3%	0.3 (0.3)	6.7%	0.7 (0.3)	33.3%
5	Cossura rostrata (P)	0.7 (0.3)	6.5%	0.3 (0.3)	8.3%	1.0 (1.0)	20%		
<b>Total percent</b>			<b>71.1%</b>		<b>49.9%</b>		<b>26.7%</b>		<b>33.3%</b>
Wood 1.8 years									
1	Ophryotrocha sp. A (P)	4.7 (4.7)	19.2%						
2	Amelina sp. A (P)	2.7 (2.7)	11.0%						
3	Samytha californiensis (P)	2.0 (1.0)	8.2%	0.5 (0.4)	3.8%				
4	Cossura rostrata (P)	1.7 (0.9)	6.8%			1.0 (1.0)	16.7%		
5	Cumacea sp. K (Cr)			2.5 (2.0)	19.2%				
<b>Total percent</b>			<b>45.2%</b>		<b>23.0%</b>		<b>16.7%</b>		
Wood 3 years									
1	Ophryotrocha sp. A (P)	8.3 (5.4)	22.9%						
2	Ophryotrocha sp. G (P)	6.5 (3.9)	18.1%						
3	Samytha californiensis (P)	4.8 (0.3)	13.2%			0.3 (0.3)	5.0%		
4	Ilyarachna profunda (Cr)	2.5 (0.5)	6.9%						
5	Pseudophryotrocha sp. A (P)	1.0 (0.4)	2.8%						
6	Idas washingtonia (M)	1.0 (0.4)	2.8%						
<b>Total percent</b>			<b>66.7%</b>				<b>5.0%</b>		
Wood 5.5 years									
1	Ophryotrocha sp. A (P)	17.0 (2.5)	33.8%						
2	CRS Ampharetid sp. 18 (P)	15.0 (12.5)	29.8%						
3	Parougia sp. A (P)	7.3 (5.5)	14.6%						
4	Samytha californiensis (P)	2.0 (1.2)	4.0%						
5	Cumacea sp. K (Cr)	1.0 (0.6)	2.0%						
<b>Total percent</b>			<b>84.2%</b>						
Background 2002-2005									
		Mean density		Rel %					
1	Cossura cf. rostrata (P)	0.8 (0.3)		16.9%					
2	Chaetozone sp. D (P)	0.7 (0.2)		14.5%					
3	Monticellina sp. A (P)	0.6 (0.2)		13.3%					
4	Tharyx sp. A (P)	0.3 (0.1)		6.0%					
5	Monticellina sp. B (P)	0.3 (0.1)		6.0%					
<b>Total percent</b>				<b>56.7%</b>					

The increase in macrofaunal density at 0 m from 0.5 y to 5.5 y was followed by a significant decrease on community evenness in the latter period ( $J'$ , ANOVA,  $F=8.384$ ,  $d.f.=3$ ,  $p=0.001$ , Table 2.5), resulting from higher densities of opportunist species. At the other periods,  $J'$  was always within background levels (Table 2.5). Rarefaction diversity demonstrates a clear temporal pattern adjacent to wood parcels. At 0.5 y, rarefaction diversity adjacent to the wood parcel falls above background levels (Table 2.5, Fig. 2.9); diversity then progressively declines at 0 m from 1.8 to 5.5 y (Table 2.5, Fig. 2.9). The diversity curve at 5.5 y falls well below all other curves and is significantly lower than background (Fig. 2.9).

Table 2.5. Average number of individuals ( $M$ ) per core ( $38.5\text{cm}^2 \pm 1\text{SE}$ ), Pielou's species evenness ( $J'$ ) and expected diversity around wood parcels deployed at SCr Basin. Expected number of species  $E(S_n)$  estimated from 10 and 25 individuals ( $ES_{25}$  inside brackets).

	$M$				$J'$				$ES_{10} (ES_{25})$			
	0.5yr	1.8yr	3yr	5.5yr	0.5yr	1.8yr	3yr	5.5yr	0.5yr	1.8yr	3yr	5.5yr
<b>0m</b>	10.0 (0.9)	16.0 (5.0)	30.5 (5.6)	48.3 (7.6)	0.92	0.83	0.84	0.67	6.3 (10.9)	6.0 (9.5)	5.8 (9.2)	4.6 (7.3)
<b>0.5m</b>	3.3 (0.8)	7.5 (0.4)	5.3 (0.5)	6.0 (0.9)	0.93	0.87	0.93	0.95	7.0	7.2	5.6	7.3
<b>1m</b>	2.7 (0.5)	5.0 (0.0)	3.0 (0.3)	5.7 (0.2)	0.91	0.97	0.98	0.93	7.0		7.5	6.8
<b>2m</b>	1.7 (0.2)	-	6.0 (0.3)	4.7 (1.6)	1.00	-	0.96	0.97			5.4	6.7
<b>Bkgd</b>	3.3 (0.1)				0.94				6.2 (9.8)			



*Figure 2.9. Hulbert's rarefaction plot detailing the temporal variability on macroinfaunal diversity at the wood parcels (0 meters only).*

Functional group analysis also indicated dramatic wood parcel effects on the adjacent macrofaunal community, increasing in intensity from 0.5 – 5.5 y. At 0.5 y, the dominant trophic groups were largely typical of ambient sediments, although at 0 m the importance of the “others” group slightly increased due to the presence of the apparently opportunistic Cumacean sp. K (Fig. 2.10). By 1.8 y, omnivores at 0 m had increased to 26%, and rose to 50% of the macrofauna at 3.0 - 5.5 y (Fig. 2.10). After 3 y, the omnivores within 0.5 m from wood falls were significantly more numerous than the 0.5 y community and the background sediments (Kruskal-Wallis,  $H=12.19$ ,  $d.f.=4$ ,  $p=0.016$ ). SDF's were relatively abundant during the whole period analyzed (over 30%), with ampharetids being dominant nearby the parcels and cirratulids in background sediments. Subsurface deposit feeders usually represented less than 1% of the infauna at 0 m during all periods (Fig. 2.10). Complete abundance and distribution of species at wood parcels are presented in Appendix 2B.

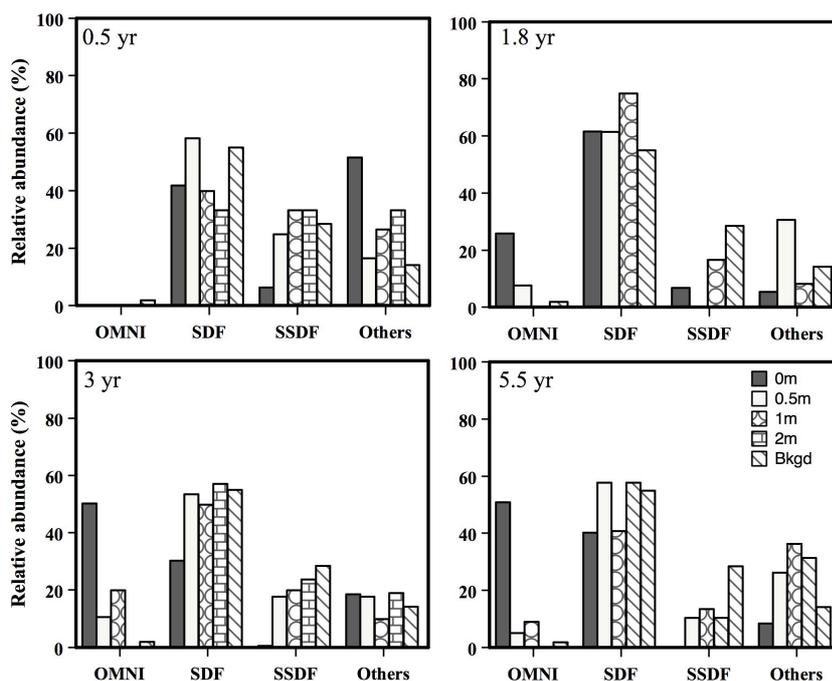


Figure 2.10. Trophic group patterns at the wood parcels implanted at SCr Basin. OMNI-Omnivores, Carnivores and Scavengers; SDF-Surface deposit feeders; SSDF-Sub-surface deposit feeders, Other-Trophic groups not determined.

#### MDS patterns of faunal distribution at Kelp and Wood parcels

Nonmetric Multidimensional Scaling (MDS) analyses further elucidate successional patterns in space and time around the kelp and wood parcels. At kelp falls, 0 m samples from 0.25 and one replicate from 0.5 y separated strongly from all other distances (Fig. 2.11, Two-way ANOSIM  $R=0.358$ ,  $P=0.001$ ). The top-ranked species at 0 m accounted for over 70% of the dissimilarity from the other samples at both time intervals (SIMPER). All other samples from 0.5 m to background, clustered with each other at 0.25 and 0.5 y, with no distinct difference between them (Fig. 2.11, ANOSIM). Overall, highest dissimilarities in sediments nearby kelp falls and background communities resulted from the dominance of apparently opportunistic

species adjacent to the kelp fall at 3 months, and the low background densities of local species.

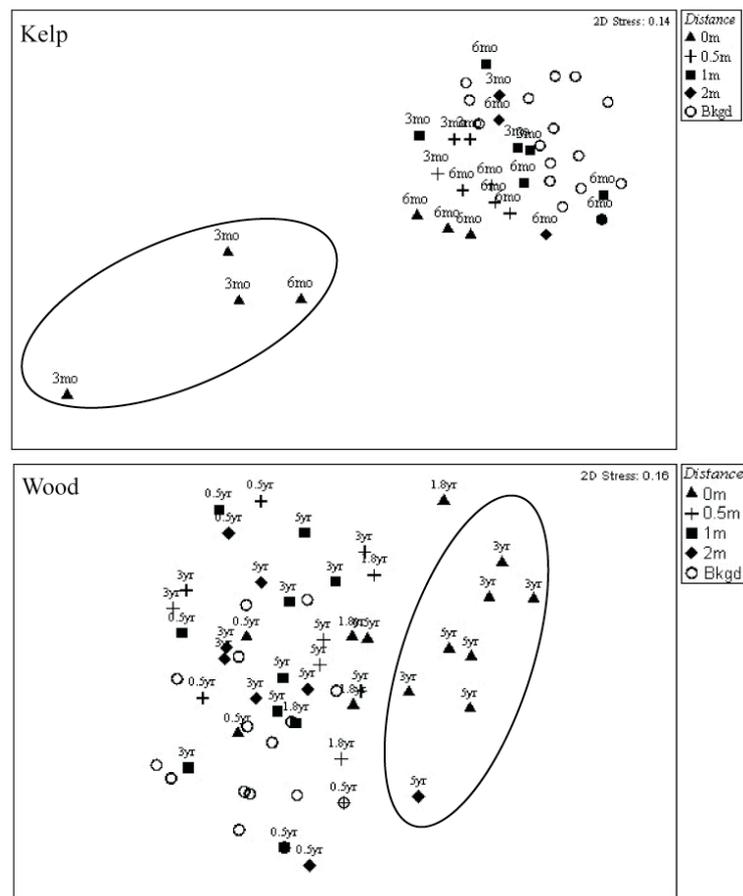


Figure 2.11. Multidimensional scaling plot (MDS) of macrofaunal communities ( $>300\mu\text{m}$ ) at Kelp (top panel) and Wood (bottom panel) parcels on SCr Basin. Grouped symbols denote stronger similarity among samples (see text).

At wood parcels, the MDS also revealed marked spatial and temporal patterns (Two-way ANOSIM,  $p < 0.01$ ). At 0.5 y and 1.8 y, all samples generally grouped together with no evident difference between treatments and background communities (Fig. 2.11, ANOSIM). A dramatic shift in macrofaunal community structure was indicated at 0 m at 3 y and persisted to 5.5 y (Fig. 2.11, ANOSIM,  $R = 0.319$ ,  $P = 0.001$ ). The top ranked macrofaunal species (including apparently opportunistic dorvilleids in the genus *Ophryotrocha*) at these two later periods accounted for over 20% of the spatial dissimilarity among samples (SIMPER). Pairwise comparisons

revealed a lower degree of dissimilarity between 5.5 y and the two first periods sampled, suggesting that macrofaunal community structure by 5.5 y may have been recovering towards background conditions (ANOSIM).

These results confirm our first hypothesis, that kelp and wood falls promote very distinct patterns of macrobenthic community succession. The dynamics of dominant species were distinct between kelps and wood, with kelps promoting early changes (scales of 1-3 months) on community composition, which lowered local diversity in the short scale as a result of a massive colonization by a few omnivore species (cumaceans and dorvilleids). On the other hand, dominant species altered community structure around wood parcels only after 1 – 2 years, but the duration of the organic enrichment promoted significant changes for over 5 years. A higher number of species colonized sediments around wood falls, likely as a consequence of increased food availability, but the fauna became less diverse with time as densities of opportunist species increased.

#### *Stable isotope analysis at Kelp and Wood parcels*

Potential primary organic matter sources at kelp and wood falls include kelp and wood biomass, sedimentary matter from deposited phytoplankton, and biomass from chemoautotrophic sulfur-oxidizing bacteria utilizing sulfide emitted from kelp or wood parcels. Kelp biomass (*Macrocystis pyrifera*) had the highest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope values from all primary organic sources (Table 2.6). Sediment organic carbon and wood fragments had very similar isotope signatures (Table 2.6), which were consistent with previous studies in the basin (Baco-Taylor 2002). Mats of sulfur-oxidizing chemoautotrophic bacteria growing on whale skeletons off southern California had  $\delta^{13}\text{C}$  signatures similar to the other organic sources but with the lowest

$\delta^{15}\text{N}$  values compared to the other sources (Table 2.6). Isotope signatures from sulfur-oxidizing bacteria can have a much wider isotopic range due to variable inorganic C sources (Ruby et al. 1987).

Table 2.6. Stable isotope values from primary organic matter sources used in this study. End members values were used in the mixing model calculations in order to account for maximum environmental variability. Values in delta notation.

	N	13C	15N	End members		Ref.
		Average (min/max)		13C(Min/Max)	15N(Min/Max)	
		-20.3				
Sedimented phytoplankton	2	(-22.1/-18.4)	6.7 (6.6/6.8)	-22.9/-17.6	6.6/6.8	This study
		-13.0				
Kelp biomass	-	(-13.8/-12.2)	9.1 (8.5/9.7)	-14.1/-11.9	8.3/9.9	Page et al., 2008
		-24.3				
Wood biomass	2	(-24.5/-24.2)	5.5 (4.9/6.1)	-24.6/-24.1	4.6/6.3	This study
		-20.4				
Sulfur-oxidizing bacterial mats	3	(-21.6/-19.3)	0.6 (-4.3/7.4)	-22.1/-18.8	-6.7/9.8	Baco and Smith, unpubl.
Chemosynthetic bacteria	-	(-46/-20)	-12			Ruby et al., 1987

The top-ranked invertebrates associated with kelp parcels generally had isotope values out of the trophic box, which indicates that they did not feed exclusively on any of those primary organic sources (Fig. 2.12). Temporal differences on isotope signatures from the specimens analyzed were minimal. Based on mixing model calculations, individuals of *Ophryotrocha* sp. A and *Cumella* sp. A from 0.25 y derived most of their nutrition on kelp biomass (57-66% and 58-63%, respectively; Table S2.1, Supp. Material). Kelp biomass contributed significantly to the isotopic signature of *Ophryotrocha* at 0.25 y if compared to sediments; but with increased contribution of sediments in the diet at 0.5 y, confirming their omnivore diet and suggesting an opportunistic feeding (Table S2.1). Due to wide temporal variability on

isotope signatures, we could not determine the contribution of food sources for Cumacean sp. K (Fig. S2.1, Suppl. Mat.). After 0.5 y, this species had isotope values (-38.1 and -11.7‰,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  respectively), strongly suggesting nutrition derived from chemoautotrophic food sources [Fig. 2.12; (Fisher et al. 1994, Deming et al. 1997)].

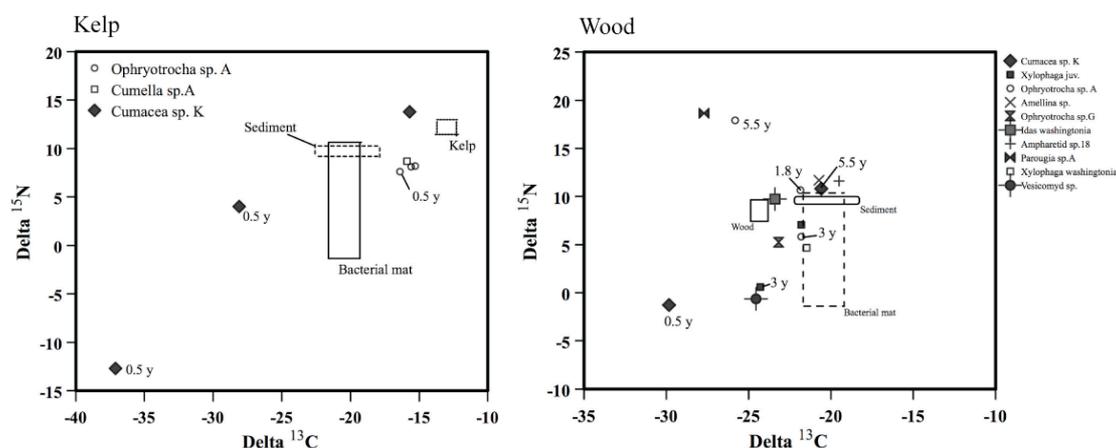


Figure 2.12.  $\Delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of top ranked organisms from kelp and wood parcels deployed in SCrB. Left: Organisms from kelp parcels (0.25 y unless otherwise indicated). Right: Organisms from wood parcels sampled from 0.5 y to 5.5 y (species with temporal replicates are indicated separately). Boxes represent one trophic shift for a given organic matter source (source indicated next to each box, see methods).

At 0.5 y wood parcels, Cumacean sp. K also had relatively light  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, once again suggesting nutrition based on chemoautotrophic production (Fig. 2.12). However, at later times at wood parcels, this species had heavier isotope ranges consistent with feeding on wood, sediment organic carbon, and/or sulfur bacteria, which may indicate an opportunistic feeding strategy. A few top ranked invertebrates at wood parcels fell into, or were close to the trophic shift box for sulfur bacteria, sediment organic matter, wood or some combination of these (Fig. 2.12). Surface deposit feeders such as the ampharetids *Amelina* sp. and Ampharetid sp.18 appear to feed primarily on sediments, while the other dorvilleids fall into areas which may

reflect a mixture of sources that were out of the range for mixing calculations, but still suggesting that they are omnivorous (Fig. 2.12, Suppl. Material Fig. S2.1). There is no evidence that any animals fed exclusively on the wood material, but the wide  $\delta^{15}\text{N}$  signatures on the fauna highlight the additional availability of food sources (e.g. dead faunal biomass) and trophic levels (i.e. scavengers) that existed around these parcels. Mixing model calculations constrained contributions of primary food sources for only three species at wood falls (Fig. S2.1). The diet of the dorvilleids *Ophryotrocha* sp. A and *Ophryotrocha* sp. G had a stronger contribution of bacteria and wood at 3 y (28-32% and 41-60%, respectively), with *Ophryotrocha* sp. G ingesting lower amounts of sediments (Table S2.1). An augmented signal of wood biomass in the fauna after 3 y is consistent with the increased input of organic carbon originated from the break up of the wooden matrix from the parcels to the sediments nearby. The opportunistic feeding on abundant organic sources within the sediments may also have promoted ingestion of bacterial biomass, but its close isotopic signature with the wood material limits further assumptions about its importance to the fauna (Suppl. Mat.). The wood borer *Xylophaga* had a larger contribution of wood material as a juvenile (> 50%), but the adult specimen had increased bacterial carbon contribution, probably reflecting its microbial symbiosis (Distel & Roberts 1997); Table S2.1).

#### *Biodiversity and species overlap between kelp, wood and other chemosynthetic habitats*

This study collected a total of 64 identified macrofaunal species, of which 27 were found at kelp falls and 53 at wood falls. The dominant species found at kelp and wood falls were not sampled in ambient sediments (Tables 2.2 and 2.4). From the dominant species sampled at the parcels (> 5% rel. abundance), there were 6 species

that overlap between kelp and wood parcels (9%), and four of those species (*Samytha californiensis*, *Ophryotrocha* sp. A, *Cumacea* sp. K and *Cumella* sp. A) are likely to be enrichment opportunists (Table 2.7). In addition, one species of polynoid (*Subadyte mexicana*), which is likely a predator, was also shared between wood and kelp parcels, evidencing the increased effect of organic enrichment on the composition of both communities (Table 2.7). Kelp and wood parcels also attracted unique species absent from background communities and the other parcel type. From the 7 species exclusively found at kelps, the gastropod *Hyalogyrina* n.sp. was the most abundant, and is a new species first encountered on whale-falls (Smith & Baco 2003). The other 6 species did not achieve high densities at kelp falls, making it difficult to distinguish their kelp “endemism” from undersampling of the background assemblage.

The wood attracted 17 species not found on kelp falls or in ambient sediments, including six dorvilleid species, three ampharetid species, the isopod *Ilyarachna profunda*, and the bivalves *Idas washingtonia* and *Xylophaga washingtona*. As for kelp falls, many species were rare, again making it difficult to resolve wood-fall endemism from rarity in the background community (Table 2.7). The wood-borer *Xylophaga*, the ampharetids *Amelinna* sp. A and Ampharetid sp. 18 reached massive abundances around wood falls, probably either raining down from the parcel or exploiting rich organic conditions. The high number of dorvilleids in the wood parcels suggests these habitats provide analogous organic conditions (but less intense) to whale carcasses, which host over 40 dorvilleid species (Smith & Baco 2003).

Kelp and wood parcels share together 21 species with whale falls (25%), of which 13 species exhibit enrichment response nearby both organic islands (Smith et al. in prep). The level of overlap between kelp, wood and the San Clemente seep is

lower (2 species) including to the ampharetid *Amelinna sp. A.* and the chemoautotrophic-symbiont-containing mytilid *Idas washingtonia*. Other dorvilleid species (or genus) are shared with seep and hydrothermal vent habitats (Table 2.7), summing to a total of 9 shared species (14%, Table 2.7). Kelp and wood overlaps with seeps and vents include the dorvilleids (*Parougia*, *Ophryotrocha* and *Schistomeringos*) and the polynoids *Subadyte mexicana* and *Bathykurila guaymasensis* (Levin et al. 2000, MacAvoy et al. 2002, Smith & Baco 2003).

Table 2.7. Species present at Kelp and wood fall in this study (0-0.5m) that overlap with whale carcasses, cold seeps and hydrothermal vent habitats. X- 1-5% relative abundance, XX- over 5% of relative abundance in this study. \*kelp-wood overlap (>5% rel. Abundance at any fall, see methods). P-present at a specific habitat.

Species	Kelp	Wood	Whale	Seep	Vent	References
CRS Ampharetid sp. 16		XX	P			Smith et al., in prep
CRS Ampharetid sp. 18		XX				
<i>Amelinna sp. A</i>		XX		P		Bernardino and Smith, in prep.
<i>Samytha cf. californiensis</i>	XX*	XX*	P			Smith and Baco, 2003
CRS Cirratulid. sp. 2		XX	P			Smith et al., in prep
<i>Parougia sp. A</i>		XX	P	P	P (genus)	Smith and Baco, 2003, Levin et al. 2003, Levin 2005, Blake & Hilbig, 1990
<i>Pseudophryotrocha sp. A</i>		X	P	P		Smith and Baco, 2003, Levin et al. 2003
<i>Ophryotrocha sp. A</i>	XX*	XX*	P	P (genus)	P (genus)	Smith and Baco, 2003, Levin 2005
<i>Ophryotrocha sp. B</i>		X	P	P (genus)	P (genus)	Smith and Baco, 2003, Levin 2005
<i>Ophryotrocha sp. G</i>		XX	P	P (genus)	P (genus)	Smith and Baco, 2003, Levin 2005
<i>Schistomeringos longicornis</i>		X	P	P		Smith and Baco, 2003, Levin 2005
CRS Dorvilleid 22		X				
<i>Gyptis sp. A</i>	X		P			Smith et al., in prep
<i>Sige cf. brunea</i>		X	P			Smith et al., in prep
<i>Subadyte mexicana</i>	X*	XX*	P			Smith and Baco, 2003
CRS Polynoid sp. 10	X		P			Smith et al., in prep
<i>Macellicephaloides sp. B</i>	X					
<i>Spiophanes sp. A</i>		X				
Lysianassid sp. F	X					
<i>Cumella sp. A</i>	XX*	X*	P			Smith and Baco, 2003
Cumacea sp. K	XX*	XX*	P			Smith and Baco, 2003
<i>Ilyarachna profunda</i>		XX	P			Smith and Baco, 2003
Flabellifera sp. 2	X					
Flabellifera sp. 3	X					
<i>Munidopsis quadrata</i>		XX	P			Smith and Baco, 2003
<i>Idas washingtonia</i>		X	P	P	P	Smith and Baco, 2003, Levin 2005, Tunnicliffe, 1998
<i>Xylophaga cf. washingtonia</i>		XX				
Thyasiridae sp. 2		X		P		Bernardino and Smith, in prep.
<i>Hyalogyrina n. sp.</i>	XX		P			Smith and Baco, 2003
Bivalve sp. Q	XX*	XX*	P			Smith et al., in prep

The low level of species overlap between kelp, wood and the other reducing habitats gives support to our second hypothesis. At the species level, we found higher overlap between our organic parcels and whale falls, if compared to seeps and vents. The low levels of colonization by a specialized fauna, typically chemosynthetic species, were only represented by one mytilid *Idas* at wood falls. Our successional study revealed that colonizers of kelp and wood falls include a community massively dominated by enrichment opportunists (Grassle & Grassle 1974), but includes species responding to habitat-specific characteristics which may contribute to higher regional diversity in the deep-sea.

## 2.4. Discussion

The transfer of organic detritus to the sediments around kelp and wood falls exhibited distinct dynamics. The distinct temporal scales of sediment enrichment created by kelp and wood falls was influenced by differences in their composition (Snelgrove et al. 1996, Hannides 2008). At kelp falls, kelp detritus left from detritivores activities and the remaining faunal biomass was rapidly ( $< 0.25$  y) transferred to nearby sediments (0 m). The organic input lead to the development of sedimentary reducing conditions around kelp treatments, evidenced by the growth of bacterial carpets on the sediment surface (0.25 – 0.5 y kelp parcels; Fig. 2-C), concurrently with high sulfide levels within the top few centimeters of sediment (Hannides 2008, Treude et al. 2009). The rapid organic enrichment around kelp falls indicates the efficient transfer of kelp detritus and its utilization by microbes and metazoans. The nutritional value of kelp biomass might facilitate its biological remineralization by deep-sea scavengers, and by microbes that decompose the detritus left behind by the fauna (de Leeuw & Largeau 1993, Vetter 1994, Boetius & Lochte

1996, Vetter & Dayton 1998, 1999). Direct faunal utilization of macroalgae in the deep-sea is apparently linked to its high quality within an impoverished background (Vetter 1994, Vetter & Dayton 1999). The massive numbers of metazoans that rapidly recruited after kelp deployment, evidences the attractive affect that kelp detritus have on deep-sea detritivores. An efficient remineralization and rapid energy transfer of organic detritus to the food web is also observed at rich organic packages, such as fish falls (Smith 1985, 1986). The efficient degradation of kelp biomass is additionally supported by its fast decomposition rates in deep submarine canyons [aprox.  $2.5\% \text{ d}^{-1}$ ; (Harrold et al. 1998)], and evidences the importance of macroalgae detritus to deep-sea ecosystems (Vetter & Dayton 1998).

On the other hand, the sluggish decomposition of wooden material, which is primarily carried by *Xylophaga* borers in Santa Cruz Basin, appears to delay the transfer of organic carbon to the sediments for at least 1.8 y. After initial degradation of the wood parcels, fecal pellets (Turner 1977), faunal biomass [e.g. *Xylophaga* juveniles that rained down to the sediments; (Wolff 1979)] and principally wooden material, promoted high levels of refractory organic carbon in the sediments after 3 y. At wood falls, its refractory composition likely delays the sedimentary organic enrichment by *a*) diminishing scavenging by larger animals thus restricting sloppy-feeding, *b*) requiring wood falls specialists to initiate the break up of the refractory wooden material and *c*) limiting sedimentary microbial remineralization (Turner 1977, Boetius & Lochte 1996, Hannides 2008). The organic enrichment around wood falls culminated at 3 y and was sustained significantly above background levels for a period of at least 2.5 years. The levels of organic carbon in the sediments encountered at 3 y wood falls may be one of the highest measured at these depths, but the limited microbial degradation of the refractory wood detritus was indicated by low levels of

sulfide in surface sediments (Hannides 2008), and by the visual absence of bacterial mats around the wood falls. Sediments covered with bacterial mats at cold seeps and around whale carcasses commonly exhibit high sulfate reduction rates, which is a primary source of free sulfide at the sediments (Sahling et al. 2002, Treude et al. 2003, Treude et al. 2009).

Considering the high availability of organic carbon but low pore-water sulfides, wood falls may serve as an intermediate-scale organic and sulfide rich habitat between kelp falls and whale carcasses. Kelp falls sustain very rapid increases in organic enrichment, eliciting small-scale increases on free sulfide, which limits its utilization by organisms with rapid development such as sulfur-oxidizing bacteria. Although microbial degradation of wood-carbon do not sustain significant sulfide levels in the sediments, the long-term availability of enrichment conditions may increase habitat utilization by organisms able to harvest low sulfide levels, or with facultative chemoautotrophy [e.g. mytilid bivalves; (Fisher 1999, Dubilier et al. 2008, Duperron et al. 2008, Lorion et al. 2009)]. At the other end, whale carcasses may serve as end members of organic islands, where massive amounts of fresh organic matter take years to be eaten by scavengers (Smith & Baco 2003, Smith 2006), and high bacterial degradation of sedimentary whale biomass provide sulfide levels comparable to other deep-sea reducing habitats (Treude et al. 2009).

We observed marked differences in macrofaunal abundance, composition and succession between kelp and wood parcels, preventing rejection of our first hypothesis. The patchy disturbance caused by kelp and wood falls promoted distinct magnitudes of increased macrofaunal abundance within 0.5 meters from the treatments, and reduced community diversity. The colonization patterns at kelp and wood parcels exhibited some similarities to other deep-sea enrichment experiments,

with several species being rare or absent at background communities but abundant at the disturbed areas. At 0.25 y kelp falls, cumaceans and dorvilleids were responsible for the increased macrofaunal abundance and low community diversity, a pattern similar to other small-scale enrichment experiments in deep-sea areas (Smith 1986, Grassle & Morse-Porteous 1987, Snelgrove et al. 1996, Snelgrove & Smith 2002, Levin et al. 2006). Macrofaunal abundance at wood falls was still increasing between 1.8 and 5.5 years of deployment, with an increase in species richness from several species not seen in ambient sediments, which were likely colonizing new microhabitats created by the falls (Dayton & Hessler 1972, Snelgrove et al. 1996). However, diversity at kelp and wood falls was not enhanced locally and gradually decreased with the temporal increase in the dominance of opportunists at the wood falls, consistent with the intermediate disturbance hypothesis (Grassle & Morse-Porteous 1987, Smith et al. 1998). Typical ambient species were also dominant at 0.5 y wood falls and their high density suggests that they may have been responding to low-intensity organic enrichment from the wood parcel (Kukert & Smith 1992). Background species were always observed over 0.5 meters from kelp, and many species returned to previously disturbed areas after 0.5 y revealing that the succession was leading toward convergence with background conditions.

Wood falls attracted specialists prior to any sedimentary organic enrichment, with juvenile *Xylophaga* bivalves being dominant at 0.5 y. *Xylophaga* bivalves process the wood matrix as they grow (Distel & Roberts 1997, Turner 2002), and the early recruitment of these wood-borers supports that they are widely distributed at deep-sea depths, favoring their rapid settlement onto any available piece of wood (Grassle & Morse-Porteous 1987, Pailleret et al. 2007, Voight 2007b, a). It was remarkable to detect *Xylophaga* juveniles down on the sediments after 0.5 y, and to

confirm that many of these individuals came from a brooding species present on the wood falls. The fact that besides *X. washingtonia*, another brooding species of *Xylophaga* colonized our wood falls before a period of 3 months, suggests either a remarkable dispersal ability for the brooding *Xylophaga* species or simultaneous hermaphroditism, an adaptation to the ephemeral habitat (Tyler et al. 2007, Voight 2007a, Tyler et al. 2009). Due to rapid and massive colonization, *Xylophaga* populations illustrate well mass effect dynamics with densities controlled by immigration and enhanced by local birth rates (Leibold et al. 2004).

The high density of opportunists modified the macrofaunal functional structure nearby the parcels from a typical deposit feeding to scavenger-omnivore predominance, with broad reductions in subsurface deposit feeders. The significant reductions in this group suggests that organic disturbance strongly affected subsurface sediments (Kukert & Smith 1992). The functional change at kelp and wood parcels was strikingly similar, albeit followed the distinct time scales from disturbance. At both parcels, we observed an early increase on the proportion of omnivores due to opportunist colonization. At sediments where no significant organic input was observed, there were minimal changes on the abundance of surface and subsurface deposit feeders, spatially associated with the persistence of background polychaetes species close to the parcels. The long-term changes in trophic structure were restricted to sediments under the influence of high organic input, and lasted for over 5 years at wood parcels.

Most top-ranked species from both organic falls had isotope signatures indicative of higher nutrition on detritus, reflecting species-specific preferences but with wide ranges of dependence at a variety of organic sources. *Cumella* sp. A appeared to feed primarily on kelp at 0.25 y but Cumacea sp. K had isotopic

composition indicative of nutrition from chemoautotrophic production (Fisher et al. 1994, Levin & Michener 2002). At 0.5 y wood parcels the same species had  $\delta^{13}\text{C}$  signatures indicating a source of chemoautotrophic biomass before any sedimentary enrichment, possibly through sulfur-oxidizing bacteria filaments growing on wood surfaces (Ruby et al. 1987, Laurent et al. 2009). Chemosynthetic energy sources could be particularly important to opportunist organisms prior to the sedimentary enrichment around the parcels, as observed for detritivores growing on forest litter (McLeod & Wing 2007). The other specimens at wood parcels had wide isotope signatures suggesting nutrition on many different organic sources and occupying high trophic positions, with low contribution from chemosynthetic carbon. Low dependence on chemosynthesis has been observed at reducing habitats that have greater availability of organic sources (Levin et al. 2000). Two dorvilleid species had slightly distinct contributions of wood detritus to their diet, probably a consequence of a patchy supply of organic carbon around the parcels. *Xylophaga* juveniles had a stronger contribution of wood carbon to their diet but the adults had increased bacterial carbon, possibly reflecting the signatures of their symbiotic bacteria (Distel & Roberts 1997, Nishimoto et al. 2009).

Biodiversity comparisons among deep-sea habitats face inherent difficulties associated with the taxonomy of poorly known faunas, and comparisons between studies that used very distinct sampling methodologies. We also could not reject our second hypothesis of low levels of species overlap between wood, kelp and the macrofauna of other reducing habitats. The macrofauna at kelp and wood falls exhibited higher taxonomic affinity with nearby whale-falls than to other deep-sea chemosynthetic communities, although this could easily be a location effect (the whale fall was only ~100 m away, while seeps and vents were 100's to 1000's of km

away). For example, cumaceans were top-ranked individuals at our organic falls and at whale carcasses, but were not found at the San Clemente seep (Smith et al. in prep). Vent taxa present on kelp and wood falls were mostly restricted to polychaete predators and microbial mat grazers, such as polynoids and some dorvilleids, which also occur at whale carcasses and Californian seeps (Smith & Baco 2003, Levin 2005). The dorvilleids found at kelp and wood parcels typically colonize whale-falls and other sulfide-rich sediments, including the nearby San Clemente seep (Levin et al. 2006, Smith et al. in prep). The high number of dorvilleid species at wood parcels was surprising, with 6 species representing at least 3 genera often found at seeps, vents and whale-falls, and suggesting that wood islands may create a complex of niches for this group (Baco-Taylor & Smith 2003, Levin et al. 2003). The longer persistence of wood-falls and their stronger organic enrichment compared to kelps may further facilitate the colonization of species attracted to sulfide around these parcels (Smith et al. 1989, Smith & Baco 2003). The wood also attracted a few *Idas washingtonia*, which contains chemoautrophic endosymbionts, and occurs in abundance at whale falls (Deming et al. 1997, Smith & Baco 2003).

The colonization by seep and whale fall species suggests that wood islands have low potential to sustain chemosynthesis, but demonstrates that these parcels can increase the local heterogeneity of the deep seafloor for long periods of time. It is hard to estimate the frequency of occurrence of such organic islands across deep-sea habitats, but it is clear that they provide food and substrate to deep-sea opportunists (Wolff 1979, Kiel & Goedert 2006, McLeod & Wing 2007). The frequent biogeographic occurrence of these habitats are supported by recent explorations in the West Pacific that have collected an abundant wood associated fauna with several new

specimens at depths far from continental margins (Pailleret et al. 2007, Samadi et al. 2009).

The frequent occurrence of wood and kelp falls, together with whale carcasses and other organic habitats may increase regional species diversity and favor stepping stone dispersal for some species (Levin et al. 2001, Snelgrove & Smith 2002, Van Dover et al. 2002). Our study indicates that wood falls create organic rich islands that are exploited by both opportunists and a few specialized taxa, evidencing the role of patch disturbance and habitat variability in maintaining regional diversity (Snelgrove & Smith 2002). Wood falls host many dorvilleid polychaetes, which are typical of seeps and vents and are a remarkable species rich group in whale falls (Smith & Baco 2003). Bathymodiolin mussels show specialization on organic falls and are abundant on several reducing habitats (e.g. seeps and vents). Molecular and phylogenetic analyzes indicate that the high diversity of the Bathymodiolinae and their successful colonization of seeps and vents may have resulted from their ability to exploit wide geochemical conditions at organic islands (Fisher et al. 1987, Distel et al. 2000, Jones et al. 2006, Lorion et al. 2009). Hence, the strongest evidence for wood falls to increase regional levels of biodiversity comes from the fact that multiple species colonize these habitats from other areas. Consequently, wood falls may increase habitat and population connectivity across wide ocean basins (Smith et al. 1989, Distel et al. 2000, Van Dover et al. 2002, Kritzer & Sale 2006), as suggested from their wide distribution and the fact they sustain organic rich islands for extended periods of time.

## **2.5. Conclusions**

This study showed that kelp and wood falls contribute to the heterogeneity of habitats in the deep-sea, as evidenced by the colonization of several organic-enrichment species not present on ambient deep-sea communities. The long-term organic enrichment created by wood falls increased macrofaunal colonization for several opportunist and specialized species, with evidence of mass effects dynamics for wood-borers. As a result of abundant organic sources and low sulfide levels, chemoautotrophy is limited in sediments around wood falls. Wood and kelp falls host a limited number of species that overlap with vents and seeps, with higher similarity with whale carcasses at similar ocean depths, but this could be a result of low spatial isolation. Our study gives support that wood falls create favorable habitats for the stepping stone dispersal of species adapted to use these low sulfide but organic rich parcels in deep-sea areas.

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Appendix 2A. Kelp macroinfauna at 3 and 6 months after deployment. Average density per core (37.8 cm<sup>2</sup>) and relative percentage from total number of organisms. SCrB- Santa Cruz Basin background macroinfauna (average of n=9 core samples).

	Kelp 0.25 years								Kelp 0.5 years								SCrB	
	0 m		0.5 m		1 m		2 m		0 m		0.5 m		1 m		2 m		Background	
	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %
<b>POLYCHAETA</b>																		
<b>Ampharetidae</b>																		
Ampharetid indet.					0.3 (0.2)	5.3					0.5 (0.3)	5.1					0.4 (0.2)	7.2
CRS Ampharetid sp. 6									0.3 (0.1)	1.4								
CRS Ampharetid sp. 16																	0.2 (0.1)	3.6
<i>Mugga</i> sp. A																	0.1 (0.1)	2.4
<i>Samytha</i> cf. <i>californiensis</i>	1.3 (0.5)	6.5	0.3 (0.2)	2.6					0.5 (0.1)	2.7	0.3 (0.3)	2.6						
<b>Cirratulidae</b>																		
Cirratulid indet.			0.3 (0.2)	2.6	1.0 (0.6)	15.8	0.3 (0.2)	8.3			0.3 (0.3)	2.6	0.7 (0.7)	20	0.3 (0.3)	7.7	0.4 (0.1)	8.4
<i>Monticellina</i> sp. A			0.3 (0.2)	2.6	1.0 (0.6)	15.8	1.3 (0.8)	33.2	0.8 (0.1)	4.1	0.8 (0.5)	7.7	0.7 (0.7)	20	0.3 (0.3)	7.7	0.6 (0.2)	13.3
<i>Monticellina</i> sp. B																	0.3 (0.2)	6.0
<i>Tharyx</i> sp. A			1.0 (0.0)	7.9	0.7 (0.2)	10.5	0.3 (0.2)	8.3			0.3 (0.3)	2.6			0.7 (0.3)	15.4	0.3 (0.1)	6.0
<i>Chaetozone</i> sp. C											0.3 (0.3)	2.6			0.3 (0.3)	7.7		
<i>Chaetozone</i> sp. D					0.7 (0.2)	10.5			1.3 (0.4)	6.8	1.3 (0.3)	12.8	0.7 (0.3)	20	1.7 (1.2)	38.5	0.7 (0.2)	14.5
<b>Cossuridae</b>																		
Cossurid indet.					0.7 (0.2)	10.5							0.3 (0.3)	10	0.3 (0.3)	7.7	0.2 (0.1)	3.6
<i>Cossura</i> cf. <i>rostrata</i>			2.3 (0.2)	18.4	1.0 (0.3)	15.8	0.3 (0.2)	8.3			1.8 (0.5)	17.9	0.3 (0.3)	10	0.3 (0.3)	7.7	0.8 (0.3)	16.9
<b>Dorvilleidae</b>																		
Dorvilleid indet.									0.8 (0.4)	4.1								
<i>Ophryotrocha</i> sp. A	7.3 (2.3)	36.1							2.3 (1.1)	12.2								
<b>Hesionidae</b>																		
Hesionid indet.	0.3 (0.2)	1.6																
<i>Gyptis</i> sp. A									0.3 (0.1)	1.4								
<b>Paraonidae</b>																		
<i>Aricidea</i> <i>lopezi</i>																	0.1 (0.1)	1.2
<i>Levinsenia</i> <i>oculata</i>											0.3 (0.3)	2.6						
<b>Polynoidae</b>																		
<i>Subadyte</i> <i>mexicana</i>									0.3 (0.1)	1.4	0.3 (0.3)	2.6						
<i>Harmathoe</i> <i>fragilis</i>									0.3 (0.1)	1.4							0.1 (0.1)	1.2
CRS Polynoid sp. 10											0.3 (0.3)	2.6						
<i>Macellicephaloides</i> sp. B									0.3 (0.1)	1.4								
<b>Spionidae</b>																		
<i>Spiophanes</i> cf. <i>anoculata</i>											0.3 (0.3)	2.6						
<i>Spiophanes</i> cf. <i>berkeleyorum</i>									0.3 (0.1)	1.4							0.1 (0.1)	1.2
<b>Sternaspidae</b>																		
CRS Sternaspid sp. 1							0.3 (0.2)	8.3										
<b>CRUSTACEA</b>																		
<b>Amphipoda indet.</b>																		
<i>Ampelisca</i> sp. 1							0.3 (0.2)	8.3									0.1 (0.1)	1.2
<b>Phoxocephalidae sp. A</b>																		
Phoxocephalidae sp. B											0.3 (0.3)	2.6					0.1 (0.1)	2.4
<b>Amphipoda sp. E</b>																		
Lysianassid sp. F	0.3 (0.2)	1.6																
<b>Cumacea indet.</b>																		
<i>Cumella</i> sp. A	7.0 (1.5)	34.4																0.1 (0.1)
Cumacea sp. K			7.0 (3.8)	55.3	0.3 (0.2)	5.3			9.8 (1.7)	52.7	2.8 (0.6)	28.2	0.3 (0.3)	10				
Flabellifera sp. 2	0.7 (0.4)	3.3																
Flabellifera sp. 3	0.7 (0.4)	3.3																
<i>Carpoapseudes</i> sp.			0.3 (0.2)	2.6			0.7 (0.4)	16.7					0.3 (0.3)	10			0.2 (0.1)	3.6
<b>MOLLUSCA</b>																		
Bivalve indet.			0.3 (0.2)	2.6											0.3 (0.3)	7.7	0.1 (0.1)	1.2
Bivalve juv. Indet.	0.3 (0.2)	1.6															0.1 (0.1)	1.2
Bivalve sp. Q									1.0 (0.2)	5.4	0.3 (0.3)	2.6						
<i>Hyalogyrina</i> sp.	2.3 (1.0)	11.5							0.5 (0.3)	2.7								
<b>OTHER</b>																		
Anthozoa					0.3 (0.2)	5.3												
Nemertea			0.7 (0.4)	5.3			0.3 (0.2)	8.3	0.3 (0.1)	1.4								
Ophiuroidea																	0.1 (0.1)	1.2
<b>Total Polychaetes</b>	9.0 (2.8)	44.3	4.3 (0.5)	34.2	5.3 (0.5)	84.2	2.7 (1.3)	66.7	7.0 (1.3)	37.8	6.3 (1.3)	64.1	2.7 (1.2)	80	4.0 (1.7)	92.3	4.2 (0.2)	85.5
<b>Total Other Macrofauna</b>	11.3 (1.0)	55.7	8.3 (3.4)	65.8	1.0 (0.6)	15.8	1.3 (0.4)	33.3	11.5 (1.3)	62.2	3.5 (0.5)	35.9	0.7 (0.3)	20	0.3 (0.3)	7.7	0.6 (0.1)	14.5

Appendix 2B Wood sediment macrofauna at 0.5, 1.8, 3 and 5.5 years after deployment. Average density per core (37.8 cm<sup>2</sup>) and relative percentage from total number of organisms. SCrB- Santa Cruz Basin background macrofauna (average of n=17 core samples).

	WOOD 0.5 yr										WOOD 1.8 yr				SCrB	
	0 m		0.5 m		1 m		2 m		0 m		0.5 m		1 m		Background	
	Average (ISE)	Rel %														
<b>POLYCHAETA</b>																
<b>Ampharetidae</b>																
Ampharetid indet.					0.3(0.3)	6.7			6.3(3.2)	26	5.5(4.5)	42.3	1.0(1.0)	16.7	0.4(0.2)	7.2
CRS Ampharetid sp. 10															0.2(0.1)	3.6
CRS Ampharetid sp. 16									1.3(1.3)	5.5						
CRS Ampharetid sp. 18																
CRS Ampharetid sp. 19																
<i>Samytha cf. californiensis</i>	0.3(0.3)	3.2							2.0(1.0)	8.2	0.5(0.4)	3.8				
<i>Amelinna</i> sp. A									2.7(2.7)	11						
<i>Amphicteis</i> sp. A																
<i>Mugga</i> sp. A			0.3(0.3)	8.3											0.1(0.1)	2.4
<b>Capitellidae</b>																
<i>Capitella cf. capitata</i>																
<b>Chaetopteridae</b>																
<i>Phyllochaetopterus limicolus</i>	0.3(0.3)	3.2														
<b>Cirratulidae</b>																
Cirratulid indet.	0.3(0.3)	3.2			1.0(0.6)	20			0.7(0.7)	2.7			1.0(0)	16.7	0.4(0.1)	8.4
CRS Cirratulid sp. 2																
CRS Cirratulid sp. 13	0.7(0.7)	6.5	0.3(0.3)	8.3					1.3(0.9)	5.5	0.5(0.4)	3.8	1.0(1.0)	16.7		
<i>Chaetozone</i> sp. C																
<i>Chaetozone</i> sp. D	0.7(0.7)	6.5	0.3(0.3)	8.3	0.3(0.3)	6.7	0.7(0.3)	33.3	0.3(0.3)	1.4	1.0(0.8)	7.7	1.0(1.0)	16.7	0.7(0.2)	14.5
<i>Chaetozone</i> sp. E																
<i>Chaetozone</i> sp. F																
<i>Monticellina</i> sp. A	1.7(0.9)	16.1	1.3(1.3)						0.3(0.3)	1.4	0.5(0.4)	3.8	0.5(0.5)	8.3	0.6(0.2)	13.3
<i>Monticellina</i> sp. B															0.3(0.2)	6.0
<i>Tharyx</i> sp. A	0.3(0.3)	3.2													0.3(0.1)	6.0
<b>Cossuridae</b>																
Cossurid indet.			0.7(0.3)	16.7	0.7(0.3)	13.3	0.3(0.3)	16.7							0.2(0.1)	3.6
<i>Cossura cf. rostrata</i>	0.7(0.3)	6.5	0.3(0.3)	8.3	1.0(1.0)	20			1.7(0.9)	6.8					0.8(0.3)	16.9
<b>Dorvilleidae</b>																
Dorvilleid indet.									1.3(1.3)	5.5						
<i>Parougia</i> sp. A									0.3(0.3)	1.4						
<i>Pseudophryotrocha</i> sp. A																
<i>Ophryotrocha</i> sp. A									4.7(4.7)	19.2						
<i>Ophryotrocha</i> sp. B																
<i>Ophryotrocha</i> sp. G																
<i>Schistomeringos longicornis</i>																
CRS Dorvilleid 22																
<b>Glyceridae</b>																
<i>Glycera cf. branchiopoda</i>																
<b>Paraonidae</b>																
<i>Aricidea cf. lopezi</i>							0.3(0.3)	16.7							0.1(0.1)	1.2
<b>Phyllodocidae</b>																
<i>Sige cf. brunea</i>											0.5(0.4)	3.8				
<b>Polynoidae</b>																
<i>Subadyte mexicana</i>																
<i>Bathyrurila guaymasensis</i>																
<i>Harmothoe fragilis</i>											0.5(0.4)	3.8			0.1(0.1)	1.2
<b>Spionidae</b>																
<i>Spiophanes</i> sp. A					0.3(0.3)	6.7										
<i>Spiophanes cf. berkeleyorum</i>															0.1(0.1)	1.2
<i>Spiophanes kroeyeri</i>																
<b>CRUSTACEA</b>																
Amphipoda indet.															0.1(0.1)	1.2
Amphipod sp. E	0.3(0.3)	3.2													0.1(0.1)	1.2
Phoxocephalidae sp. A	0.3(0.3)	3.2	0.3(0.3)	8.3			0.3(0.3)	16.7	0.7(0.7)	2.7	0.5(0.4)	3.8			0.1(0.1)	2.4
Phoxocephalidae sp. B															0.1(0.1)	1.2
Lysianassid sp. F																
<i>Eyakia cf. robusta</i>																
<i>Munidopsis quadrata</i>			0.3(0.3)	8.3												
Cumacea indet.															0.1(0.1)	1.2
<i>Cumella</i> sp. A									0.3(0.3)	1.4						
Cumacea sp. K	3.3(1.3)	32.3									2.5(2.0)	19.2				
Asellote Isopod sp. A							0.3(0.3)	16.7								
Asellote Isopod sp. G					0.3(0.3)	6.7										
<i>Ilyarachna profunda</i>									0.3(0.3)	1.4						
Cirolanidae sp.1																
<i>Carpopapseudes</i> sp.											0.5(0.4)	3.8	0.5(0.5)	8.3	0.2(0.1)	3.6
<b>MOLLUSCA</b>																
Bivalve indet.															0.1(0.1)	1.2
Bivalve juv. indet.															0.1(0.1)	1.2
Bivalve sp. Q	1.0(0.6)	9.7									0.5(0.4)	3.8				
<i>Idas washingtonia</i>																
Thyasiridae sp. 1																
Thyasiridae sp. 2	0.3(0.3)	3.2			0.3(0.3)	6.7										
Vesicomysid sp.																
<i>Xylophaga cf. washingtonia</i>																
<b>ECHINODERMATA</b>																
Asteroidea					0.3(0.3)	6.7										
Ophiuroidea															0.1(0.1)	1.2
<b>CNIDARIA</b>																
Anthozoa																
<b>Total polychaeta</b>	5.0(1.5)	48.4	3.3(1.5)	83.3	3.7(1.7)	73.3	1.3(0.3)	66.7	23.0(12.4)	94.5	9.0(2.4)	69.2	5.5(4.5)	91.7	4.2(0.2)	85.5
<b>Total Other macrof</b>	5.3(0.3)	51.6	0.7(0.7)	16.7	1.3(0.3)	26.7	0.7(0.3)	33.3	1.3(1.3)	5.5	4.0(2.4)	30.8	0.5(0.5)	8.3	0.6(0.1)	14.5

Cont.	WOOD 3 yr								WOOD5.5 yr							
	0 m		0.5 m		1 m		2 m		0 m		0.5 m		1 m		2 m	
	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %	Average (ISE)	Rel %						
<b>POLYCHAETA</b>																
<b>Ampharetidae</b>																
Ampharetid indet.	5.0(1.9)	13.9	1.7(0.9)	17.9					1.3(0.9)	2.6	0.3(0.3)	5.3	1.0(0.6)	13.6	0.3(0.3)	5.3
CRS Ampharetid sp. 10								0.3(0.3)	0.7							
CRS Ampharetid sp. 16										0.3(0.3)	5.3					
CRS Ampharetid sp. 18								15.0(12.5)	29.8	0.3(0.3)	5.3	0.3(0.3)	4.5			
CRS Ampharetid sp. 19								0.3(0.3)	0.7							
<i>Samytha cf. californiensis</i>	4.8(0.3)	13.2	0.3(0.3)	3.6	0.3(0.3)	5			2.0(1.2)	4	0.3(0.3)	5.3				
<i>Amelinna</i> sp. A	0.5(0.5)	1.4											0.3(0.3)	4.5		
<i>Amphicteis</i> sp. A																
<i>Mugga</i> sp. A																
<b>Capitellidae</b>																
<i>Capitella cf. capitata</i>	0.3(0.3)	0.7														
<b>Chaetopteridae</b>																
<i>Phyllochaetopterus limicolus</i>																
<b>Cirratulidae</b>																
Cirratulid indet.			1.3(1.3)	14.3	2.3(0.3)	35	1.0(1.0)	14.3					0.3(0.3)	4.5	1.3(0.9)	21.1
CRS Cirratulid. sp. 2			1.3(0.7)	14.3												
CRS Cirratulid. sp. 13											1.7(0.7)	26.3			1.3(0.9)	21.1
<i>Chaetozone</i> sp. C									0.3(0.3)	4.8						
<i>Chaetozone</i> sp. D									0.7(0.7)	9.5						
<i>Chaetozone</i> sp. E											0.7(0.7)	10.5	1.0(0.6)	13.6	0.7(0.7)	10.5
<i>Chaetozone</i> sp. F									0.3(0.3)	0.7						
<i>Monticellina</i> sp. A	0.5(0.5)	1.4			0.3(0.3)	5	1.7(0.3)	23.8			0.7(0.7)	1.3				
<i>Monticellina</i> sp. B																
<i>Tharyx</i> sp. A					0.3(0.3)	5										
<b>Cossuridae</b>																
Cossuridae indet.					0.7(0.7)	10										
<i>Cossura cf. rostrata</i>			1.7(1.2)	17.9	0.7(0.3)	10	1.3(0.3)	19			0.7(0.7)	10.5	0.7(0.3)	9.1	0.7(0.3)	10.5
<b>Dorvilleidae</b>																
Dorvilleid indet.																
<i>Parougia</i> sp. A	0.8(0.3)	2.1			0.3(0.3)	5			7.3(5.5)	14.6	0.3(0.3)	5.3				
<i>Pseudophrytrocha</i> sp. A	1.0(0.4)	2.8														
<i>Ophryotrocha</i> sp. A	8.3(5.4)	22.9							17.0(2.5)	33.8						
<i>Ophryotrocha</i> sp. B									0.7(0.3)	1.3						
<i>Ophryotrocha</i> sp. G	6.5(3.9)	18.1														
<i>Schistomeringos longicornis</i>	0.5(0.3)	1.4			0.7(0.7)	10										
CRS Dorvilleid 22	0.5(0.3)	1.4														
<b>Glyceridae</b>																
<i>Glycera cf. branchiopoda</i>									0.3(0.3)	0.7						
<b>Paraonidae</b>																
<i>Aricidea cf. lopezi</i>							0.3(0.3)	4.8					0.3(0.3)	4.5		
<b>Phyllodocidae</b>																
<i>Sige cf. brunea</i>			0.3(0.3)	3.6	0.3(0.3)	5			0.3(0.3)	0.7			0.7(0.7)	9.1		
<b>Polynoidae</b>																
<i>Subadyte mexicana</i>			0.7(0.3)	7.1												
<i>Bathyrkila guaymasensis</i>	0.3(0.3)	0.7														
<i>Harmothoe fragilis</i>	0.5(0.3)	1.4														
<b>Spionidae</b>																
<i>Spiophanes</i> sp. A			0.3(0.3)	3.6			0.3(0.3)	4.8								
<i>Spiophanes cf. berkeleyorum</i>																
<i>Spiophanes kroeyeri</i>									0.3(0.3)	0.7						
<b>CRUSTACEA</b>																
Amphipoda indet.																
Amphipod sp. E																
Phoxocephalidae sp. A			0.7(0.7)	7.1			1.3(0.3)	19			1.0(1.0)	15.8	1.3(0.9)	18.2	0.3(0.3)	5.3
Phoxocephalidae sp. B											0.3(0.3)	5.3				
Lysianassid sp. F															0.3(0.3)	5.3
<i>Eyakia cf. robusta</i>									0.3(0.3)	0.7					0.7(0.3)	10.5
<i>Munidopsis quadrata</i>																
Cumacea indet.																
<i>Cumella</i> sp. A																
Cumacea sp. K	0.3(0.3)	0.7							1.0(0.6)	2						
Asellote Isopod sp. A																
Asellote Isopod sp. G																
<i>Ilyarachna profunda</i>	2.5(0.5)	6.9							0.7(0.7)	1.3						
Cirolanidae sp.1	0.3(0.3)	0.7														
<i>Carpoapseudes</i> sp.											0.3(0.3)	5.3	0.7(0.3)	9.1	0.3(0.3)	5.3
<b>MOLLUSCA</b>																
Bivalve indet.	0.3(0.3)	0.7	1.0(0.6)	10.7	0.3(0.3)	5										
Bivalve juv. indet.																
Bivalve sp. Q																
<i>Idas washingtonia</i>	1.0(0.4)	2.8														
Thyasiridae sp. 1									0.3(0.3)	0.7			0.3(0.3)	4.5	0.3(0.3)	5.3
Thyasiridae sp. 2																
Vesicomyd sp.									0.7(0.7)	1.3						
<i>Xylophaga cf. washingtona</i>	2.3(1.3)	6.3							1.3(0.9)	2.6						
<b>ECHINODERMATA</b>																
Asteroidea																
Ophiuroidea					0.3(0.3)	5							0.3(0.3)	4.5		
<b>CNIDARIA</b>																
Anthozoa	0.3(0.3)	0.7														
<b>Total polychaeta</b>	29.3(10.8)	81.3	7.7(3.3)	82.1	6.0(0.6)	90	5.7(1.7)	81	46.0(13.1)	91.4	4.7(1.2)	73.7	4.7(1.9)	63.6	4.3(2.9)	68.4
<b>Total Other macrof</b>	6.8(2.0)	18.8	1.7(1.2)	17.9	0.7(0.3)	10	1.3(0.3)	19	4.3(0.3)	8.6	1.7(0.7)	26.3	2.7(0.7)	36.4	2.0(1.2)	31.6

## 2.7. Supplementary Material

Figure. S2.1. Triangle delineating the area in fauna isotope values that can be calculated from mixing models that have the three main organic sources represented in the apices (sediment, kelp/wood, and sulfur bacteria). Species out of the triangle may feed substantially on other sources not constrained in our sampling (see Phillips and Gregg, 2003). Mean values for each organic source represent end members on their isotope signature.

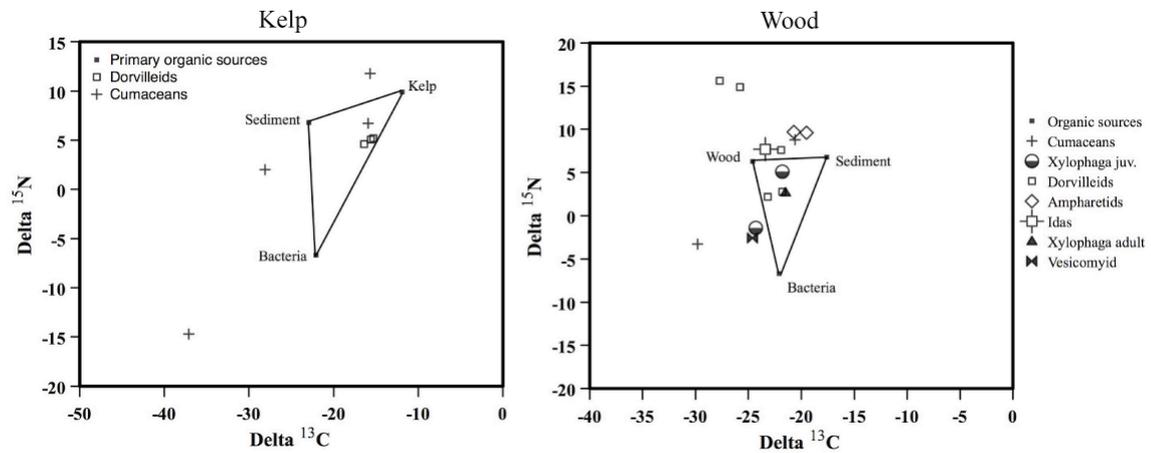


Table S2.1. Mixing organic source contributions from species at kelp and wood parcels. Isotope signatures (corrected) of species entered in the mixing model. Range and mean (SE) in source proportions presented for each species. Values in **bold** indicate significant differences in the contribution of a source (95% Conf. Limit).

KELP	Species	N	Avrg 13C	Avrg 15N	Source proportions [%] range (95% Conf. Lim)			Mean source proportions [%](SE)		
					Sediment	Kelp	Bacteria	Sediment	Kelp	Bacteria
	<i>Cumella</i> sp.A (0.25y)	1	-15.9 (0.1)	6.7 (0.1)	10-22 (0-100)	58-63 (22.9-98.1)	20-28 (0-100)	15.7 (27.1)	60.5 (8.7)	23.8 (24)
	<i>Ophryotrocha</i> sp.A(0.25y)	2	-15.5 (0.2)	5.2 (0.1)	7-8 (0-63.8)	66-65 (40.4-90.2)	27 (0-100)	7.8 (13)	<b>65.3 (5.7)</b>	26.8 (11.9)
	<i>Ophryotrocha</i> sp.A (0.5y)	1	-16.4 (0.1)	4.6 (0.1)	13-14 (0-100)	57 (32.6-81.3)	29-30 (0-100)	13.7 (13.7)	56.9 (5.6)	29.3 (12.8)
WOOD	Species	N	Avrg 13C	Avrg 15N	Sediment	Wood	Bacteria	Sediment	Wood	Bacteria
	<i>Xylophaga</i> juv. (0.5y)	1	-21.8 (0.1)	5.1 (0.1)	35-37 (0-100)	52-53 (0-100)	10-11 (0-79.4)	36.1 (9.9)	53 (10.6)	10.8 (5.4)
	<i>Xylophaga</i> <i>washingtonia</i> (5.5y)	1	-21.5 (0.1)	2.7 (0.1)	33-35(0-81.7)	36-38(0-94.6)	29 (0-100)	33.9 (11.1)	37 (13.4)	29.1 (13)
	<i>Ophryotrocha</i> sp.A (3y)	1	-21.8 (0.1)	2.8 (0.1)	29-31(0-73.8)	41-43 (0-95.5)	28 (0-100)	29.9 (10.2)	41.8 (12.5)	28.2 (0.12)
	<i>Ophryotrocha</i> sp.G (3y)	1	-23.2 (0.1)	2.2 (0.1)	8-10 (0-42.2)	58-60 (0-100)	32 (0-100)	8.5 (7.8)	<b>59.4 (11.1)</b>	32.1 (14.5)



## Chapter 3. Infaunal community structure and succession during the sulfophilic stage of a whale carcass in the deep NE Pacific

### **Abstract**

Although natural and experimental deep-sea whale falls have been studied in several ocean basins, the impact of whale falls on sediment macrofaunal community structure has rarely been studied. Here we evaluate macrofaunal community structure and succession around a 30-ton deep-sea whale-fall artificially implanted at 1670 m in the Santa Cruz Basin (SCr; NE Pacific). This study focused on macrofaunal community patterns from 4.5 to 6.8 y after carcass deployment in order to evaluate: (1) patterns of organic enrichment around the whale-fall sediments; (2) spatial and temporal patterns of macrofaunal species structure and (3) level of species overlap with other artificial and natural reducing habitats at similar depths on the Californian margin. Sediment organic enrichment around the whale-fall during the period studied was highly patchy, with some sediments adjacent the whale fall exhibiting intense organic loading (11% organic carbon by weight). Macrofaunal abundance was highly elevated from 4.5 to 6.8 y, and was dominated by enrichment opportunist and sulfophilic species. Dorvilleids, ampharetids and cumaceans accounted for over 50% of macrofaunal abundance nearby the whale-fall (0 – 0.5 m) at all times sampled. Bacterial mats were abundant over the sediment surface for over 6.8 y, indicating the presence of pore-water sulfides and organic enrichment, and indicating that the sulfophilic stage had started by 4.5 y. The macrofaunal community was composed of chemoautotrophic-symbiont-hosting and heterotrophic species grazing sulfur-oxidizing bacterial mats. Whale-fall sediments harbor many species and trophic types not present in background sediments, and significantly increase beta diversity for at least 7 yrs. There were low levels of species overlap between the whale-fall, cold seeps and hydrothermal vents, explained by differences in biogeochemistry and food webs among these habitats. The sediment macrofauna around the SCr whale-fall was more diverse than in other similar habitats at cold seeps and hydrothermal vents, indicating that whale-falls may be significant oases of enhanced alpha diversity in the deep-sea.

### 3.1. Introduction

Nekton carcasses (*e.g.* fishes, dolphins, whales) provide an important source of energy to the deep sea, introducing large quantities of fresh organic material into a food-poor environment (Stockton & DeLaca 1982, Smith 1985, 1986, Smith & Demopoulos 2005). The arrival of a carcass of the largest living animal on Earth at the deep-sea floor has received considerable attention and the succession of organisms exploiting whale-carcasses have been studied in numerous deep-sea and shallow water sites (Smith 1985, Naganuma et al. 1996, Smith et al. 1998, Smith & Baco 2003, Goffredi et al. 2004, Fujiwara et al. 2007). The amount of carbon that is exported as whale-detritus is lower than the flux of particulate organic carbon (POC) to deep-sea oceans, but dead whales sink as enormous organic-rich packages that disturb a very limited area of the sea-bottom (Smith 2006). For example, the seabed immediately underlying a typical whale fall (*i.e.* roughly 50 m<sup>2</sup>), can experience the equivalent of 2000 yr of background POC flux in a single pulse of organic enrichment (Smith & Baco 2003). Consequently, whale carcasses provide an immense and very rich source of labile organic matter to the food limited deep-sea ecosystems. This enormous food bonanza attracts numerous macro- and megafaunal organisms that exploit the rich organic sources for years to decades (Smith et al. 1998, Baco-Taylor & Smith 2003, Smith 2006).

Modern whale carcasses in the deep sea host a dense and species-rich macrofaunal assemblage attached to bone surfaces and living in the underlying soft sediments (Bennett et al. 1994, Smith et al. 1998, Baco-Taylor & Smith 2003, Smith & Baco 2003, Goffredi et al. 2004). Experimental studies on the Californian slope revealed that whale carcasses typically support a diverse succession of macrofaunal assemblages that include: *i*) a first stage of mobile scavengers; *ii*) an enrichment

opportunistic stage; *iii*) a sulfophilic or chemoautotrophic stage and *iv*) a reef stage (Smith & Baco 2003). Along the successional stages, considerable attention has been given to the epifaunal macrofauna associated with the whale bones, revealing that whale skeletons can harbor the highest local species richness of any deep-sea hard-substrata habitats, such as cold seeps and vents (Baco-Taylor & Smith 2003).

Highlights of the extreme richness associated with these habitats comes from the limited number of overlapping species inhabiting similar whale-fall ecosystems, considering that a relatively limited number of whale-falls have been studied globally (Smith & Baco 2003, Fujiwara et al. 2007). The high number of whale-fall specialists described recently further suggests that much of the biodiversity associated with these habitats remains to be discovered, and that carcasses of large animals may have promoted evolutionary reducing habitats in the deep sea for long periods of time (Dahlgren et al. 2004, Glover et al. 2005b, Kiel & Goedert 2006).

Whale skeletons generate organic-rich conditions and sustain a reducing habitat for years in the deep-sea. The long-term persistence of reducing conditions at whale carcasses create habitat for specialized species that depend on chemosynthesis for their nutrition, which may increase the overlap of species between whale skeletons and other chemosynthetic habitats (Smith et al. 1989). The whale fall chemosynthesis is principally based on anaerobic microbial degradation of bone lipids and on the degradation of organic carbon in sediments around the carcass; releasing high levels of hydrogen sulfide (Goffredi et al. 2008, Treude et al. 2009). Biogeochemical processes in a whale fall can sustain similar chemosynthetic conditions to other rich reducing habitats such as cold seeps and hydrothermal vents; and taxonomic affinities among species inhabiting whale carcasses and chemoautotrophic-based communities supports the similar ecological niche found between them (Smith & Baco 2003,

Treude et al. 2009). The broad range of ecological niches present at whale carcasses attracts organisms that host chemoautotrophic symbionts, including whale-fall “specialists”, heterotrophic opportunists and also background species (Baco-Taylor & Smith 2003, Smith & Baco 2003).

The importance of chemosynthetic food-webs at many reducing habitats in the deep-sea and the encounter of chemosynthesis-based species colonizing both whales, seeps and vents, led to the stepping stone hypothesis that whale carcasses serve as intermediate habitats for the dispersal of vent and seep species (Smith et al. 1989). Overlapped species between whale-vent-seep habitats include both specialized organisms that first evolved at a specific habitat and then invaded others, and other generalist and opportunist species that are able to exploit rich organic conditions over vast areas (Bennett et al. 1994, Van Dover et al. 2002). For example, molecular evidence suggests that the Bathymodiolinae (Bivalvia: Mytilidae), which are symbiont-containing mussels dependent on chemosynthesis, diversified first on whale and wood remains before invading vents and seeps (Fisher 1999, Distel et al. 2000, Van Dover 2000). On the other hand, molecular data from distinct groups evidenced that many evolved first at seep or vent habitats and then colonized deep-sea organic habitats (Baco-Taylor et al. 1999, Shank et al. 1999). Phylogenetic patterns also suggests that many overlapping species are ubiquitous to whale falls, wood islands, vents and seeps, which also supports the stepping stone hypothesis (Distel et al. 2000, Van Dover et al. 2002, Smith & Baco 2003, Lorion et al. 2009). However, whale falls, hydrothermal vents and cold seeps each harbor many endemic species that have specific physiological and reproductive adaptations to its environment (Dahlgren et al. 2004, Glover et al. 2005b). Until sufficient sampling and experimentation is carried

on these habitats, knowledge about ecology and the evolutionary potential of these habitats will be incomplete.

The ecology of epifaunal organisms associated with whale carcasses has been studied in a few sites, but the effort to study the macrofaunal community thriving at sediments nearby whale skeletons has been limited (Naganuma et al. 1996, Smith et al. 1998). A whale fall study sampled macrofaunal communities in sediments around natural whale skeletons in the Pacific, where at the time of sampling the carcasses had been in the seafloor for at least 4 years (Smith et al. 1998). The macrofaunal community at these carcasses were at an advanced stage in the predicted ecological succession, where most of the whale soft tissue and organic matter had been decomposed, with very weak evidence of sulfides within the sediments (Smith et al. 1998). Consequently, the number of specialized organisms in the macrofaunal community could not be well evaluated, although a species rich bone-associated fauna was described from the same carcass (Bennett et al. 1994, Smith et al. 1998). Therefore, long term studies focusing on the sediment macrofauna at whale falls along variable stages of succession and biogeochemical conditions can help to evaluate long-term patterns on community succession and structure, and further investigate levels of species overlap with other chemosynthetic ecosystems.

This study will focus at the sediment macrofauna around the Santa Cruz whale carcass, which was artificially implanted at 1800 m depth in 1998. Following deployment, the whale carcass was revisited and sampled at 0.1, 1.5, 4.5, 5.8 and 6.8 years, corresponding to the most comprehensive and long-term ecological study of a whole whale carcass to date. In this study, we will focus on the macrofauna community at sediments around the whale carcass during the sulfophilic stage (i.e. from 4.5 to 6.8 years after the whale implantation). Therefore, patterns of community

structure, succession and species overlap will be evaluated in sediments directly influenced by the organic impact from the whale carcass, and available data from sediment geochemistry will be used to support our observations (Treude et al. 2009). We also compared levels of species overlap between the whale fall macrofauna to other organic islands (i.e. kelp and wood parcels) artificially implanted at similar ocean depths nearby, and to the San Clemente seep macrofauna situated about 200 Km southeast. We aimed to address the following hypothesis:

- 1) *Whale falls create sulfophilic habitats with intense organic enrichment in sediments nearby, which will affect the macrofaunal community structure and succession;*
- 2) *A low degree of species overlap will be observed between the Santa Cruz whale fall and other assemblages at southern California seeps, kelp falls and wood falls at similar ocean depths.*

### **3.2. Materials and methods**

#### *Study site and sampling*

The whale fall was experimentally implanted in Santa Cruz Basin, California (33°27'N, 119°22'W, 1675 m, bottom temperature ~ 4°C, bottom-water oxygen concentration 260 µM) in April of 1998 (Figure 1.1-Point A). It is a carcass of a 13-m mature gray whale (*Eschrichtius robustus* Gray, 1864), which weighted approximately 30 x 10<sup>3</sup> kg at the time it was sunk (C. Smith, pers. communication). This whale carcass was visited on multiple occasions since its deployment, but this study focus on the macrofauna community at sediments from 4.5 y, 5.8 y and 6.8y

after whale implantation. Sediment samples were collected using the remotely operated vehicle (ROV) *Tiburón* during three cruises (Oct.2002, Mar.2004 and Feb-Mar.2005) on board the *R/V Western Flyer*, Monterey Bay Aquarium Research Institute (Appendix 3A). Sampling cores were taken from five randomly radiating sampling transects starting immediately close to the carcass, and samples were collected at distances of 0, 1, 3, and 9 m from the whale. The sediment macrofauna was sampled with 3-5 replicate tube cores from each distance during each cruise. The background macrofauna was sampled during 3 distinct cruises in 2002, 2004 and 2005, totaling 17 replicate tube cores distant at least 9-100 meters from any organic island (i.e. whale-fall, kelp or wood parcels). Corers sampled at 9 m from 2005 were pooled with the background thus increasing our temporal replication. Based on macrofaunal abundance and species composition, there was no evidence of any influence from the whale carcass at distances of over 3 m in 2005. All cores assigned to macrofauna analysis were sliced on board at 0-1, 1-5 and 5-10 depth intervals and preserved in 4% seawater buffered formalin. Replicate cores were also taken at each distance to be analyzed for total organic carbon and nitrogen.

#### *Laboratory analysis*

In laboratory, samples were sieved in a 300 $\mu$ m mesh and all organisms were stained, sorted and identified at the lowest attainable taxonomic level. Distribution of taxa among feeding guilds helped to distinguish temporal and spatial patterns of trophic succession around the whale-fall (Fauchald & Jumars 1979, Kukert & Smith 1992). Sediment samples for organic carbon and nitrogen analysis were acidified to remove carbonates by repeated additions of sulfurous acid (8 % v/v) until no effervescence was observed (Verardo et al. 1990) and then analyzed using a Perkin-

Elmer 2400 CHN Elemental Analyzer, with a limit of detection of 1 mg and 1.2 mg for C and N, respectively, while the precision was 0.3 % and 0.4 % for C and N, respectively. CHN standards were made with acetanilide and blanks were made of both non-acidified and acidified cups, both giving minor signals of TOC and TN.

A few top-ranked macrofaunal organisms at representative stages of whale-fall community succession were selected for stable isotope analysis. Selected individuals were sorted using methanol-cleaned forceps and specimens were then rinsed and cleaned of attached debris in DI water. Calcareous shelled organisms were decalcified with phosphoric acid and placed in pre-weighed tin cups for overnight drying (35-40 °C). Multiple individuals of the same species Calcareous shelled organisms were decalcified with phosphoric acid and placed in pre-weighed tin cups for overnight drying (35-40 °C). Multiple individuals of single species were combined in one sample to make up the necessary dry weight of 0.5-2 mg for analysis. Samples were combusted in a Eurovector elemental analyzer and resulting N<sub>2</sub> and CO<sub>2</sub> gases were separated by gas chromatography and admitted into an isotope ratio mass spectrometer (IRMS) for determination of <sup>15</sup>N/<sup>14</sup>N and <sup>13</sup>C/<sup>12</sup>C ratios (reproducibility: ±0.5 ‰ for δ<sup>15</sup>N and ±0.2 ‰ for δ<sup>13</sup>C). Macrofaunal C-isotopic ratios were measured against a Pee Dee Belemnite (PDB) standard for δ<sup>13</sup>C and atmospheric nitrogen for δ<sup>15</sup>N. Results are expressed as delta (δ) notation representing the relative difference between sample and standard, where  $\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$ , where  $R = {}^{15}\text{N}/{}^{14}\text{N}$  or  $R = {}^{14}\text{C}/{}^{13}\text{C}$ .

Macroinfaunal organisms were fixed in 4% formaldehyde solution, potentially introducing artifacts in stable isotope values, although shifts in carbon isotope ratio are usually small compared to the wide natural C variability in marine food sources (Fry & Sherr 1989, Edwards et al. 2002, Sarakinos et al. 2002). In this study, we

corrected for preservation artifacts by adding 1‰ to  $\delta^{13}\text{C}$  (Baco-Taylor 2002, Sarakinos et al. 2002, Demopoulos et al. 2007). Trophic shift boxes from potential organic matter sources at the whale-fall helped to determine organisms' food sources (Fry 2006). Trophic changes of +3 ‰ were added to the range of  $\delta^{15}\text{N}$  values obtained for each organic matter source (DeNiro & Epstein 1978, Minagawa & Wada 1984, Fisher et al. 1994). Species that exclusively use a particular organic-matter source for nutrition are expected to fall within the appropriate trophic-shift box.

### *Video Analysis*

Prior to sampling transects, the *ROV Tiburon* flew over the carcass along transects paralleling the long axis of the skeleton. Surveys were conducted at the beginning of the first dive series to avoid ROV disturbance of bacterial mats and sediments prior to the survey. Digital pictures were taken from the camera oriented vertically downward and helped to estimate bacterial mat and black sediment cover at the whale carcass and on the surrounding sediments. ROV altitude was held constant at approximately 2 m during transects, and the camera was fired to obtain overlapping images. Visual maps of the whale fall were created in 2004 and 2005 from selected images that were combined in a mosaic using the computer software Inkscape (Freeware). Overlapping portions of images were adjusted according to identifiable bone and sediment features, and images were sequentially stretched to correct for parallax. The image mosaic was calibrated from images that contained a known scale, using the computer software ImageJ (NIH, USA). An absolute length scale was obtained from 10-cm diameter circular scale markers dropped on the sediment surface around the whale carcass by the ROV. In images with no visual scale, similar structures were overlaid at the sediment-water interface (e.g., bone outlines,

bioturbation features, sessile benthos) in successive images, and laser scale markers were used to calibrate length scales throughout the mosaic. Errors in length estimates due to scale calibration at the sediment-water interface are estimated to be  $\leq 10\%$ .

Estimates of absolute areal coverage of microbial mats, and black (presumably sulphidic) sediments were for the sediment-water interface within a 50 cm radius of the skeleton. We restricted mat measurements to the 50-cm radius because video surveys indicated that all mats occurred within 50 cm of the skeleton in both 2004 and 2005. In one area, black sediments extended 60 to 70 cm from the skeleton, but this was a very small proportion of the total black-sediment area. To facilitate mapping, the carcass was divided in three regions: head, thorax and lumbar. The percentage cover in each area was subsequently used to estimate the absolute area of sediment coverage in each section from the total sediment area measured in each region in 2004 and 2005.

The protrusion of bones above the sediment-water interface and their irregular shapes hindered estimates of mat area on the skeleton. Thus, we could only estimate percentage cover of bone plan areas by bacterial mats, and skeleton plan area. The use of a real scale was hindered by very variable angles of observation and due to the bones arrangement above the seafloor. Direct comparisons of individual bones between cruises were also generally not possible. To compare mat coverage between 2004 and 2005, bones were grouped by type, including mandibles/maxillae, head bones, scapulas, ribs and vertebrae. Vertebrae were subsequently subdivided in thoracic, lumbar and caudal vertebrae.

### *Statistical methods*

Total faunal abundance, faunal similarity and diversity were compared among distances and time for each treatment type. Total densities around treatments and in ambient sediments were examined with One-Way ANOVA if normality of variances was present. If homogeneity of variances was not attained, a non-parametric Kruskal-Wallis test was performed. For significant ANOVA and Kruskal-Wallis results, *post-hoc* tests were used to examine difference in means [statistical package BioEstat© (Zar 1996)]. Species diversity was evaluated for pooled replicate cores at each distance sampled (n=1-5) due to the low density of metazoans. Hulbert's rarefaction curves ( $ES_n$ ) were used to compare species diversity between treatments (see Chapter 1). Values of  $ES_n$  were compared at n=10, n=25 and n=50, where possible. Near treatment samples were compared at higher n values, because these samples contained higher abundances and were particularly interesting as they were directly influenced by the treatments. Background replicate cores (n=17) from 2002, 2004 and 2005 were combined to calculate a composite diversity from the background community; with confidence limits calculated from pooled cores. A one-tailed 95% confidence interval obtained from rarefaction "knots" obtained in each pooled sample was used to determine statistical significance [Chapter 2; (Smith 1986)]. Pielou's evenness ( $J'$ ) is given to provide further information on community structure (Clarke & Warwick 2001). The relative abundance of trophic guilds at each period sampled was integrated by distance in order to increase the power of statistical analyses. Samples from 0 m and 0.5 m were compared against the background in order to evaluate changes in trophic structure nearby the whale-fall; and examined with ANOVA or Kruskal-Wallis.

Cluster analyses and non-metric multi-dimensional scaling (MDS) based on species-abundance data from standardized quantitative samples [PRIMER v6, (Clarke

& Gorley 2006)] were used to compare community structure across distance and time. Square-root transformations were used prior to multivariate analysis in order to balance the importance of common and rare species (Clarke & Warwick 2001). Clustering and ordination analyses were often combined to verify mutual consistency. Analysis of similarities (ANOSIM) was performed on standardized quantitative samples to determine significant differences between groups (distance and time) and dissimilarity percentages identified species contributions to these patterns (SIMPER analysis, (Clarke & Warwick 2001).

Levels of species overlap between the whale-fall, kelp, wood, and other reducing habitats were restricted to direct comparisons of relatively abundant species (i.e., over 1% rel. ab.) collected within 0.5 m of the whale carcass, excluding species sampled at ambient sediments (i.e. total of 17 background cores from 2002, 2004 and 2004). We also excluded rare species (less than 1% rel. abundance) from our comparisons in order to account for difficulties in taxonomic identification and undersampling biases. Comparisons of species overlap with other published studies were carried out at the genus and species level in order to account for differences in taxonomic identification between studies.

### **3.3. Results**

#### *Organic enrichment around the whale-fall*

We found evidence of highly patchy organic enrichment in surface sediments around the whale during the period analyzed (Fig. 3.1). At 4.5y, sedimentary organic carbon was within background levels but reached 11% TOC at 0 m, evidencing the meter-scale patchiness of organic enrichment around the carcass (Fig. 3.1). Total organic nitrogen (TON) was slightly higher nearby the carcass, but with no significant

differences from background sediments (Fig. 3.1). The temporal patterns of TOC and TON suggests that the organic enrichment stage in the whale carcass had finished by 4.5 y (Smith & Baco 2003).

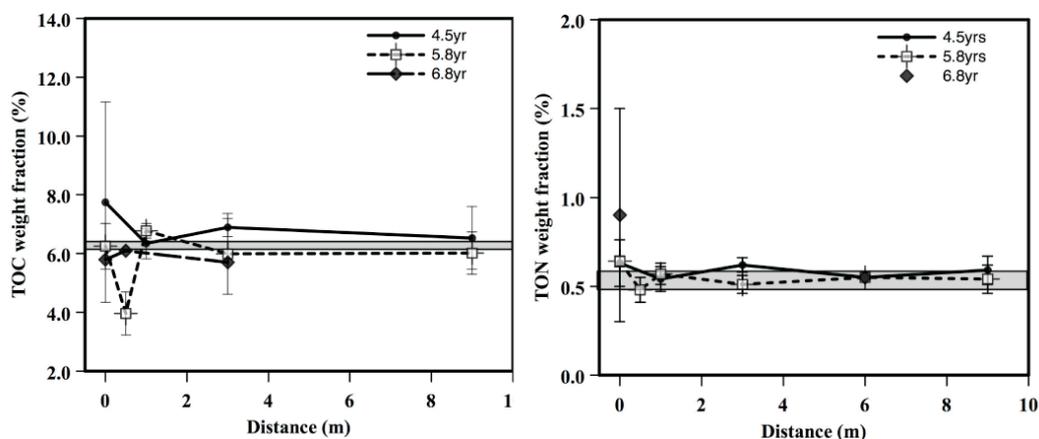


Figure 3.1. Left: TOC ( $\pm 1SD$ ) in surface sediments around the whale fall. Right: surface TON ( $\pm 1SD$ ). Background values ( $\pm 1SD$ ) displayed in shaded gray.

#### *Macrofauna density, composition and patterns of succession*

Macrofaunal abundance showed marked spatial and temporal differences in sediments around the Santa Cruz (SCr) whale fall. Macrofaunal density decreased significantly towards background sediments, with spatial differences being observed at all periods sampled. At all periods, macrofaunal density at 0 m was over 10 times background values ( $1269 \pm 189 \text{ ind. m}^{-2}$ ;  $p < 0.05$ ; Fig. 3.2, Table 3.1). At 4.5y, macrofaunal abundance at 1 m was also higher than background sediments (Kruskal-Wallis; Table 3.1). Significant higher abundances at 0 to 1 meters from the carcass were still evident after 5.8 and 6.8 years of deployment (Kruskal-Wallis test,  $p < 0.05$ , Fig. 3.2, Table 3.1). From 4.5 to 6.8 years, macrofaunal density was still increasing, being over 27 times background values in the last period (Fig. 3.2). At all periods

evaluated, macrofaunal abundance over 1 m was not different from background sediments (Fig. 3.2).

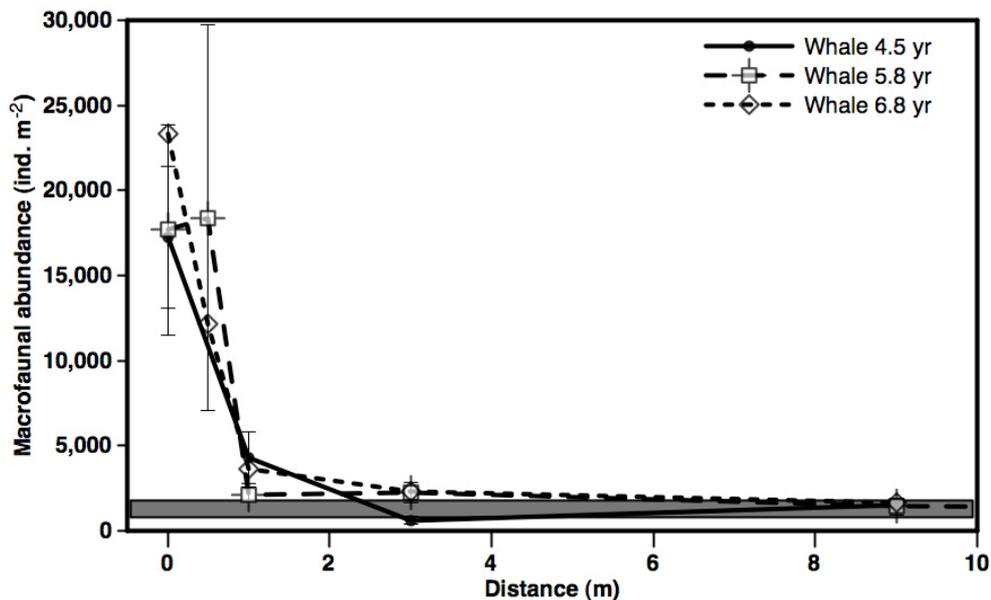


Figure 3.2. Total macrofaunal abundance around the SCr Whale in 2002, 2004 and 2005. Background density ( $\pm 1SE$ ) in shaded gray.

Table 3.1. *P* values from Kruskal-Wallis test of total macrofaunal abundances between 4.5y and 6.8y.

Distance	4.5y				5.8y				6.8y				
	0m	1m	3m	9m	0m	0.5m	1m	3m	9m	0m	0.5m	1m	3m
0m													
0.5m													
1m					0.042					0.020			
3m	0.000	0.011			0.050	0.012				0.047			
9m	0.018				0.006					<0.01			
Bkgd	0.000	0.038			0.000	0.041	0.080			0.000	0.002	0.010	

Polychaetes and cumaceans predominantly dominated the macrofauna around the carcass (0-1 m) during the three periods sampled (Fig 3.3). At 4.5y, dorvilleid and ampharetids polychaetes were massively present in the nearest 0 meters, representing near 80% of the macrofaunal individuals (Fig. 3.3). Two dorvilleid species (*Ophryotrocha* sp.A and *Ophryotrocha platykephale*) were among the top-ranked species at 0 m after 4.5y (Table 3.2). A new species of gastropod (*Hyalogyrina* n.sp.) was another dominant species at 0 m, and is extremely abundant in the whale carcass

(Smith & Baco 2003). The chemoautotrophic symbiont-containing mytilid bivalve *Idas washingtonia* was an abundant species at 0 m and up to 3 meters from the carcass at 4.5 y. At 0.5 meters, Cumacean sp. K was the dominant species (>50% relative abundance), together with dorvilleids, ampharetids and cirratulids (Fig. 3.3). The macrofauna over 3 m at 4.5y was typically composed of background species, dominated by cirratulid polychaetes. At 5.8 years, dorvilleids and cumaceans dominated the macrofauna at 0 m, with ampharetids also abundant at 0.5 m (Fig. 3.3). The dominant species at 0 m were the dorvilleids *Ophryotrocha* (2 spp.) and *Parougia* sp., and two cumacean species, which all accounted for over 60% of all individuals (Table 3.2). At 1 m, the macrofauna was dominated by cirratulids typical of background sediments, but ampharetids, dorvilleids, and vesicomys bivalves reached high abundances (Fig. 3.3). Sediments at 3 m exhibited a particular mixture of background (cirratulids and cossurids) and opportunist species (*Parougia* sp. A and cumaceans; Fig. 3.3). After 6.8 years, the macrofauna within 0 - 0.5 m meters was closely similar, with dorvilleids, cumaceans and ampharetids being the dominant organisms (Fig. 3.3). The top-ranked species at 0 m included three dorvilleid species, two ampharetids and Cumacean sp. K (Table 3.2). Sediments at 1 meter had a lower proportion of opportunistic species if compared to the previous period (5.8 y), indicating succession towards background conditions (Fig. 3.3). Background species dominated sediments over 3 meters from the whale fall, but some sediment patches contained species not typically found on background sediments (Fig. 3.3).

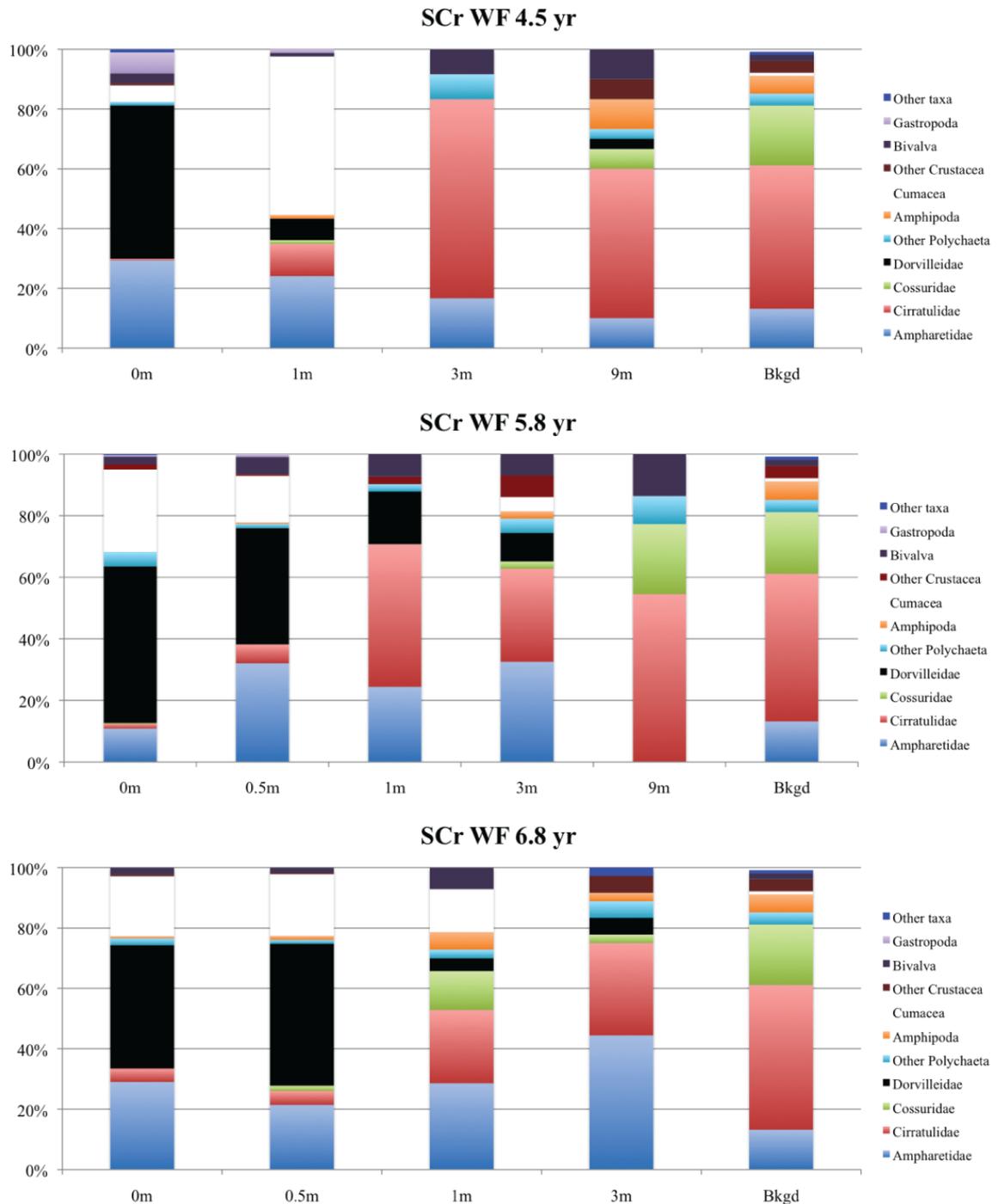


Figure 3.3. Macrofaunal composition around the whale fall at 4.5y, 5.8y and 6.8y.

Many of the dominant species exhibited significant increases in abundance nearby the whale fall but were not found on background areas, which is characteristic of disturbance opportunistic species (Grassle & Grassle 1974). From the total of 14 dorvilleid species identified in sediments nearby the whale carcass, at least 4 species

responded opportunistically to the arrival of the whale carcass (Table 3.2). The genus *Ophryotrocha* included at least 3 species that were particularly dominant up to 0.5 meters and cumaceans were also among the dominant species up to 1 meter from the whale-fall but not at background sediments. Two ampharetid species were also opportunist species that were exploiting disturbance conditions nearby the whale fall (Table 3.2).

Table 3.2. Top-ranked species at 0 m sediments from the macrofauna at 4.5, 5.8 and 6.8 y. Dominant species from background samples also indicated. Density in (ind. m<sup>-2</sup> ( $\pm 1$  SE)).

Whale 4.5 years											
Rank	Species	0 m		0.5m		1m		3m		9m	
		Mean density	Rel %								
1	<i>Ophryotrocha</i> sp. A (P)	26.2(3.7)	39.4%								
2	CRS Ampharetid sp. 14 (P)	9.7(1.1)	14.6%								
3	CRS Ampharetid sp. 12 (P)	5.2(1.0)	7.8%			0.2(0.1)	1.2%				
4	<i>Ophryotrocha platykephale</i> (P)	4.8(0.8)	7.3%								
5	<i>Hyalogyrina</i> n.sp. (M)	4.7(0.8)	7.0%			0.2(0.1)	1.2%				
6	<i>Cumela</i> sp. A (Cr)	3.0(0.8)	4.5%								
<b>Total percent</b>			80.6%				2.4%		8.3%		
Whale 5.8 years											
1	<i>Ophryotrocha</i> sp. A (P)	15.4(3.8)	22.6%	16.3(7.2)	23.1%						
2	Cumacea sp. K (Cr)	14.2(3.8)	20.9%	5.0(2.9)	7.1%			0.2(0.1)	2.3%		
3	<i>Ophryotrocha</i> sp. E (P)	7.4(3.1)	10.9%	1.3(0.5)	1.9%						
4	<i>Cumela</i> sp. A (Cr)	4.0(1.1)	5.9%	5.3(2.8)	7.5%			0.2(0.1)	2.3%		
5	CRS Ampharetid sp. 14 (P)	2.8(0.8)	4.1%	7.7(4.4)	10.8%						
6	<i>Parougia</i> sp. A (P)	1.2(0.3)	1.8%	3.0(1.5)	4.2%	0.8(0.1)	9.8%	0.6(0.2)	7.0%		
<b>Total percent</b>			66.2%		54.6%		9.8%		11.6%		
Whale 6.8 years											
1	Cumacea sp. K (Cr)	17.4(3.3)	19.4%	7.2(1.9)	15.4%	1.2(0.2)	8.6%				
2	<i>Ophryotrocha</i> sp. A (P)	10.2(1.3)	11.4%	2.2(0.7)	4.7%	0.2(0.1)	1.4%				
3	<i>Ophryotrocha</i> sp. E (P)	7.6(3.4)	8.5%	9.4(4.2)	20.1%						
4	CRS Ampharetid sp. 14 (P)	8.4(2.2)	9.4%	1.6(0.7)	3.4%						
5	CRS Ampharetid sp. 12 (P)	7.6(1)	8.5%	3.4(1.3)	7.3%						
6	<i>Exallopus</i> sp. A (P)	2.8(1.1)	3.1%	4.0(1.8)	8.5%						
<b>Total percent</b>			60.3%		59.4%		10.0%				
Background (2002-2005)											
		Mean density		Rel %							
1	<i>Cossura cf. rostrata</i> (P)	0.8 (0.3)		16.9%							
2	<i>Chaetozone</i> sp. D (P)	0.7 (0.2)		14.5%							
3	<i>Monticellina</i> sp. A (P)	0.6 (0.2)		13.3%							
4	<i>Tharyx</i> sp. A (P)	0.3 (0.1)		6.0%							
5	<i>Monticellina</i> sp. B (P)	0.3 (0.2)		6.0%							
<b>Total percent</b>				56.7%							

Areas with high dominance of opportunist species within 0 – 0.5 m of the whale-fall showed significant decreases on community evenness ( $J'$ ; Fig. 3.4). At 4.5y,  $J'$  was significantly lower in sediments within 0 - 1 m from the whale-fall, due to the dominance of dorvilleids, ampharetids (0 m) and cumaceans (1m, Fig. 3.3;

ANOVA,  $F=4.15$ ,  $d.f.= 2$ ,  $p=0.027$ ). At 5.8 y, sediments at 0 m were still heavily colonized by opportunistic polychaetes and cumaceans and presented significant lower evenness than areas over 0.5 m and background sediments (Fig. 3.3., ANOVA,  $F=7.55$ ,  $d.f.= 5$ ,  $p=0.002$ ). The same pattern was observed after 6.8 y, with 0 m sediments showing lower community evenness than 0.5 m and areas further away from the whale-fall (ANOVA,  $F=6.11$ ,  $d.f.= 4$ ,  $p=0.001$ ). Community evenness also varied markedly from 4.5y to 6.8 y at sediments nearby the whale-fall (0 - 1 m). At 1 m,  $J'$  was significantly lower at 4.5 y if compared to the other periods (5.8 y and 6.8 y) and background areas (ANOVA,  $F=3.07$ ,  $d.f.= 3$ ,  $p=0.044$ ). Sediments at 0 m had higher dominance than background areas during all periods studied (ANOVA,  $F=8.00$ ,  $d.f.= 3$ ,  $p=0.001$ ). The spatial and temporal patterns on macrofaunal community evenness described above, clearly indicates that areas within 1 m from the whale-fall were strongly disturbed at 4.5 y, but not after 5.8 y when significant changes in  $J'$  were mainly observed at 0 m.

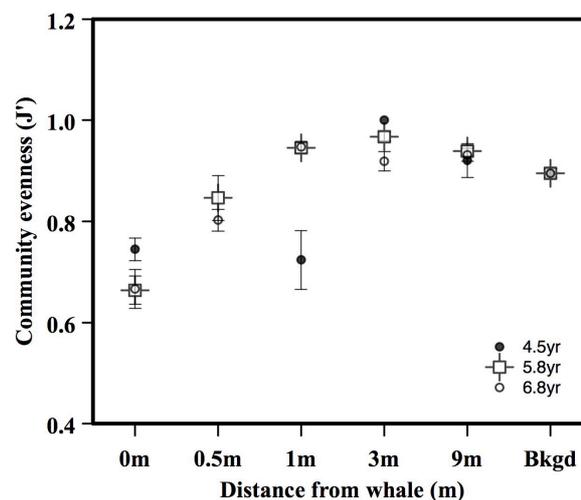


Figure 3.4. Macrofaunal community evenness (Pielou's  $J'$ ) at the Santa Cruz whale-fall.

Although significant spatial and temporal differences in community evenness were observed, macrofaunal diversity nearby the whale-fall was generally higher than

background sediments between 4.5 y and 6.8 y (Fig. 3.5). At 4.5 y, diversity at 1 m was significantly lower than background sediments (Fig. 3.5-a). However, 0 m sediments and areas further 3 m from the whale-fall at 4.5y had similar or higher diversity levels than background (Fig. 3.5-a). At 5.8y, diversity at 1 m markedly increased if compared to the previous period and nearby the whale-fall (0 - 1m), diversity was comparable to background sediments (Fig. 3.5 -b). In the last period analyzed, levels of diversity were higher than background at all distances sampled (Fig. 3.5-c). There was evidence of a temporal increase on species diversity around the whale-fall (0 m), from 4.5 y to 6.8 y (Fig. 3.6). The temporal increase in species diversity nearby the whale-fall was concurrent with the recolonization of previously disturbed areas by background species and possibly the arrival of new and rare species. At the two latter periods, levels of diversity also increased from 0 m to 3 m (Fig. 3.6), likely in response to the augmented richness in those areas which were colonized by whale-fall and background species. The augmented diversity towards latter stages of succession (6.8 y) supports a change on the habitat structure within the continuous of a slightly disturbed community (2002) to a more species rich fauna in 2005 (Fig. 3.6).

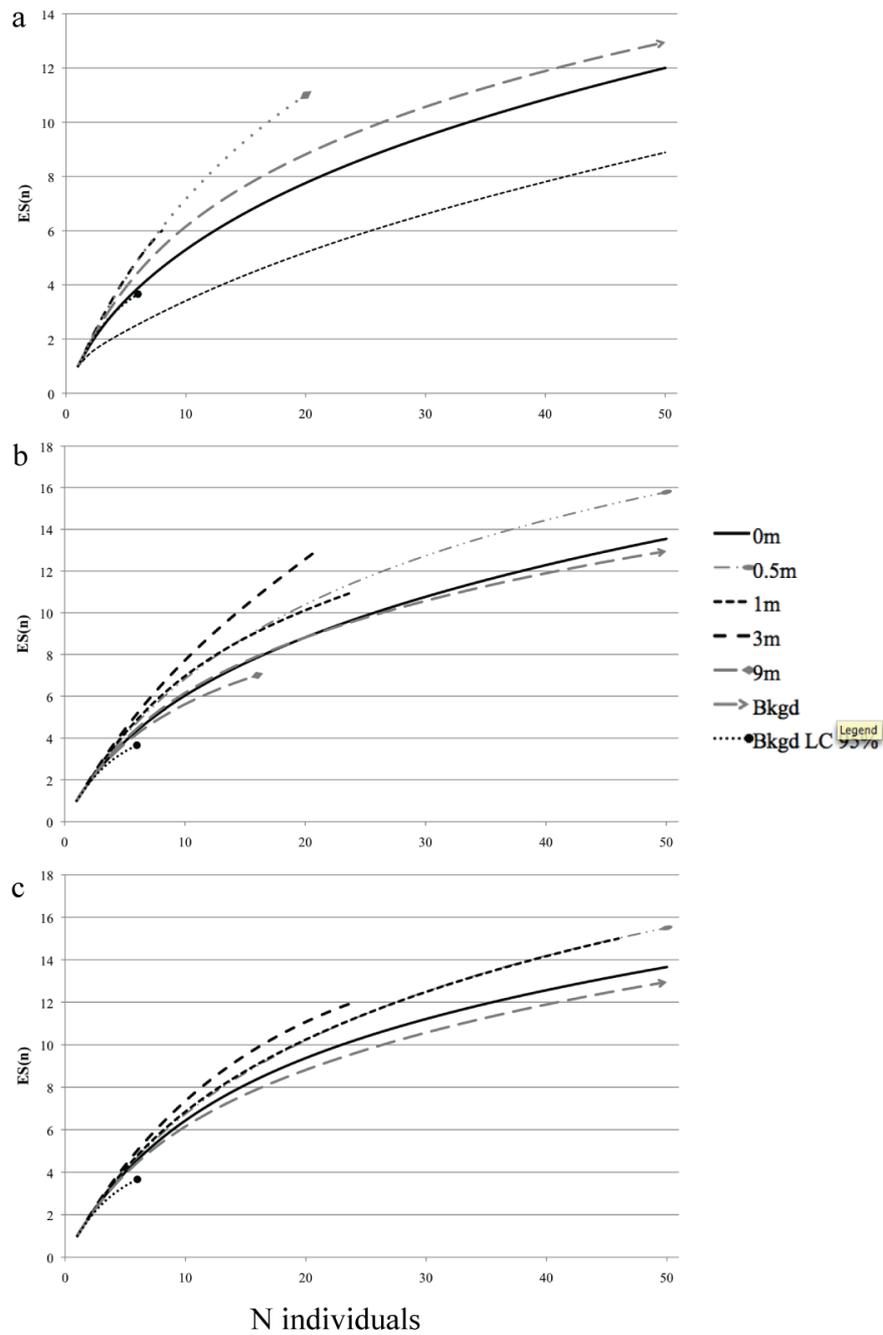


Figure 3.5. Hulbert diversity plot from all periods sampled at the SCr whale-fall. a) 4.5 y; b) 5.8 y and c) 6.8 y. Bkgd LC = 95% background confidence limit.

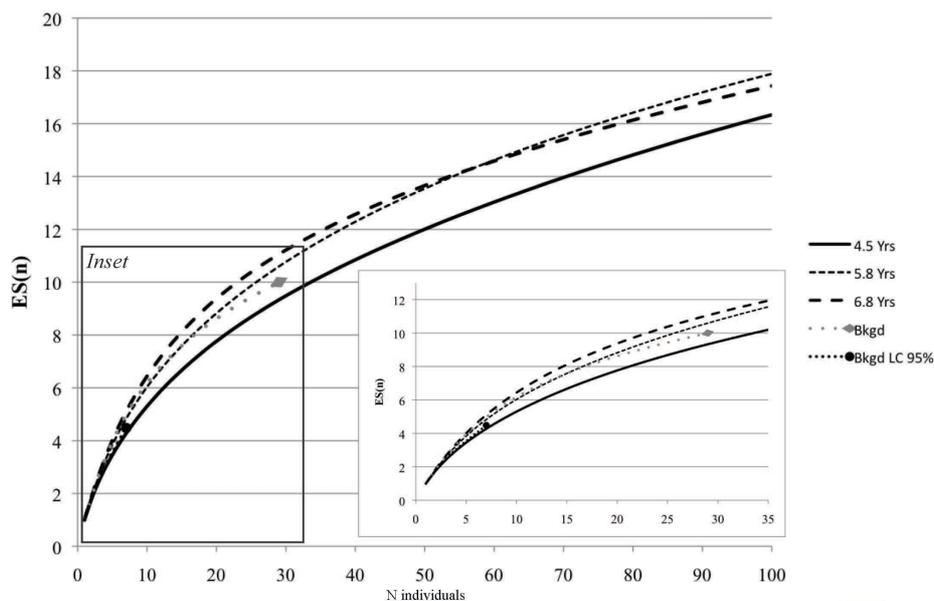


Figure 3.6. Hulbert rarefaction diversity at 0 m sediments in the SCr whale-fall after 4.5y, 5.8y and 6.8y.

### *Trophic group analysis*

There were significant changes on trophic-group dominance between the whale-fall and background sediments, and from 4.5 y to 6.8 y (Fig. 3.7). At 4.5y, omnivores (OMNI) were significantly more abundant at 0 m (Kruskal-Wallis,  $H=47.674$ , d.f.=11,  $p=0.001$ ), and subsurface deposit feeders (SSDF) were absent up to 1 m sediments ( $p<0.01$ ; Fig. 3.7). At 1 m, cumaceans were responsible for the highest proportion of OTHER trophic group, with significant differences from background sediments (Fig. 3.7; Kruskal-Wallis,  $p=0.02$ ). From 3 meters towards background areas, surface deposit feeders (SDF) predominate, being typically represented by polychaetes. At 5.8 y, the number of OMNI increased from the previous period at 0, 0.5 and 1 m sediments, being significantly higher than background (Fig. 3.7; Kruskal-Wallis,  $H=48.262$ , d.f.=15,  $p=0.001$ ). The increase in OMNI was due to an increased abundance of dorvilleids at sediments within 1 m from the whale-fall in these areas if compared to 4.5 y (Fig. 3.3). In the last period (6.8 y),

the dominance of OMNI still predominated in areas within 0 – 0.5 meters from the whale-fall (Fig. 3.7; Kruskal-Wallis,  $H=54.3$ ,  $d.f.=15$ ,  $p=0.001$ ). The high number of cumaceans at 0 m significantly increased the proportion of OTHER relative to background areas (Kruskal-Wallis,  $p<0.05$ ). The elevated contribution of SDF's at 0 – 3 m sediments in all periods sampled was typically due to the presence of ampharetid polychaetes, whereas cirratulids were the dominant deposit feeders in background areas.

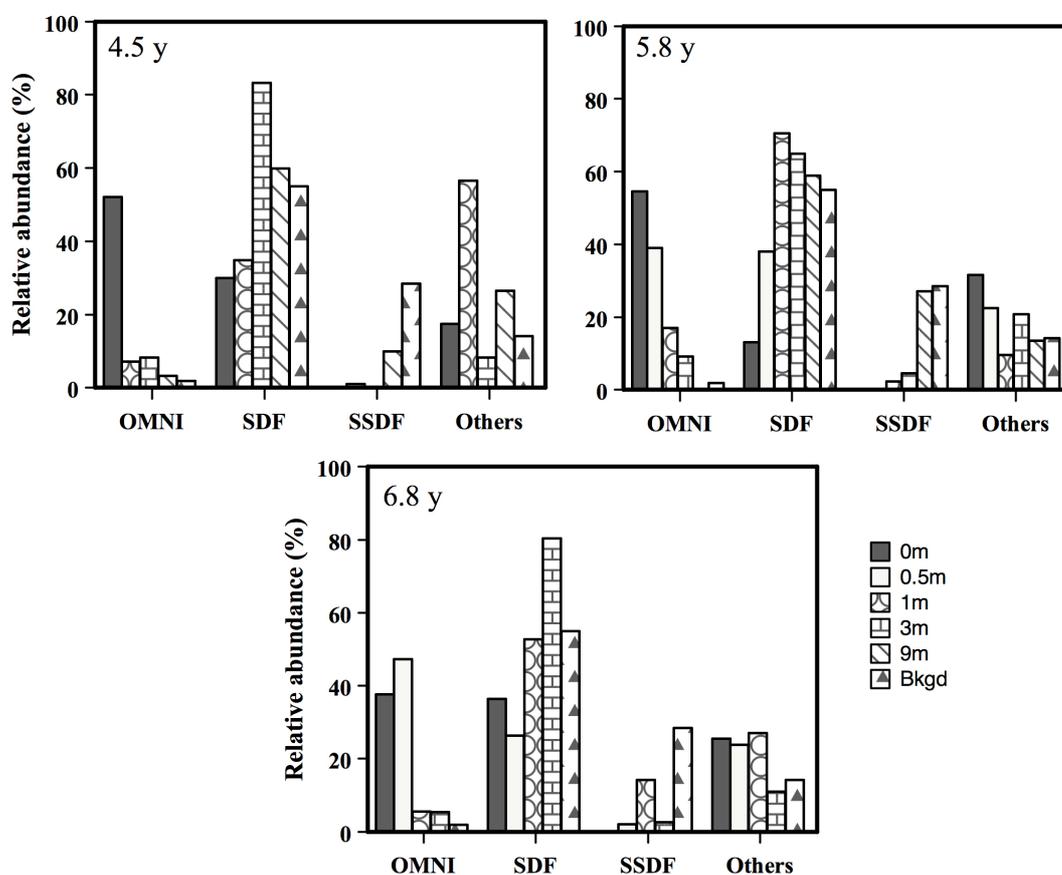


Figure 3.7. Trophic group distribution around the SCr whale-fall during 4.5, 5.8 and 6.8y.

*Video analysis – coverage of bones and sediment by bacterial mats*

Bone coverage was estimated from 39 and 46 bones in 2004 and 2005 images, 5.8 and 6.8y respectively (Table 3.3). The lower number of bones observed in 2004 is partly due to the larger extent of bacterial mats at this time, often preventing a clear delineation of bones. Percent bacterial mat coverage as function of bone type and was generally lower in 2005 than in 2004, with statistically significant reductions occurring on vertebrae (Table 3.3).

Head bones and ribs had the highest and most stable coverage of bacterial mats (Table 3.3). These bones were positioned further over the seafloor, which made them very visible in all images and possibly prevented the accumulation of sediments stirred up by ROV activities in 2004. Such sediment deposition was observed on flat bones near the seafloor, such as jaws and scapulae, probably resulting from sediment resuspension from the ROV thrusters during the previous dive series in 2002 or 2004. There was a significant difference in bacterial mat coverage on the vertebrae between years, with thoracic and caudal vertebrae sustaining a ~30% decrease in mat coverage from 2004 to 2005 (Table 3.3). Pooling of all vertebrae also yielded a significant 31% decrease in mat coverage from 2004 to 2005 ( $p=0.001$ ; Kruskal-Wallis test; Table 3.3).

*Table 3.3. Percentages of bone plan area covered by bacterial mat on the Santa Cruz whale skeleton in 2004 and 2005. N = number of bones analyzed. 1. Vertebrae were divided according to its structure and position on skeleton. \* Statistically significant differences in percent coverage present between years ( $p<0.05$ ). \*\* Highly statistically significant differences in percentage coverage between years ( $p<0.01$ )*

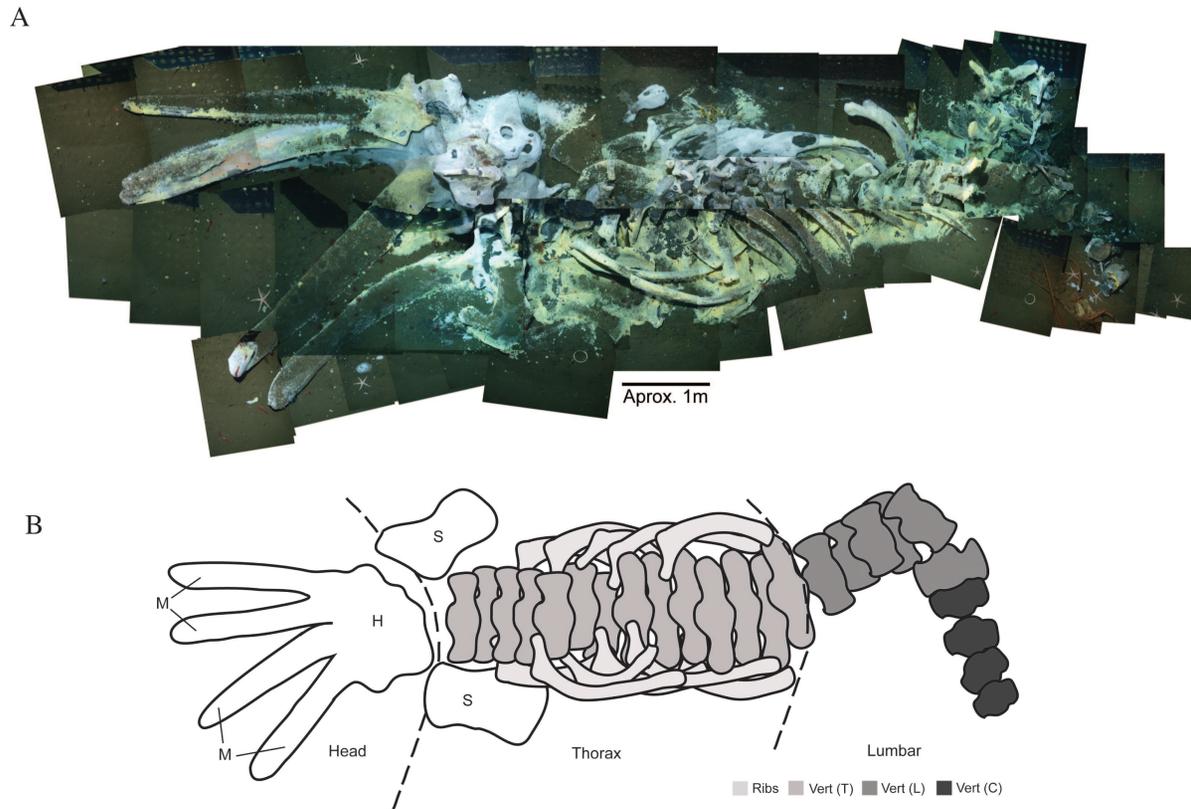
Bone Class	Region <sup>1</sup>	2004			2005		
		N	% Coverage	SE (%)	N	% Coverage	SE (%)
Skull		3	97	2	4	94	3
Mandibles/maxillae		4	61	10	4	45	15
Scapula		2	44	16	2	60	19
Rib		12	78	6	14	76	8
Vertebrae	Thorax	8	94**	3	11	58**	11
	Lumbar	5	77	8	6	55	9
	Caudal	5	67*	9	5	32*	5
	All Vert	18	82**	4	22	51**	6

Bacterial mats and black sediments were concentrated in the head and thoracic regions of the skeleton (Fig. 3.8; Table 3.4). Around the head, bacterial mat decreased in area by over 45%, and black sediments decreased by ~50% between 2004 and 2005 (Table 3.4). In the thoracic region, the area of black sediment decreased by over 60% while the area of bacterial mat increased by 45%. This suggests that bacterial mats continued to grow in the thoracic region between 2004 and 2005, i.e., 6.8 years after carcass emplacement. The lumbar region had the smallest area of bacterial mat and black sediments, with an observed 30% decrease in the area of bacterial mats between 2004 and 2005. Over the whole carcass, the area of bacterial mats was relatively stable, decreasing slightly (7%) between 2004 and 2005. The decrease in mat coverage in the head and lumbar regions was offset by the large increase in mat area in the thoracic region. However, the area of black sediments patches around the entire skeleton decreased by more than 50% between 2004 and 2005. The combined area of bacterial mat and black sediments declined ~ 23% between 2004 and 2005, suggesting a gradual decline in the availability of sulfide-rich around the carcass over this period.

*Table 3.4. Estimated surface area (m<sup>2</sup>) of total sediment, bacterial mats and black sediments within 0.5 m of the whale skeleton in 2004 and 2005. “Total sediment area” includes bacterial mats, black sediments, and other sediments. “Error” is the difference percentage difference in total sediment area between 2004 and 2005.*

Carcass region	Total sediment area			Bacterial Mat				Black Sediment			
	2004	2005	Error	2004		2005		2004		2005	
				Area	% cover	Area	% cover	Area	% cover	Area	% cover
Head	8.6	8.7	<1%	0.9	11%	0.4	4%	0.6	7%	0.3	4%
Thorax	6.5	7.0	8%	1.0	16%	1.6	23%	1.0	15%	0.4	5%
Lumbar	2.6	2.4	9%	0.3	14%	0.3	11%	0.1	5%	0.2	10%
Whole carcass	17.7	18.1	2%	2.3	13%	2.2	12%	1.7	10%	0.9	5%

Figure 3.8. **A.** Color mosaic of the SCrB whale carcass in 2005. **B.** Schematic of the whale skeleton showing the major bones and the three regions of the carcass used to measure the bacterial mat coverage (head, thorax and lumbar). M: mandibles/maxillae; H: head bones; S: scapulae. Color legend: Vert (T): thoracic vertebrae; Vert (L): lumbar vertebrae; Vert (C): caudal vertebrae. Not all skeleton components were visible so the number of bones depicted is not anatomically accurate.



*Multidimensional patterns of macrofaunal distribution*

The temporal macrofaunal successional around the whale-fall is clearly evidenced by the Nonmetric Multidimensional Scaling (MDS) analysis. Broad patterns of dissimilarity at all periods were evident between areas around the whale carcass and background sediments (Fig. 3.9). Considering the whole period analyzed (4.5y to 6.8y), the macrofauna exhibited significant differences between 0 m and areas further 1 m from the carcass (ANOSIM,  $R= 0.445$ ,  $p=0.001$ ). At 0 m the dominant species *Ophryotrocha* sp. A and Ampharetid sp. 14 responded for over 40% of group similarity (SIMPER). The macrofaunal structure at 0.5 m at the two latter periods (5.8 and 6.8y) was also clearly distinct from background areas (ANOSIM,  $p=0.002$ ).

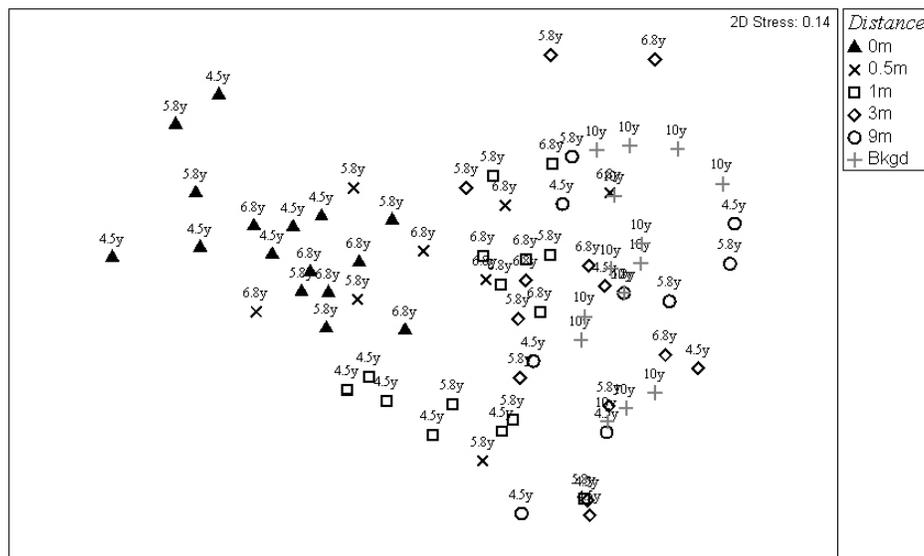


Figure 3.9. Multidimensional scaling plot (MDS) of macrofaunal communities (>300µm) at the SCr whale-fall.

The spatial differences between communities nearby the whale-fall and background sediments confirm the strong heterogeneity in their dominant species, where a mosaic of species from both ends colonized sediments between 1 m and 3 m. At 0 m,

temporal differences in community structure were not significant within the period analyzed.

Our first hypothesis that whale-falls create sulfophilic and organic rich habitats with influence on macrofaunal community structure was partially supported. After 4.5y, the whale-fall sustained a low sediment organic enrichment, but with high sulfide availability from 5.8 to 6.8y (i.e. from 2004 to 2005). The distinct sediment geochemistry within 0 – 0.5 m from the whale-fall continuously supported a distinct set of species after 6.8 years. The data suggests that after 4.5y, the minor variability in sediment organic carbon between 0 m and background areas could not explain the marked differences on community structure. However, the effects of a significant organic input from the whale-carcass before 4.5 y evidently favored the massive colonization of opportunist species and induced higher sedimentary microbial activity (Treude et al. 2009). The spatial distribution of bacterial mats over sediments nearby the whale-fall supports that after 5.8 y, the gradient on sulfide availability has a fundamental role structuring the macrofaunal community.

#### *Food web analysis on the macroinfauna around the Santa Cruz Whale carcass*

Potential food sources for the macrofauna around whale-falls include sediment organic matter (i.e. phytoplankton), metazoan biomass, whale soft tissue and sulfur-oxidizing bacteria growing over reducing sediments. Whale tissue and bacterial mats growing over bones were sampled in the SCr whale-fall, and exhibited very distinct  $\delta^{15}\text{N}$  values if compared to the other organic sources (Table 3.5). The most depleted carbon

and nitrogen values come from sulfur-oxidizing bacteria, which indicates an organic matter of local origin [Table 3.5; (Ruby et al. 1987)].

Table 3.5. Stable isotope signatures of potential organic sources around the SCr whale-fall.

	N	<sup>13</sup> C Average (min/max)	<sup>15</sup> N	Ref.
Sedimented phytoplankton	2	-20.3 (-22.1/-18.4)	6.7 (6.6/6.8)	This study
Sulfur-oxidizing bacterial mats	3	-20.4 (-21.6/-19.3)	0.6 (-4.3/7.4)	Baco and Smith, unpubl.
Whale soft tissue	2	-16.1 (-16.1/-16.1)	12.8 (12.8/12.8)	Baco and Smith, unpubl.
Other chemosynthetic bacteria	-	-33 (-46/-20)	-12	Ruby et al., 1987

The whale-fall macrofauna exhibited wide ranges on carbon and nitrogen isotope signatures. There was no difference on macrofaunal carbon isotope values between years sampled (average  $\delta^{13}\text{C}$  of -22.2, -19.7, and -23.6 ‰, for 4.5, 5.8 and 6.8y, respectively). Average macrofaunal  $\delta^{15}\text{N}$  was also similar and ranged from 4.3 to 6.1 ‰ (from 4.5 to 6.8y). Carbon isotope values ranged from -27.6 ‰ for the dorvilleid *Ophryotrocha* sp.A, to -14.4 ‰ for the Dorvilleid n.sp. (Fig. 3.10). Macrofaunal  $\delta^{15}\text{N}$  ranged from -0.6 ‰ to 12.5 ‰ for the dorvilleid *Parougia* sp.A and Cumacea sp.K, respectively. The wide ranges on isotope signatures for dorvilleids suggest opportunistic feeding on many organic sources, including chemoautotrophic sources of nitrogen and carbon for those species. The dominant macrofaunal species associated with the whale-fall from 4.5y to 6.8y had isotope values consistent with primary feeding on the abundant carpets of bacteria growing around the whale carcass (Fig. 3.10). Besides sulfur-oxidizing bacteria, sediment organic carbon was a potential primary food source for *Cumella* sp.A and dorvilleids (Fig. 3.10). The dominant macrofaunal species did not feed directly on whale biomass, and the major reliance on bacterial biomass (i.e. directly or through the food

web) suggests that the community was heavily dependent on chemosynthetic derived carbon from 4.5 y to 6.8 y.

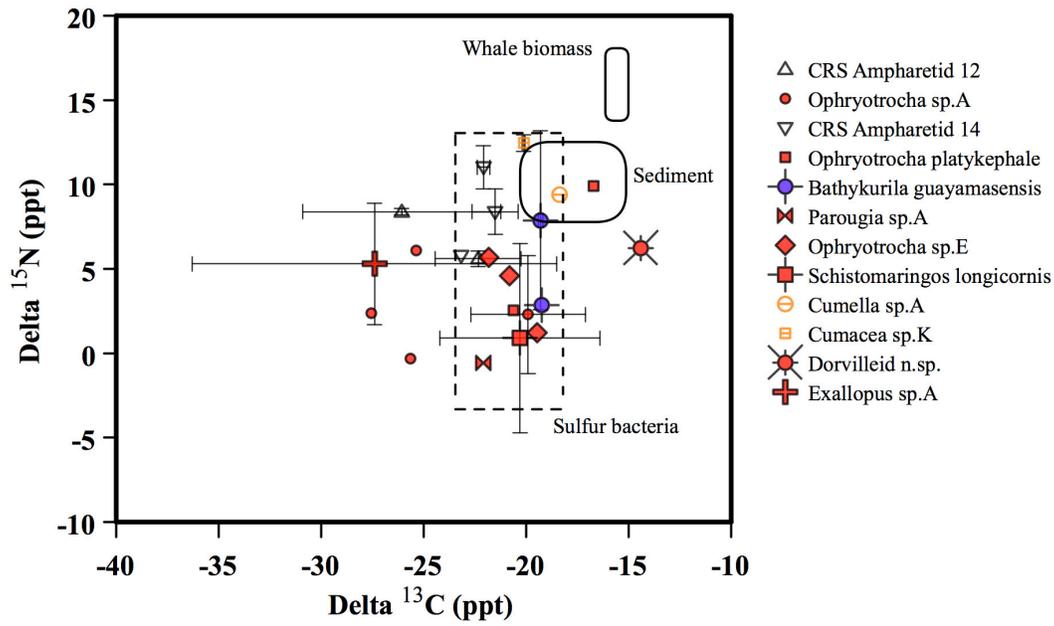


Figure 3.10. Stable isotope values for the whale-fall macrofauna. Standard error values are shown for replicated measurements. Colors indicate distinct macrofaunal groups: white: Ampharetids, red: Dorvilleids, blue: Polynoids, orange: Cumaceans. Symbols differentiate species within each group. Boxes indicate range on isotope values for one trophic shift for each organic source indicated in the plot.

### Species overlap

A total of 68 macrofaunal species were collected around the SCr whale-fall (4.5y to 6.8y), including 18 species present in background sediments. Rare species (i.e. not including bkgd spp. and with < 1.0% rel. abundance) accounted for 38% of the total species richness (25 spp.). Species that were exclusively found in sediments within 0 – 0.5 m of the whale-fall represented 22% (15 spp.) of the total richness, increasing to 25 species when 1 m samples are included. Polychaetes contributed to the majority of the species around (up to 0.5m) the whale carcass, with ampharetids (3 spp.), cirratulids (2

spp.), dorvilleids (7 spp.) and polynoids (1 spp.) being the most representative. The asellote isopod *Ilyarachna profunda* and the mytilid *Idas washingtonia* were also exclusively sampled within 0.5 m sediments and these species are both abundant on whale bones (Smith & Baco 2003).

Table 3.6. Whale-fall sediment macrofaunal species that overlap with other chemosynthetic communities in the deep-sea. Species included reached > 1.0% rel. abundance within 0-0.5m from the whale carcass (see methods). X- 1-5% rel. abundance, XX- over 5% rel. abundance in this study. P – present. References: 1- Smith and Baco, 2003, 2- Bernardino et al., in prep, 3- Levin et al., 2003, 4- Levin, 2005, 5- Blake and Hilbig, 1990, 6- Smith et al., in prep, 7- Tunnicliffe, 1998, 8- Bernardino and Smith, in prep.

Species	SCr whale	Kelp	Wood	Seep	Vent	References
CRS Ampharetid sp. 1	XX					
CRS Ampharetid sp. 6	X					
CRS Ampharetid sp. 12	XX					
CRS Ampharetid sp. 14	XX					
<i>Sosanopsis</i> sp.A	X					
<i>Samytha</i> cf. <i>californiensis</i>	XX	XX	XX			1, 2
CRS Cirratulid. sp. 1	XX					
CRS Cirratulid. sp. 2	XX		XX			2
<i>Parougia</i> sp. A	XX		XX	P	P (genus)	1, 2, 3, 4, 5
<i>Ophryotrocha</i> sp. A	XX	XX	XX	P (genus)	P (genus)	1, 2, 4
<i>Ophryotrocha</i> sp. B	X		X	P (genus)	P (genus)	1, 2, 4
<i>Ophryotrocha</i> sp. E	XX			P (genus)	P (genus)	1, 4
<i>Ophryotrocha</i> sp. H	X			P (genus)	P (genus)	1, 4
<i>Ophryotrocha</i> sp. K	X			P (genus)	P (genus)	1, 4
<i>Schistomeringos longicornis</i>	XX		X	P		1, 4
<i>Ophryotrocha platycephale</i>	XX			P	P	3, 5
<i>Exallopus</i> sp. A	XX			P (genus)	P (genus)	1, 4, 5
<i>Bathykurila guaymasensis</i>	XX		X		P	1, 2, 5
<i>Cumella</i> sp. A	XX	XX	X			1, 2
Cumacea sp. K	XX	XX	XX			1, 2
<i>Ilyarachna profunda</i>	X		XX			1, 2
<i>Idas washingtonia</i>	XX		X	P	P	1, 2, 4, 7, 8
Bivalve sp. Q	X	XX	XX			1, 2
<i>Hyalogyrina</i> n. sp.	P	XX				1, 2

Our second hypothesis was supported by the low level of species overlap between the SCr whale-fall macrofauna and other reducing habitats in the deep Californian slope. Whale and kelp overlap (9% from total spp.), included the ampharetid *Samytha cf. californiensis*, the dorvilleid *Ophryotrocha* sp.A, two cumaceans (*Cumella* sp.A and Cumacean sp.K), one bivalve species and the gastropod *Hyalogirina* n.sp. (Table 3.6). The number of overlapping species between whale-fall and wood macrofauna was the highest among the habitats compared, with 18% of the total species present on both habitats (Table 3.6). A few organic respondent opportunist species present at wood and kelp falls were also present in the SCr whale fall, which contributed to higher species overlap between these organic islands. The number of species common to whale, seeps and vents was low (6 and 4%, respectively), and was mostly restricted to the dorvilleids *Parougia* sp.A, *Ophryotrocha platycephale*, *Schistomaringos longicornis* and the polynoid *Bathypurila guaymasensis* (Table 3.6). Many of the dorvilleids present in the SCr whale-fall are genera that occur in seep and vent ecosystems (e.g. *Parougia*, *Ophryotrocha*, *Exallopus*), which highlights the extreme diversity and adaptation of this group to reducing environments [Table 3.6, (Dahlgren et al. 2001, Thornhill et al. 2009, Wiklund et al. 2009a)]. The overlap of *Idas washingtonia* between whale, wood, seep and vent ecosystems supports the suitability of whale and wood parcels to sustain chemoautotrophic symbiosis and the stepping stone hypothesis to dispersal of vesicomylid species (Smith et al. 1989, Distel et al. 2000).

### 3.4. Discussion

Any systematic organic enrichment in surface sediments around the whale-fall had disappeared after 4.5y, indicating that the transition between the *organic-enrichment* (OE) and *sulfophilic* (SU) stages had started (Smith & Baco 2003). The absence of a broad-scale organic enrichment at the SCr whale-fall is consistent with sedimentary patterns around other natural deep-sea whale carcasses sampled during similar successional stages [i.e > 4 y; (Naganuma et al. 1996, Smith et al. 1998)]. Although the whale carcass studied by Smith et al. (1998) was larger than the SCr whale, the former had been in the seafloor for much longer at the time of sampling [4 – 36y; (Bennett et al. 1994)]. The sediments around the SCr whale-fall (0 m) exhibited a strong spatial heterogeneity in organic content at 4.5 y, with patches sustaining high organic levels (up to 11 % w/w TOC). Visual observations of samples collected in the thoracic whale-fall region at 5.8 y also revealed the presence of pieces of whale blubber in a highly anoxic sediment (Treude et al. 2009), thus evidencing that sediment patches were still enriched organically after 5.8 y. The temporal patterns of organic enrichment from this study indicated that organically enriched sediments may persist for > 6 y around large-sized (i.e. > 10 ton) whale-falls.

The broad spatial distribution of bacterial mats around the whale skeleton for over 6 years evidenced the wide availability of pore water sulfides. Bacterial mats and black (i.e. anoxic and sulphidic) sediments occupied a larger area around the thoracic and head regions, which is spatially consistent with pore-water sulfide fluxes and bacterial abundance around the SCr whale-fall (Treude et al. 2009). The temporal decrease on the

covered area by bacterial mats and black sediments indicated a decline in the overall availability of free sulfides between 5.8 to 6.8 y (Treude et al. 2009). The bacterial mat coverage around the SCr whale-fall is strikingly higher than other whale skeletons at similar successional stages. In the Santa Catalina whale-fall (SC), concentrations of sediment sulfides and abundance of sediment bacteria were low by 4 y, but bone surfaces were still covered by bacterial mats (Bennett et al. 1994, Smith et al. 1998). Sediments sustain faster microbial degradation rates of any available organic source, as opposed to the degradation of bone lipids from the hard bone matrix, which is mediated in the skeleton by specialized bone eating worms (Smith et al. 1998, Glover et al. 2005b, Treude et al. 2009). As a result, the lower degradation of bone lipids may extend the temporal availability of sulfides sustaining bacterial growth on bone surfaces for periods of over 6 y (Bennett et al. 1994, Smith & Baco 2003). Although sediments around whale-falls sustain sulfate reduction and methanogenesis for over 6.8 y (Treude et al. 2009), the processes over bone surfaces likely lasts longer. Consequently, differences in the access to organic sources, microbial sulfate reduction rates and sulfide release are markedly distinct between bones and sediments around whale carcasses, and have primary importance controlling the magnitude and durability of reducing conditions.

The large area of sediments covered by bacterial carpets nearby the whale-fall is equivalent in size to visual measurements from typical chemosynthetic habitats such as seeps and vents (Sahling et al. 2002, Treude et al. 2003, Levin et al. 2009). This suggests that whale carcasses impact similar areas of deep seafloor than cold seeps and hydrothermal vents (Bennett et al. 1994, Deming et al. 1997, Treude et al. 2009). Due to the rapid degradation of whale organics in the sediments, the production of sulfide is

shorter in whales if compared to seeps and vents (Treude et al. 2009). However, if compared to other organic reducing habitats (e.g. wood, kelp and other mammalian carcasses), whale-falls may provide the best conditions to sustain chemosynthesis-based nutrition (Smith 2006, Glover et al. 2008). Whale carcasses typically are larger and composed of high quality organic matter, which includes about 60 % of lipids by weight (Smith 2006). In the lower range of organic enrichment are kelp and wood falls that sustain only low levels of sulfide production in nearby sediments and for very short periods (Hannides 2008). The same occur with small-sized mammalian bones (e.g. cetaceans, cow bones), which are rapidly processed by scavengers and do not typically go through the EO and SU stages (Jones et al. 1998, Smith & Baco 2003). Although the mammalian skeletons might have enough lipid reserves to sustain minor bacterial growth and chemosynthesis, their potential to sustain macrofaunal chemosynthesis is clearly limited (Jones et al. 1998, Smith & Baco 2003, Glover et al. 2008, Jones et al. 2008).

Macrofaunal abundance was enhanced by 5-10 times around the whale-fall for over 6 y, despite any broad scale organic enrichment after 4.5 y. Macrofaunal density had a pronounced small scale variability within 0.5 m, which is consistent with the patchy organic enrichment encountered. The macrofauna was dominated by organic opportunist species, defined by individuals that attained significant abundances at the whale carcass but were rarely present in background sediments (Grassle & Grassle 1974). The lower community evenness nearby the whale-fall is a similar pattern observed at point sources of organic enrichment in shallow water sediments (Pearson & Rosenberg 1978). Sulfophilic species (e.g. *Idas washingtonia*) were additionally abundant from 4.5 to 6.8 y, which indicates the temporal overlap between the EO and the SU stages (Smith & Baco

2003, Treude et al. 2009). The long temporal development of the EO stage within the sediments ( $> 4.5$  yrs) could be a function of the large size of the whale carcass, and suggests that sediment communities may experience a longer enrichment stage than typically observed on whale-bones [about 2 y; (Smith & Baco 2003)]. At the whale epifauna, the EO stage ends with a rapid removal of whale tissue attached to bones, which becomes unavailable to large scavengers as they fall over surrounding sediments. The SCr whale-fall had a prolonged duration of the EO stage than other carcasses located from depths of 200 to 2800 m (Smith & Baco 2003, Goffredi et al. 2004, Smith 2006, Fujiwara et al. 2007). The difference on successional dynamics are driven by factors influencing the whale-fall degradation including carcass size, distinct colonizing species (e.g. scavenger communities), and depth (Smith & Baco 2003). Although each whale-fall is apparently colonized by several distinct species (Fujiwara et al. 2007), the macrofaunal succession is driven by facilitation models and organic availability, which maintains the ecological function within each stage of succession (Smith & Baco 2003).

Background species were rarely seen nearby the whale-fall, possibly avoiding extreme sedimentary reducing conditions and enhanced predation (Smith et al. 1998). The recolonization of background species (e.g. *Cossura rostrata*) to sediments at 1 m and 0.5 m after 6.8 y, suggests that the succession was leading towards background conditions as observed at other deep-sea whale carcass in the North Pacific (Smith et al. 1998). The dominant species colonizing sediments near the whale-fall were never sampled in background areas. High densities of dorvilleid polychaetes, cumaceans and ampharetids lowered species diversity near the SCr whale-fall at 4.5 y, similar to observed at hydrothermal vents and cold seep sediments (Grassle et al. 1985, Levin 2005,

Levin et al. 2009). This suggests that the flux of reduced chemicals at the whale-fall at 4.5 y had a similar effect on the species structure as in other reducing habitats. Besides continuous sulfide availability at 5.8 and 6.8 y (Treude et al. 2009), the macrofaunal community was more diverse than background sediments, which was influenced by increased richness of organic enrichment species (i.e. dorvilleids and ampharetids). At least 14 dorvilleid species colonized whale-fall sediments (i.e. 0 – 0.5 m) between 4.5 and 6.8 y, rivaling or even exceeding the high species richness for this group at cold seeps (Levin et al. 2000, Levin et al. 2003). This supports the hypothesis for resource partitioning among dorvilleids within sulfide rich habitats (Levin et al. 2003) and suggests that whale-falls provide optimal evolutionary microhabitats for this group. This indicates that the sediment macrofauna can host an unexpected diverse community if compared to background assemblages, as observed for the whale-fall epifauna (Baco-Taylor & Smith 2003).

Whale falls exhibit higher macrofaunal diversity than soft sediment habitats at hydrothermal vents and cold seeps. A comparison of active and inactive hydrothermal vent sediments in the Pacific revealed low rarefaction diversity levels at all sites, with microbial mat sediments sustaining the highest diversity (Levin et al. 2009). Microbial mat habitats at cold seep sites typically exhibit higher diversity than vent sediments (Sahling et al. 2002, Levin et al. 2003)], and can exhibit similar diversity levels than the SCr whale-fall [(ES(100)=16 – 17 at 0 m]. However, mat habitats at seep and vent sediments typically have lower diversity if compared to background areas, whereas the SCr whale-fall sustained higher species diversity than ambient sediments after 5.8 y. Diversity levels at whale fall sediments are also significantly higher than sediments

around wood and kelp-falls at similar ocean depths. The higher diversity at later stages of succession is explained by the colonization of sulfophilic and heterotrophic species, many of those supported by chemosynthetic organic carbon and exhibiting a wide range of feeding modes.

The broad range of carbon sources and trophic levels found in the SCr macrofaunal community supports the existence of complex food webs in the sediments (Smith & Baco 2003). Chemosynthetic-symbiont species such as the mytilid *Idas washigtonia* were abundant in sediments from 4.5 to 6.8 y, but did not reach the high densities observed over whale bones (Baco-Taylor & Smith 2003, Smith & Baco 2003). Several trophic levels derived their nutrition direct or indirectly from sulfur-oxidizing bacterial mats, evidencing that whale-falls provide abundant sources of chemosynthetic carbon like cold seeps and hot vents (Levin & Michener 2002, Levin et al. 2009, Thurber et al. 2009). The broad isotopic range in multiple congeneric dorvilleid species supports the existence of sedimentary microhabitats with abundant sulfur-bacterial carbon. Californian seeps may also offer broad evolutionary habitats for dorvilleids based on wide ranges in carbon isotopic signatures and coexistence of several congeneric species feeding on bacteria (Levin & Michener 2002, Levin et al. 2003). The strong reliance on chemosynthetic carbon sources at the SCr whale-fall between 4.5 and 6.8 y is in sharp contrast to the simple sediment food webs encountered at younger whale skeletons (< 1.5 y) at the Californian slope (Baco & Smith in prep). At large whale carcasses, the fauna shifts from an early dependence on whale organics (i.e. bone lipids and soft tissue) towards a late reliance on chemosynthesis (Smith & Baco 2003). The reliance on

bacterial carbon between 4.5 and 6.8 y for most of the sediment macrofauna supports that during the SU stage the food web is similar to the whale epifauna.

At higher taxonomic levels (i.e. family or genus), the macrofauna from the SCr whale fall exhibits stronger commonalities to other organic islands, seeps and vents. Dorvilleids and ampharetids polychaetes are common at a few Pacific hydrothermal sediments and at many cold seep sites in the Californian and Alaskan margins (Levin 2005, Levin et al. 2009). At the species level, the SCr whale-fall exhibited limited species overlap with other chemosynthetic habitats in the deep Californian slope, confirming our second hypothesis. Levels of species overlap were higher between whale- and wood-falls, possibly due to closer proximity between the two (~ 100 m) and to the longer organic enrichment effects created by wood parcels if compared to kelps. Wood and kelp overlaps with the SCr whale fall were mainly represented by cumaceans (*Cumella* and *Cumacea* sp.K), and the dorvilleids *Ophryotrocha* sp.A and *Parougia* sp.A. The dorvilleid *Ophryotrocha platikephale* and the polynoid *Bathykurila guaymasensis* occur at whale-falls and vents, but the latter represent potentially two genetically distinct species (Glover et al. 2005a). The dorvilleid genus *Parougia*, *Ophryotrocha* and *Schistomaringos* common to whale, seeps and vents, are mostly represented by heterotrophic dorvilleid polychaetes, but these include a broad range of morphologically distinct species and supports the limited species overlap. The overlaps between whales, seeps and vents are typically species that are attracted to concentrations of free sulfides and that exploit sulfur-based microbial chemoautotrophic production. These conditions are typically encountered at microbial mat habitats and supports that the flux of sulfides may be qualitatively similar between whale-falls and seeps or vents (Treude et al. 2009).

The overlap of chemosynthetic-symbiont species was limited to *Idas washingtonia*, which is part of a mytilid group abundant at both seeps, vents and wood-falls (Samadi et al. 2007, Lorion et al. 2009). Other unidentified vesicomys bivalves colonized whale-fall sediments, but the macrofauna had a limited number of chemoautotrophic species if compared to the epifaunal communities (Smith & Baco 2003). These could be a function of limited sampling on the whale-fall macrofauna, due to the unfavorable and patchy biogeochemical gradients within the sediments and even intense predation limiting the development of adult symbiont-containing species.

The SCr whale-fall sediment macrofauna appeared to host a few endemic species that attained high abundances but were never sampled at background sediments or any other organic islands sunk nearby. At least three unidentified ampharetids, and two dorvilleid species (*Ophryotrocha* sp.E and *Exallopus* sp.A) were extremely abundant nearby the whale carcass and may represent specialists. Dorvilleids of the genus *Ophryotrocha* are commonly associated with organically enriched or polluted sediments and exhibit surprisingly high species richness (Dahlgren et al. 2001, Wiklund et al. 2009a). It is clear that further taxonomic and phylogenetic efforts on the whale-fall dorvilleids [ $> 40$  spp., (Smith & Baco 2003)], will increase the number of whale-fall endemics. Where sufficient data is available, several whale-fall specialists have been described, with at least 20 species being exclusively found on whale skeletons and many other new species await identification (Baco & Smith 2003, Smith & Baco 2003, Dahlgren et al. 2004, Glover et al. 2005b, Pleijel et al. 2008). The number of whale fall endemics inhabiting sediments will certainly grow with the use of molecular tools to differentiate cryptic species associated with whales (Vrijenhoek 2009, Wiklund et al.

2009b). Whale carcasses are one of the least studied chemosynthetic habitats in the deep-sea (Smith & Baco 2003), and the sediment macrofauna has been vastly understudied in worldwide efforts to document whale-fall diversity.

The long persistence of whale falls over the deep-sea (> 20 – 30 yrs) allied to the complex suite of niches over whale bones and in sediments, create oasis of opportunity for species able to colonize it and disperse (Smith et al. 1989, Distel et al. 2000, Baco & Smith 2003, Schuller et al. 2004). Whale carcasses are undoubtedly among the most species rich hard-substrata habitat in the deep-sea (Baco & Smith 2003), and this study evidenced that the sediment macrofauna is more diverse than other similar reducing habitats and background sediments. Whale-falls are colonized by a broad range of abundant sulfophilic and organic respondent species that are not seen over vast areas of deep-sea sediments. As observed at seep and vent habitats, many species colonizing modern whale falls have irradiated from other reducing habitats (e.g. seeps, vents, wood-falls, fish farms), supporting the stepping stone theory (Smith et al. 1989, Baco-Taylor et al. 1999, Distel et al. 2000, Van Dover et al. 2002, Lorion et al. 2009, Wiklund et al. 2009b). The fact that the whale-fall macrofauna sustain higher diversity levels than similar sulfide rich communities in the deep-sea, suggests that carcasses concentrate a vast and unknown number of species that exploit reducing habitats in the deep-sea.

The evolutionary story of whale-falls dates back at least 11-30 Myr, and together with seeps and wood-falls provided habitats for a broad range of taxonomic and functional groups in deep-sea (Baco et al. 1999, Kiel & Goedert 2006, Smith 2006). Several species only found at whale carcasses are directly dependent on bones [e.g. *Osedax* worms, (Rouse et al. 2004)], and many others that attain extremely high densities

at carcasses are also likely specialists on this habitat (Smith & Baco 2003, Smith 2006). This suggests that during the evolutionary lifetime, whale-falls harbored a variety of taxa and trophic types that may be adapted to the resources provided by its habitats (Smith 2006). Consequently, as whale-fall communities harbor one of the most species rich deep-sea reducing habitats, whale exploitation by humans may pose great impact on global deep-sea diversity (Smith 2006).

### **3.5. Conclusions**

In contrast to the sediment macrofauna studied around other deep-sea whale-skeletons, the SCr macrofauna appeared to host a remarkable species diversity composed of many rare species not seen on background assemblages. The whale-fall supported large opportunist and chemoautotrophic communities for over 6.8 y, with only modest organic enrichment but with marked chemical impacts on the surrounding sediments. After 4.5 y, the SCr whale-fall macrofauna appeared to match the transition between the organic-enrichment and the sulfophilic stages described by Smith and Baco (2003), possibly indicating a longer successional periods at the sediments. The whale-fall macrofauna had high numbers of species not present in background sediments, and significantly increase local species diversity for at least 7 yrs. The sediments around the SCr whale-fall exhibited higher diversity levels than other similar habitats at cold seeps and hydrothermal vents, with low level of species overlap, confirming that whale-falls are unique oasis of deep-sea biodiversity across wide ocean basis.

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Appendix 3A. List of sediment samples collected from 2002, 2004 and 2005 at the Santa Cruz whale carcass.

<b>Whale-fall samples</b>				
<i>Year sampled</i>	<i>Parcel Type</i>	<i>Tiburón Dive no.</i>	<i>Approx. age at the time of sampling</i>	<i>Distances sampled from parcel (m)</i>
2002	Whale	490, 491	4.5 years	0, 1, 3, 9 and Bkgd
2004	Whale	653, 654, 655	5.8 years	0, 0.5, 1, 3, 9 and Bkgd
2005	Whale	822, 823, 824	6.8 years	0, 0.5, 1, 3 and Bkgd
<b>Background samples</b>				
<i>Year sampled</i>		<i>ROV Dive</i>		<i>N cores/Distance sampled</i>
2002		490, 502, 505		9 / 100m
2004		655		3 / 20m
2005		822, 823, 824		5 / 9m

## Appendix 3 B. List of macrofaunal species collected in this study.

	WF 4.5 YEARS									
	0 m		1 m		3 m		9 m		SCrB	
	Average (ISE)	Rel %								
<b>Ampharetidae</b>										
Ampharetidae indet.	2.3 (0.8)	3.5%	3.4 (0.8)	20.5%	0.4 (0.1)	16.7%	0.6 (0.2)	10.0%	0.4 (0.2)	7.2
CRS Ampharetid sp. 1	0.3 (0.1)	0.5%								
<i>Sosanopsis</i> sp. A	0.3 (0.1)	0.5%								
CRS Ampharetid sp. 6	0.7 (0.3)	1.0%	0.2 (0.1)	1.2%						
<i>Samytha cf. californiensis</i>	1 (0.2)	1.5%								
<i>Paralysippe</i> sp.			0.2 (0.1)	1.2%						
CRS Ampharetid sp. 12	5.2 (1.0)	7.8%	0.2 (0.1)	1.2%						
CRS Ampharetid sp. 14	9.7 (1.1)	14.6%								
CRS Ampharetid sp. 16									0.2 (0.1)	3.6
<i>Mugga</i> sp. A									0.1 (0.1)	2.4
<b>Cirratulidae</b>										
Cirratulidae indet.			0.6 (0.2)	3.6%	0.4 (0.2)	16.7%	1.2 (0.2)	20.0%	0.4 (0.1)	8.4
<i>Chaetozone</i> sp. A	0.2 (0.1)	0.3%								
<i>Monticellina</i> sp. A					0.4 (0.1)	16.7%	0.6 (0.2)	10.0%	0.6 (0.2)	13.3
<i>Monticellina</i> sp. B									0.3 (0.2)	6.0
<i>Tharyx</i> sp. A			1.0 (0.2)	6.0%	0.4 (0.1)	16.7%	1.0 (0.3)	16.7%	0.3 (0.1)	6.0
<i>Chaetozone</i> sp. C			0.2 (0.1)	1.2%	0.3 (0.1)	8.3%				
<i>Chaetozone</i> sp. D					0.2 (0.1)	8.3%	0.2 (0.1)	3.3%	0.7 (0.2)	14.5
<i>Chaetozone</i> sp. H	0.2 (0.1)	0.3%								
<b>Cossuridae</b>										
Cossuridae indet.			0.2 (0.1)	1.2%					0.2 (0.1)	3.6
<i>Cossura rostrata</i>							0.4 (0.1)	6.7%	0.8 (0.3)	16.9
<b>Dorvilleidae</b>										
Dorvilleidae indet.	0.7 (0.2)	1.0%								
<i>Parougia</i> sp. A			0.8 (0.2)	4.8%			0.2 (0.1)	3.3%		
<i>Ophryotrocha</i> sp. A	26.2 (3.7)	39.4%								
<i>Ophryotrocha</i> sp. B	0.3 (0.1)	0.5%								
<i>Ophryotrocha</i> sp. G	0.2 (0.1)	0.3%								
<i>Ophryotrocha</i> sp. H	0.5 (0.2)	0.8%								
CRS Dorvilleid sp. 16	0.5 (0.1)	0.8%								
CRS Dorvilleid sp. 17	0.2 (0.1)	0.3%								
<i>Ophryotrocha</i> sp. K	0.7 (0.3)	1.0%								
<i>Schistomeringos longicornis</i>			0.4 (0.2)	2.4%						
<i>Ophryotrocha platycephale</i>	4.8 (0.8)	7.3%								
<b>Paraonidae</b>										
<i>Aricidea cf. lopezi</i>							0.2 (0.1)	3.3%	0.1 (0.1)	1.2
<b>Phyllodocidae</b>										
<i>Sige cf. brunea</i>					0.2 (0.1)	8.3%				
<b>Polynoidae</b>										
<i>Bathyrkula guaymasensis</i>	0.5 (0.1)	0.8%								
<i>Harmothoe fragilis</i>									0.1 (0.1)	1.2
<b>Spionidae</b>										
<i>Prionospio</i> sp. B	0.2 (0.1)	0.3%								
<i>Spiophanes cf. berkeleyorum</i>									0.1 (0.1)	1.2
<b>Syllidae</b>										
<i>Eurysyllis</i> sp. A	0.2 (0.1)	0.3%								
<b>Amphipoda</b>										
Amphipoda indet.			0.2 (0.1)	1.2%					0.1 (0.1)	1.2
Amphipoda sp. E									0.1 (0.1)	1.2
Phoxocephalidae sp. A							0.4 (0.1)	6.7%	0.1 (0.1)	2.4
Phoxocephalidae sp. B									0.1 (0.1)	1.2
<i>Monoculodes</i> sp. 1							0.2 (0.1)	3.3%		
<b>Cumacea</b>										
Cumacea indet.									0.1 (0.1)	1.2
<i>Cumella</i> sp. A	3 (0.8)	4.5%								
Cumacea sp. K	0.7 (0.1)	1.0%	8.8 (2.1)	53.0%						
<b>Isopoda</b>										
Asellote Isopod sp. G							0.2 (0.1)	3.3%		
<i>Ilyarchna profunda</i>	0.5 (0.1)	0.8%								
<b>Tanaid</b>										
<i>Carpoapseudes</i> sp.							0.2 (0.1)	3.3%	0.2 (0.1)	3.6
<b>Bivalve</b>										
Bivalve indet.							0.2 (0.1)	3.3%	0.2 (0.1)	2.4
Nuculidae sp. Q	1.0 (0.4)	1.5%								
<i>Adontorhina lynnae</i>			0.2 (0.1)	1.2%						
<i>Idas washingtonia</i>	1.0 (0.4)	1.5%			0.2 (0.1)	8.3%				
Thyasiridae sp. 1	0.2 (0.1)	0.3%					0.4 (0.1)	6.7%		
<b>Gastropod</b>										
<i>Hyalogyrina</i> n. sp.	4.7 (0.8)	7.0%	0.2 (0.1)	1.2%						
<b>Ophiuroidea</b>									0.1 (0.1)	1.2
<b>Anthozoa</b>	0.7 (0.2)	1.0%								
<b>Total polychaeta</b>	54.7 (5.3)	82.4%	7.2 (0.8)	43.4%	2.2 (0.5)	91.7%	4.4 (0.6)	73.3%	4.2 (0.2)	85.5
<b>Total other macrofauna</b>	11.7 (1.3)	17.6%	9.4 (2.3)	56.6%	0.2 (0.1)	8.3%	1.6 (0.3)	26.7%	0.6 (0.1)	14.5

	WF 5.8 YEARS									
	0 m		0.5 m		1 m		3 m		9 m	
	Average (ISE)	Rel %								
<b>Ampharetidae</b>										
Ampharetidae indet.	1.2 (0.3)	1.8%	4.3 (2)	6.1%	1.0 (0.3)	12.2%	2.4 (1)	27.9%		
CRS Ampharetid sp. 1	0.4 (0.1)	0.6%	1.0 (0.6)	1.4%						
CRS Ampharetid sp. 5			0.7 (0.4)	0.9%						
CRS Ampharetid sp. 6			1.3 (0.8)	1.9%						
<i>Samytha cf. californiensis</i>	0.2 (0.1)	0.3%	1.7 (1)	2.4%						
<i>Echysippe trilobata</i>					0.4 (0.1)	4.9%				
CRS Ampharetid sp. 12	2.6 (0.8)	3.8%	5.7 (3.3)	8.0%						
CRS Ampharetid sp. 14	2.8 (0.8)	4.1%	7.7 (4.4)	10.8%						
CRS Ampharetid sp. 16	0.2 (0.1)	0.3%	0.3 (0.2)	0.5%	0.2 (0.1)	2.4%	0.2 (0.1)	2.3%		
<i>Mugga</i> sp. A					0.4 (0.2)	4.9%	0.2 (0.1)	2.3%		
<b>Chaetopteridae</b>										
<i>Phyllochaetopterus limicolus</i>									0.3 (0.1)	4.5%
<b>Cirratulidae</b>										
Cirratulidae indet.						24.4%	1.0 (0.3)	11.6%	1.5 (0.5)	27.3%
<i>Chaetozone</i> sp. A										
<i>Chaetozone</i> sp. B	1.0 (0.3)	1.5%	4.0 (2.3)	5.7%						
<i>Monticellina</i> sp. A					0.2 (0.1)	2.4%	0.8 (0.2)	9.3%	1.0 (0.3)	18.2%
CRS Cirratulid sp. 4			0.3 (0.2)	0.5%	0.8 (0.2)	9.8%	0.2 (0.1)	2.3%		
<i>Chaetozone cf. commonalis</i>					0.8 (0.3)	9.8%	0.6 (0.3)	7.0%	0.5 (0.1)	9.1%
<b>Cossuridae</b>										
Cossuridae indet.	0.2 (0.1)	0.3%								
<i>Cossura rostrata</i>							0.2 (0.1)	2.3%	1.3 (0.4)	22.7%
<b>Dorvilleidae</b>										
Dorvilleidae indet.	0.4 (0.2)	0.6%								
<i>Parungia</i> sp. A	1.2 (0.3)	1.8%	3.0 (1.5)	4.2%	0.4 (0.1)	4.9%				
<i>Ophryotrocha</i> sp. A	15.4 (3.8)	22.6%	16.3 (7.2)	23.1%	0.8 (0.1)	9.8%	0.6 (0.2)	7.0%		
<i>Ophryotrocha</i> sp. B	1.4 (0.6)	2.1%	0.3 (0.2)	0.5%			0.2 (0.1)	2.3%		
<i>Ophryotrocha</i> sp. G	7.4 (3.1)	10.9%	1.3 (0.5)	1.9%						
<i>Ophryotrocha</i> sp. H	0.8 (0.4)	1.2%								
CRS Dorv. 16			0.3 (0.2)	0.5%						
<i>Ophryotrocha notialis</i>					0.2 (0.1)	2.4%				
<i>Schistomeringos longicornis</i>	4.6 (2.1)	6.8%	2.7 (1.5)	3.8%						
<i>Parophryotrocha</i> sp. A		0.3%	0.3 (0.2)	0.5%						
<i>Ophryotrocha platycephale</i>			2.3 (1.1)	3.3%						
<i>Exallopus</i> sp. A	0.2 (0.1)	0.3%								
<b>Hesionidae</b>										
<i>Gyptis</i> sp. A	0.2 (0.1)	0.3%								
Hesionid sp. C			0.3 (0.2)	0.5%						
<b>Nereidae</b>										
<i>Neanthes cf. caudata</i>	0.2 (0.1)	0.3%								
<b>Paraonidae</b>										
Paraonidae indet.							0.2 (0.1)	2.3%		
<i>Aricidea cf. lopezi</i>					0.2 (0.1)	2.4%			0.3 (0.1)	4.5%
<b>Phyllodocidae</b>										
<i>Sige cf. brunea</i>	0.2 (0.1)	0.3%	0.3 (0.2)	0.5%						
Phyllodocidae sp. 4	0.2 (0.1)	0.3%								
<b>Polynoidae</b>										
Polynoidae indet.	0.2 (0.1)	0.3%								
<i>Lepidontopodium cf. riftense</i>	0.2 (0.1)	0.3%								
<i>Bathyrurila guaymasensis</i>	1.4 (0.4)	2.1%	0.3 (0.2)	0.5%						
<b>Sphaerodoridae</b>										
CRS Sphaerodorid sp. 4	0.6 (0.3)	0.9%								
<b>Spionidae</b>										
<i>Prionospio</i> sp.							0.2 (0.1)	2.3%		
<b>Amphipoda</b>										
Amphipod sp. E			0.3 (0.2)	0.5%						
Lysianassid sp. E							0.2 (0.1)	2.3%		
<b>Cumacea</b>										
Cumacea indet.			0.3 (0.2)	0.5%						
<i>Cumella</i> sp. A	4.0 (1.1)	5.9%	5.3 (2.8)	7.5%			0.2 (0.1)	2.3%		
Cumacea sp. K (Leucon)	14.2 (3.8)	20.9%	5.0 (2.9)	7.1%			0.2 (0.1)	2.3%		
<b>Isopoda</b>										
Isopoda indet.							0.2 (0.1)	2.3%		
<i>Ilyarchna profunda</i>	1.2 (0.5)	1.8%	0.3 (0.2)	0.5%						
<b>Tanaidae</b>										
<i>Carpoapseudes</i> sp.					0.2 (0.1)	2.4%	0.4 (0.1)	4.7%		
<b>Bivalve</b>										
Bivalve indet.	0.2 (0.1)	0.3%					0.2 (0.1)	2.3%		
Bivalve juvenile indet.	1.0 (0.4)	1.5%					0.4 (0.1)	4.7%		
Thyasiridae sp. 1									0.5 (0.1)	9.1%
Thyasiridae juv. Indet.									0.3 (0.1)	4.5%
Vesicomyd spp.	0.4 (0.2)	0.6%	4.0 (1.7)	5.7%	0.6 (0.3)	7.3%				
<b>Gastropod</b>										
<i>Hyalogyrina</i> n. sp.	0.4 (0.2)	0.6%	0.7 (0.4)	0.9%						
<b>Anthozoa</b>										
	0.2 (0.1)	0.3%								
Total polychaeta	46.4 (10.2)	68.2%	54.7 (24.6)	77.4%	7.4 (0.5)	90.2%	6.8 (1.1)	79.1%	4.8 (0.9)	86.4%
Total Other macrof	21.6 (3.9)	31.8%	16 (5.2)	22.6%	0.8 (0.3)	9.8%	1.8 (0.4)	20.9%	0.8 (0.2)	13.6%

	WF 6.8 YEARS							
	0 m		0.5 m		1 m		3 m	
	Average (1SE)	Rel %						
<b>Ampharetidae</b>								
Ampharetid - indet.	6.8 (2.3)	7.6%	2.4 (0.1)	5.1%	3.6 (0.4)	25.7%	2.0 (0.4)	27.8%
CRS Ampharetid sp. 1	0.6 (0.1)	0.7%	0.8 (0.4)	1.7%				
<i>Sosanopsis</i> sp. A	1.2 (0.4)	1.3%						
CRS Ampharetid sp. 6	0.2 (0.1)	0.2%					0.2 (0.1)	2.8%
<i>Samytha</i> cf. <i>californiensis</i>		1.1%	1.6 (0.3)	3.4%	0.2 (0.1)	1.4%		
CRS Ampharetid sp. 11	0.2 (0.1)	0.2%						
CRS Ampharetid sp. 12	7.6 (1)	8.5%	3.4 (1.3)	7.3%				
CRS Ampharetid sp. 14	8.4 (2.2)	9.4%	1.6 (0.7)	3.4%				
CRS Ampharetid sp. 16			0.2 (0.1)	0.4%	0.2 (0.1)	1.4%	0.6 (0.2)	8.3%
<i>Mugga</i> sp. A							0.4 (0.2)	5.6%
<b>Chaetopteridae</b>								
<i>Phyllochaetopterus limicolus</i>							0.4 (0.2)	5.6%
<b>Cirratulidae</b>								
Cirratulidae indet.	0.6 (0.2)	0.7%	0.4 (0.1)	0.9%	0.2 (0.1)	1.4%	0.4 (0.1)	5.6%
<i>Chaetozone</i> sp. A	3.4 (1.5)	3.8%						
<i>Monticellina</i> sp. A			0.4 (0.2)	0.9%	1.0 (0.2)	7.1%	1.0 (0.3)	13.9%
<i>Tharyx</i> sp. A					0.4 (0.1)	2.9%	0.2 (0.1)	2.8%
<i>Chaetozone</i> sp. C								
<i>Chaetozone</i> cf. <i>commonalis</i>			1.4 (0.3)	3.0%	1.8 (0.2)	12.9%	0.6 (0.2)	8.3%
<b>Cossuridae</b>								
Cossuridae indet.					0.2 (0.1)	1.4%		
<i>Cossura rostrata</i>			0.8 (0.1)	1.7%	1.6 (0.5)	11.4%	0.2 (0.1)	2.8%
<b>Dorvilleidae</b>								
Dorvilleidae indet.	0.8 (0.2)	0.9%	1.8 (0.5)	3.8%	0.4 (0.2)	2.9%		
<i>Parougia</i> sp. A	6.4 (1.9)	7.1%	1.6 (0.7)	3.4%			0.4 (0.2)	5.6%
<i>Ophryotrocha</i> sp. A	10.2 (1.3)	11.4%	2.2 (0.7)	4.7%	0.2 (0.1)	1.4%		
<i>Ophryotrocha</i> sp. B	0.8 (0.2)	0.9%	0.2 (0.1)	0.4%				
<i>Ophryotrocha</i> sp. E	7.6 (3.4)	8.5%	9.4 (4.2)	20.1%				
<i>Ophryotrocha</i> sp. H	4.0 (1.7)	4.5%						
<i>Schistomeringos longicornis</i>	4.0 (1.4)	4.5%	1.8 (0.8)	3.8%				
<i>Ophryotrocha platycephale</i>			1.0 (0.5)	2.1%				
<i>Exallopus</i> sp. A	2.8 (1.1)	3.1%	4.0 (1.8)	8.5%				
<b>Glyceridae</b>								
<i>Glycera</i> cf. <i>branchiopoda</i>	0.2 (0.1)	0.2%						
<b>Hesionidae</b>								
<i>Gyptis</i> sp. A	0.2 (0.1)	0.2%						
<b>Nereidae</b>								
Nereidae indet.	0.2 (0.1)	0.2%						
<i>Neanthes</i> sp. A	0.2 (0.1)	0.2%						
<b>Paraonidae</b>								
Paraonidae indet.					0.2 (0.1)	1.4%		
<i>Aricidea</i> cf. <i>rubra</i>			0.2 (0.1)	0.4%				
<b>Phyllococidae</b>								
<i>Sige</i> cf. <i>brunea</i>					0.2 (0.1)	1.4%		
<b>Polynoidae</b>								
<i>Bathyrkula guaymasensis</i>	1.2 (0.3)	1.3%						
CRS Polynoid sp. 10			0.2 (0.1)	0.4%				
<b>Spionidae</b>								
<i>Spiophanes</i> cf. <i>berkeleyorum</i>			0.2 (0.1)	0.4%				
<b>Amphipoda</b>								
Amphipod sp. E	0.2 (0.1)	0.2%	0.4 (0.2)	0.9%	0.6 (0.2)	4.3%	0.2 (0.1)	2.8%
Phoxocephalidae sp. A	0.4 (0.1)	0.4%	0.2 (0.1)	0.4%				
Lysianassid sp. E					0.2 (0.1)	1.4%		
<b>Cumacea</b>								
Cumacea indet.					0.2 (0.1)	1.4%		
<i>Cumella</i> sp. A	0.4 (0.1)	0.4%	2.4 (0.8)	5.1%	0.6 (0.2)	4.3%		
Cumacea sp. K	17.4 (3.3)	19.4%	7.2 (1.9)	15.4%	1.2 (0.2)	8.6%		
<b>Isopoda</b>								
<i>Ilyarachna profunda</i>	0.6 (0.1)	0.7%						
<b>Tanaidae</b>								
<i>Carpoapseudes</i> sp.			0.2 (0.1)	0.4%			0.4 (0.2)	5.6%
<b>Bivalve</b>								
Bivalve indet.			0.2 (0.1)	0.4%				
Bivalve juvenile indet.	0.8 (0.4)	0.9%			0.2 (0.1)	1.4%		
Nuculidae sp. Q					0.4 (0.2)	2.9%		
Thyasiridae sp. 1	0.4 (0.1)	0.4%			0.4 (0.1)	2.9%		
Thyasiridae sp. 3	0.2 (0.1)	0.2%						
Vesicomyd spp.	0.6 (0.3)	0.7%	0.6 (0.3)	1.3%				
<b>Ophiuroidea</b>								
							0.2 (0.1)	2.8%
<b>Total polychaeta</b>	68.6 (14.1)	76.6%	35.6 (9.6)	76.1%	10.2 (0.8)	72.9%	6.4 (0.9)	88.9%
<b>Total Other macrof</b>	21.0 (3.2)	23.4%	11.2 (2.1)	23.9%	3.8 (0.2)	27.1%	0.8 (0.3)	11.1%

## Chapter 4. The San Clemente cold seep: macrofaunal structure and species overlap with reducing habitats in the deep NE Pacific

### Abstract

The San Clemente cold seep lies within 100-200 km of other reducing environments in the NE Pacific, offering an interesting opportunity to examine diversity and species overlap among reducing habitats (i.e., whale-falls, kelp, and wood parcels) at similar ocean depths. Using Alvin, we observed clumps of tubeworms *Lamellibrachia barhami* and *Escarpia spicata* distributed as meter-scale patches among vesicomylid clam beds and black sediments. We used core samples and stable isotopic analyses to evaluate infaunal macrobenthic community structure and nutrition sources of dominant organisms at distances of 0, 1, 10 and 100 m from tubeworm thickets. Macro-infaunal abundance was elevated at 0 to 1 meters compared to seep sites 100 m away. Mats of *Siboglinum* sp. were conspicuous in sediments within 1 m of tubeworm thickets ( $2906 \pm 606$  ind  $m^{-2}$  at 0 m). Other polychaete worms dominated macrofaunal abundances at all distances sampled (over 60% of individuals), with tanaids (11%) and isopod crustaceans also being abundant. Species richness and diversity were slightly depressed at seep sites, increasing outwards from worm clumps. The San Clemente seep sediment macrofaunal community contains intermediate levels of species richness and diversity compared to other seep areas. Stable isotope data showed high variability in  $\delta C^{13}$  values for distinct faunal groups (polychaetes and crustaceans), as has been observed at other seeps. Overall, < 20% species overlap was found between the San Clemente seep and wood-, whale- and kelp-fall communities at similar ocean depths within the region.

#### 4.1. Introduction

Cold seeps occur in geologically active and passive continental margins at depths from 15m to over 7000 m, where the seepage of reduced chemical substances fuel chemosynthesis-based benthic communities (Sibuet & Olu 1998, Levin 2005). Submarine fluid venting of methane and other hydrocarbon gasses support high surface and sub-surface concentrations of hydrogen sulfide, which is produced by microbial anaerobic methane oxidation coupled to sulfate reduction (Boetius et al. 2000, Treude et al. 2003). The upward flow of sulfide and/or methane provides energy for chemosynthetically based microorganisms, either free-living or in symbiotic association with metazoans (Barry et al. 1997, Sibuet & Olu 1998, Levin 2005). Most symbiont-bearing invertebrates are megafaunal groups with broad biogeographic distributions and include vesicomysid clams, mytilid and solemyid bivalves, vestimentiferan and pogonophoran polychaetes (Sibuet & Olu 1998, Kojima 2002, Van Dover et al. 2002, Levin 2005). These organisms commonly make up the highest megafaunal biomass at seeps and are fundamentally dependent on the flux of reducing compounds for their nutrition (Barry et al. 1997). Given that sulfide and methane fluxes can be remarkably heterogeneous in space and time, distinct patterns of faunal distribution occur between habitats at each geological feature (Barry et al. 1997, Sahling et al. 2002, Levin et al. 2003, Treude et al. 2003, Van Dover et al. 2003, Levin 2005).

Several investigations have looked and compared infaunal community structure at cold seeps from shelf to abyssal depths (Sibuet & Olu 1998, Levin et al. 2000, Kojima 2002, Sahling et al. 2002, Van Dover et al. 2003, Levin 2005, Levin & Mendoza 2007). These studies revealed high habitat variability on macrofaunal

density, composition and diversity, with strong differences between seep and non-seep sediments at deeper sites (Sahling et al. 2002, Levin et al. 2003). Sediment geochemistry can vary significantly between seep microhabitats, creating very specific conditions for its associated community (Tryon & Brown 2001). For example, sulfide-rich sediments host many chemosynthetic-based and endemic species, such as *Beggiatoa* mats, bivalves, pogonophoran and other polychaetes (Barry et al. 1997, Sibuet & Olu 1998, Sahling et al. 2002, Levin et al. 2003, Thurber et al. 2009). Several heterotrophic opportunist species are also able to tolerate such high sulphidic sediments and exploit rich organic sources in some seep habitats (Sahling et al. 2002, Levin et al. 2003). Consequently, there is a broad spectrum on the utilization of chemosynthetic- or photosynthetic-derived organic carbon in the heterotrophic macrofauna, which is linked to habitat heterogeneity and availability of many organic sources within seeps (Levin & Michener 2002). Such availability is related to bathymetric variations on chemosynthetic versus ambient organic availability (Levin et al. 2000, Levin & Michener 2002).

The San Clemente cold seep was discovered in 1979 and first described as a vent ecosystem (Lonsdale 1979). It has been poorly sampled with only 3 megafaunal organisms formally described: the vestimentiferan *Lamellibrachia barhami*, a general species of vesicomid and a bryozoan (Sibuet & Olu 1998). The macroinfauna from San Clemente has not been studied in detail and its position and depth (1800 m) offer an interesting opportunity to investigate species overlap among other reducing habitats at the Southern Californian slope. The San Clemente seep lies downstream of three implanted whales, kelp and wood-falls at similar depths in the California basin, which could serve as a recruitment source for invertebrates exploiting sulfide-rich conditions (Smith & Baco 2003). Some of the enrichment opportunist and sulfide-

tolerant species are common to reducing deep-sea habitats such as vents, whale- and wood-falls (Levin et al. 2006, Smith et al. in prep). Whale-falls in particular have been proposed as dispersal stepping-stones for organisms (e.g. vesicomysids) thriving at deep reducing habitats, but so far only a limited overlap of species has been demonstrated (Smith et al. 1989, Sibuet & Olu 1998, Tunnicliffe et al. 1998, Baco-Taylor et al. 1999, Smith & Baco 2003). Difficulty in sampling has made quantitative studies of species richness in these communities rare and comparisons of species overlap between these sites at single basins are practically absent.

The primary objective of this study was to examine the structure and composition of the macroinfauna at the San Clement seep and investigate levels of species overlap with a whale-fall and other organic islands situated at about 100 km northwest of the seep site (Fig. 1.1). We also investigated nutrition sources of abundant macroinfauna at seep habitats through stable isotope analysis and compared species diversity at several distances from “seep areas” to ambient communities, and to other comparable seep environments. We ask the following questions: (1) What is the level of macrofaunal species overlap between San Clemente seep and other assemblages from reducing habitats at similar depths? (2) What is the level of macroinfaunal endemism at the San Clemente seep and how species composition change relative to ambient sediments? We hypothesized that the macroinfauna will exhibit low levels of overlap among other organic habitats at the California margin and that species composition at seep sediments will be markedly distinct from ambient assemblages.

#### **4.2. Study site and methods**

The San Clemente seep is located at 1800 m depth on a transform fault off the coast of southern California at 32° 12' N; 117° 44' W (Chapter 1 - Fig. 1.1). Large barium sulfate deposits occur as large blocks on the seafloor (Torres et al. 2002), often anchoring thickets of tubeworms *Lamellibrachia barhami* and *Escarpia spicata* (Fig. 4.1 A-B). Vesicomyid clams (*Calymene pacifica*) are also common megafaunal organisms often seen nearby worm thickets (Fig. 4.1). Worm thickets were often surrounded by clam patches, usually located within darkened sediments with presumably high sulfide levels (Fig. 4.1 C-D). Reported methane analyses at the site indicate very heterogeneous pore-water concentrations within the top 10 cm sediments, which are higher adjacent to tubeworm thickets [0m, 12-46  $\mu\text{l/ml}$ ; (Torres et al. 2000)].

Sediment macrofauna was sampled in October 1999 on board of the *R/V Atlantis* using the manned submersible *Alvin* (Table 4.1). Replicate sediment samples were collected immediately adjacent to tubeworm thickets (0 m), and at 1 m, 10 m and over 100 meters away, which represented background samples (Table 4.1). Corers were sliced at three vertical sections (0-1, 1-5 and 5-10cm) and sediments were immediately preserved in 10% v/v buffered formalin-seawater solution onboard the ship. Once in the laboratory, samples were washed on 300  $\mu\text{m}$  sieves and transferred to 80% ethanol. Macrofauna ( $>300 \mu\text{m}$ ) were sorted using dissecting microscopes and individuals were identified to the lowest taxonomic level possible using the available keys and with help from taxonomists. Reference individuals from this seep were compared directly to the southern California basin whale falls reference collection in order to evaluate the species overlap between macroinfaunal communities. Digital stills and video taken throughout the *Alvin* dives helped to visually distinguish seep habitats (Fig. 4.1).

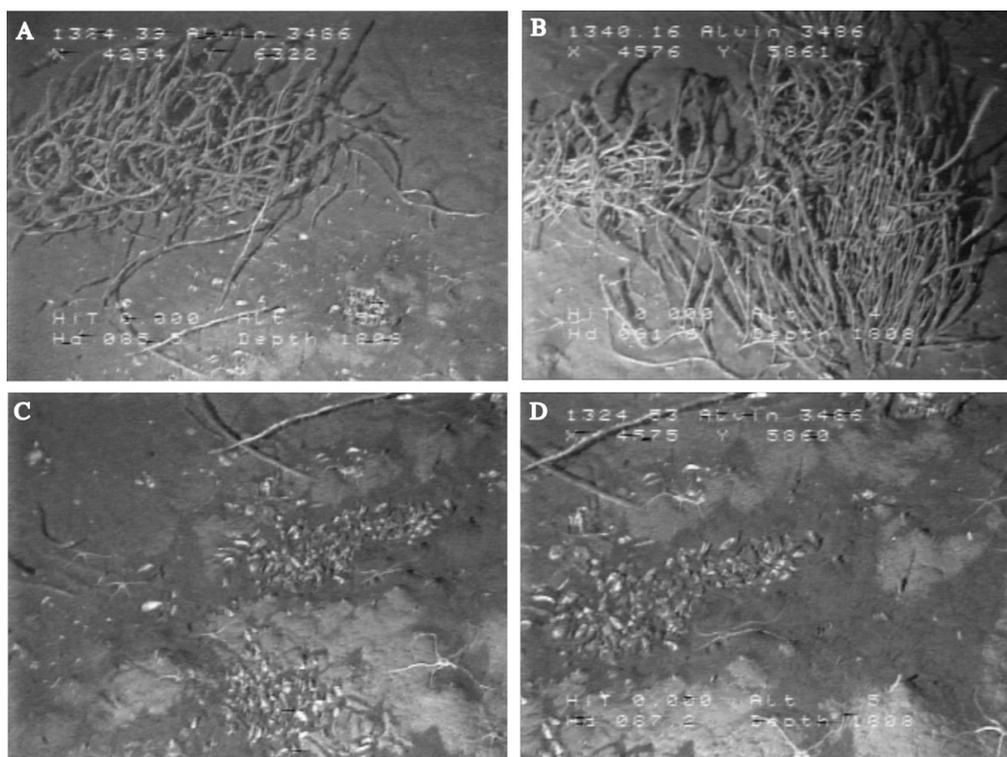


Figure 4.1. Photographs from Alvin showing characteristic megafaunal organisms from the San Clement seep. A and B. Vestimentiferan tubeworm clumps with fragmented clam shells on the sediments nearby; C and D. Clam fields within darkened, presumably reduced sediments. Large ophiuroids are also visible. Image width approx. 75cm.

Table 4.1. Summary of sediment samples collected in this study. TC. Tube core, 7cm Internal diameter; EK. Ekman core, 10cm I.D.

Alvin Dive	Distance	Core	Alvin Dive	Distance	Core
3486		TC 2	3487		TC 11
3486		TC 5	3487		TC 14
3487	0m	TC 10	3487	10m	EK 6 Veg C
3487		TC 20	3487		EK 9 Veg B
3487		EK 11 Veg D	3487		EK 9 Veg D
3486		TC 8	3486		TC 17
3486		TC 9	3486		TC 18
3487		TC 17	3487	200m	EK 10 Veg B
3487	1m	TC 18	3487		EK 10 Veg C
3487		TC 19	3487		EK 10 Veg D
3487		TC 9			
3487		EK 6 Veg B			
3487		EK 6 Veg D			

Sediment sampling consisted of typically 5 to 8 replicate cores per distance sampled. Macrofaunal density was quantified as individuals per square meter (ind. m<sup>-2</sup>)

<sup>2</sup>) due to differences in sampled areas between *Alvin* tube and *Ekman* corers (38.5 and 78.5 cm<sup>2</sup>, respectively). Community composition was assessed by sample independent analyses in order to reduce effects of sampling area on diversity estimates. Hulbert's rarefaction curve [ES(n);(Hulbert 1971)] was used to compare species diversity between treatments. Background replicate cores (n=5) were combined to calculate a composite diversity for the ambient community, and confidence limits were calculated from one-tailed 95% confidence interval [see Chapters 2 and 3; (Smith 1986)]. Pielou's evenness ( $J'$ ) was used to provide information on species structure using the statistical package PRIMER-E © (Clarke & Warwick 2001). Multi-Dimensional Scaling (MDS) was applied to macrofaunal abundance ( $\sqrt{}$  transformed) in order to emphasize similarity in species composition among habitats (Clarke & Warwick 2001). Multivariate analysis of similarities (ANOSIM) helped to differentiate samples and SIMPER analysis identified groups or species responsible for those patterns (Clarke & Warwick 2001). Statistical indexes were calculated with parametric or non-parametric methods whenever applicable, using the statistical software BioEstat©.

Dominant macrofaunal organisms from sediment samples at 0 and 1 m were selected for stable isotope analysis. All selected specimens were thoroughly rinsed in DI water and cleaned of attached debris prior to analysis. Polychaetes were removed from tubes and muscle tissues of bivalves (i.e. avoiding gills) were dissected using methanol-cleaned equipment. Samples were decalcified with phosphoric acid and placed in pre-weighed tin cups for overnight drying (35-40 °C). Multiple individuals of the same species (N= 3 to 13) were combined in one sample to make up 0.5-2 mg of dry weight, necessary for analysis. Whenever possible, replicate measurements of grouped individuals were carried and stable isotope data represent average values

within single species. We recognize the potential for nutritional variability within seep individuals (Thurber et al. 2009), but we believe that the data is still informative of food web patterns at our study site. Unfortunately, we lack isotope data from a broad number of background species and from potential organic sources from San Clemente, and therefore we do not present mixing-model calculations of organic sources for the organisms analyzed. Statistical comparisons of isotope values across species were not possible due to limited number of replicates available, therefore differences are conservatively assumed based on standard error from measurements.

Isotope samples were combusted in a Eurovector (Milan, Italy) elemental analyzer interfaced with an Isoprime isotope ratio mass spectrometer for determination of  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios (reproducibility:  $\pm 0.5\text{‰}$  for  $\delta^{15}\text{N}$  and  $\pm 0.2\text{‰}$  for  $\delta^{13}\text{C}$ ). Macrofaunal C-isotopic ratios were measured against a Pee Dee Belemnite (PDB) standard for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ . Results are expressed in units per mil (‰) as delta notation ( $\delta$ ) representing the relative difference between sample and standard, where  $\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R = ^{15}\text{N}/^{14}\text{N}$  or  $R = ^{13}\text{C}/^{12}\text{C}$ . In this study, we corrected for preservation artifacts by adding 1‰ to  $\delta^{13}\text{C}$  (Fry & Sherr 1989, Edwards et al. 2002, Sarakinos et al. 2002). Trophic changes of +3‰ were added to the range of  $\delta^{15}\text{N}$  values obtained for each organic matter source. Species that exclusively use a particular organic-matter source for nutrition are expected to fall within the appropriate trophic-shift box.

### **4.3. Results**

#### *Image observations*

Video images from *Alvin* dives permitted the identification of distinct macrohabitats, such as tubeworm thickets, vesicomid clam beds, black sediments and non-reduced sediments (Fig. 4.1). From *Alvin* images we could not visually identify the spatial distribution of siboglinid polychaetes, as these were typically small (< 3-5 cm) and not detected by video. Tubeworm thickets (i.e. vestimentiferans) were either anchored on barium sulfate rocks or aggregated within patches of blackened sediments widely distributed over the seep. Clam aggregations were commonly observed within dark sediment patches that were not colonized by tubeworms, and clamshell fragments were often seen around tubeworm patches (Fig. 4.1). Black sediments were heterogeneously distributed around tubeworm thickets, being interrupted by areas of light colored sediments without clams (Fig. 4.1). Sediments coring randomly started immediately under tubeworm thickets towards background sediments 100 meters away.

#### *Macrofauna density and composition*

Total macrofaunal densities were significantly higher at 0 and 1-meter sediments if compared to the background (Kruskal Wallis test,  $H=9.68$ ,  $p=0.02$ ). Average densities at 0 m were over two times higher than background sediments ( $2378 \pm 257$  ind.  $m^{-2}$ ; Fig. 4.2). At 0 and 1m, siboglinid polychaetes were extremely abundant ( $2906 \pm 606$  ind.  $m^{-2}$  and  $1652 \pm 257$  ind.  $m^{-2}$ , respectively), and heterogeneously distributed within patches but their abundance was not different between 0 and 1 meters (ANOVA). Sediment patches colonized by siboglinid worms were present 10 meters away from tubeworm thickets with dense mats in some areas. The vertical macrofaunal distribution within the sediments did not reveal any

significant patterns between distances sampled, suggesting weak gradients of pore-water sulfides in the area (Kruskal Wallis test).

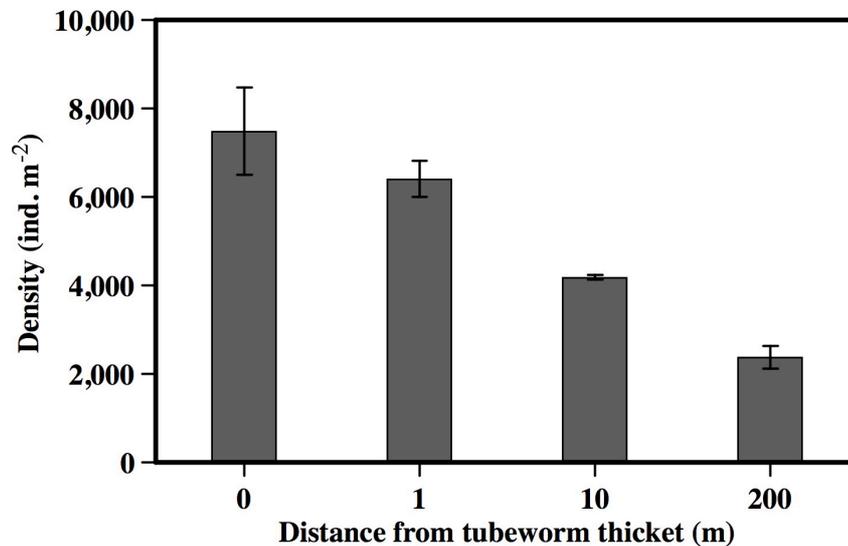


Figure 4.2. Total macrofaunal density at seep and background sites (Average  $\pm$  1 SE).

Polychaete worms typically dominated the macrofauna at all distances sampled with over 65% of all individuals. Polychaete dominance changed markedly across the seep areas sampled. At 0 and 1 m, *Siboglinum* sp. 1 represented over 25% of the fauna (Fig. 4.3), and was among the top-ranked species up to 10 meters (Table 4.2). A tanaid species, one syllid polychaete and one thyasirid bivalve were among the top ranked species at 0 meters, and together with *Siboglinum* sp. 1 comprised over 58% of all individuals. The dorvilleids *Meiodorvillea* and *Pinniphitima* were found at San Clemente seep but never reached high abundances, always comprising 1-5% of the macrofauna from 0 to 10 meters (Appendix 4A). At 10 meters, spionid (15%), cirratulid (12%) and other polychaete worms (25%) were dominant, a pattern similar to background sediments (Fig. 4.3). Tanaids were abundant at 0 to 1m sediments (8-13% of rel. abundance) and thyasirid bivalves were common at all seep and non-seep areas sampled, comprising 4-7% of the infauna at all sites (Fig. 4.3; Appendix 4A).

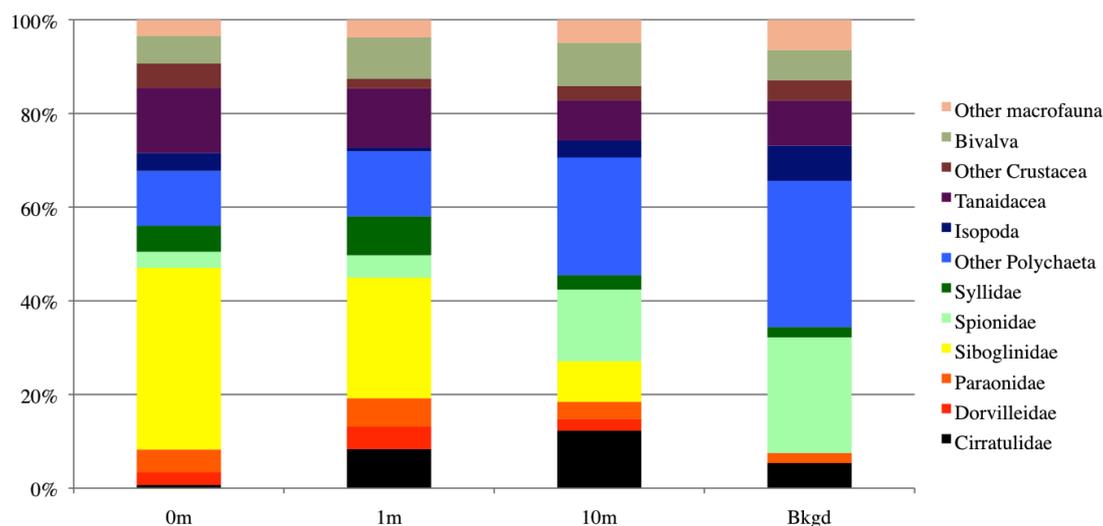


Figure 4.3 Macrofaunal composition at San Clemente cold seep.

Table 4.2. Top ranked species from San Clemente seep macrofauna. Density as mean ind.  $m^{-2}$  ( $\pm 1SE$ ). (P) Polychaete, (Cr) Crustacea, (M) Mollusca.

SAN CLEMENTE SEEP							
		0 m		1 m		10 m	
Rank	Species	Density	Rel %	Density	Rel %	Density	Rel %
1	<i>Siboglinum</i> sp. 1 (P)	2905 (606)	38.8%	2018 (267)	28.1%	364 (163)	8.7%
2	Tanaid sp. 1 (Cr)	988 (442)	13.2%	567 (118)	7.9%	155 (22)	3.7%
3	<i>Sphaerosyllis</i> sp. A (P)	415 (94)	5.5%	321 (51)	4.5%	52 (23)	1.2%
4	Thyasirid sp. 4 (M)	362 (46)	0.7%	144 (39)	0.4%	180 (23)	0.6%
<b>Total percent</b>			<b>58.2%</b>	<b>40.9%</b>	<b>14.2%</b>		
Background (200m)							
Rank	Species	Density		Rel %			
1	<i>Aurospio</i> cf. <i>dibranchiata</i> (P)	229 (46)		9.6%			
2	Tanaid sp. E (Cr)	229 (52)		9.6%			
3	<i>Prionospio</i> sp. C (P)	205 (29)		8.6%			
4	Thyasirid sp. 4 (M)	153 (33)		6.4%			
<b>Total percent</b>			<b>34.2%</b>				

*Macrofaunal diversity, species overlap and multidimensional community analysis*

Macrofaunal diversity increased from 0 m towards background sediments, peaking at 10 meters ( $ES_{50}=24.6$ ). Low diversity levels at 0 m sediments were associated with significant lower community evenness at 0 m (ANOVA,  $F=4.985$ ,  $d.f.=3$ ,  $p=0.011$ ; Table 4.3) if compared to all other distances sampled. At 1 - 10 meters, community evenness exhibited background values, but diversity was higher due to the presence of seep (e.g. *Siboglinum* sp. 1) and ambient species. Rarefaction diversity did not reveal significant differences between seep and non-seep areas, although 0 m curves fell below background (Table 4.3; Fig. 4.4). Expected species diversity suggests that the number of species at 0 meters was still increasing rapidly with the increase in the number of individuals (Fig. 4.4). Although  $E(S_{50})$  was lower at 0 m if compared to 1 m, the former curve is steeper than all other sampled areas and suggests higher species richness at 0 m (Fig. 4.4).

Table 4.3. Species evenness [ $J'$  ( $\pm 1 SE$ )], Shannon-Wiener diversity ( $H'$  base 2 and 10), and rarefaction diversity  $E(S_{50}$  and  $S_{100})$  from the seep macroinfauna at San Clemente.

Distance	$J'$	$H' \log_2$	$H' \log_{10}$	$ES(50)$	$ES(100)$
0m	0.7 (0)	3.4	1.0	17.6	27.8
1m	0.8 (0)	4.1	1.2	20.3	29.2
10m	0.9 (0)	4.7	1.4	24.6	-
Bkgd	0.9 (0)	4.0	1.2	21.1	-

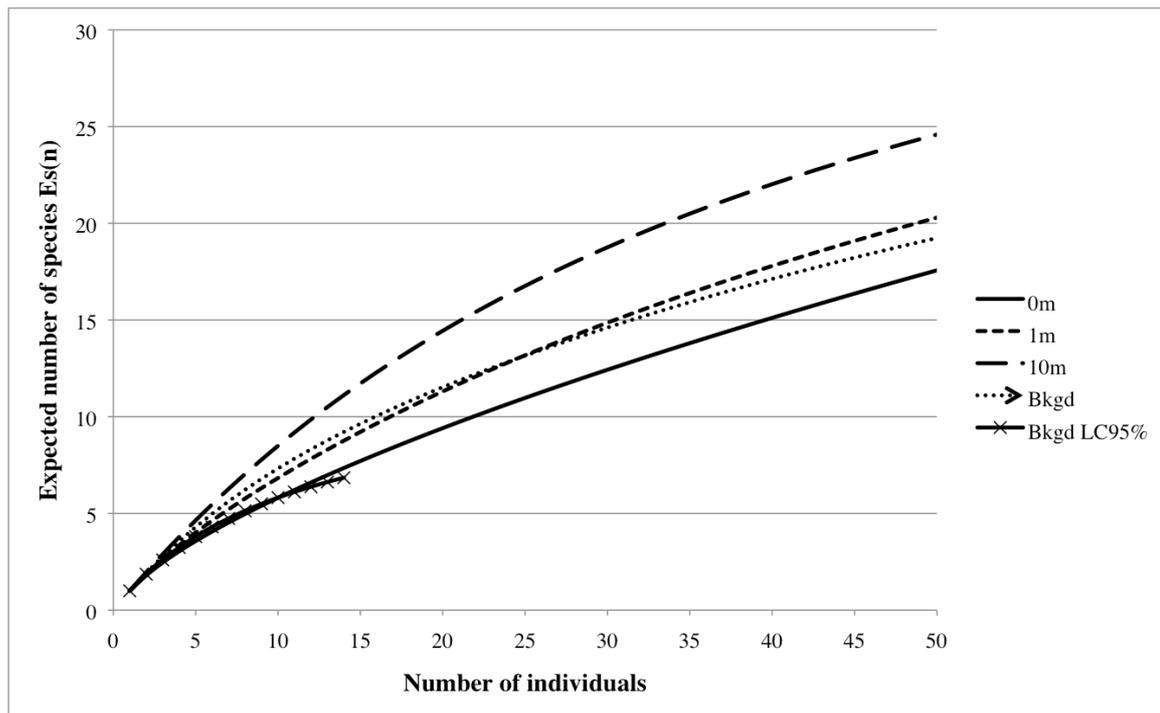


Figure 4.4. Rarefaction species diversity at San Clemente cold seep.

Multidimensional analysis revealed a strong spatial heterogeneity in macrofaunal composition at the San Clemente seep sediments (Fig. 4.5). In general, seep habitats were very heterogeneous with no significant difference in species composition (ANOSIM test,  $r = 0.303$ ), although samples from 0 m and 1 m grouped distinctly from the others (Fig. 4.5). Similarities within seep sites were low (25 - 30%, at 0 m and 1 m), and increased towards background areas (54%, SIMPER). Higher dissimilarity at seep areas closer to tubeworm thickets corroborates the strong spatial sediment heterogeneity observed. The top-ranked species responded for most of the observed dissimilarity. *Siboglinum* sp. 1 was a key species determining high dissimilarities between 0 and 1 m sediments, and spionids, cossurids and tanaidaceans were most significant at 10 m and background sediments (SIMPER).

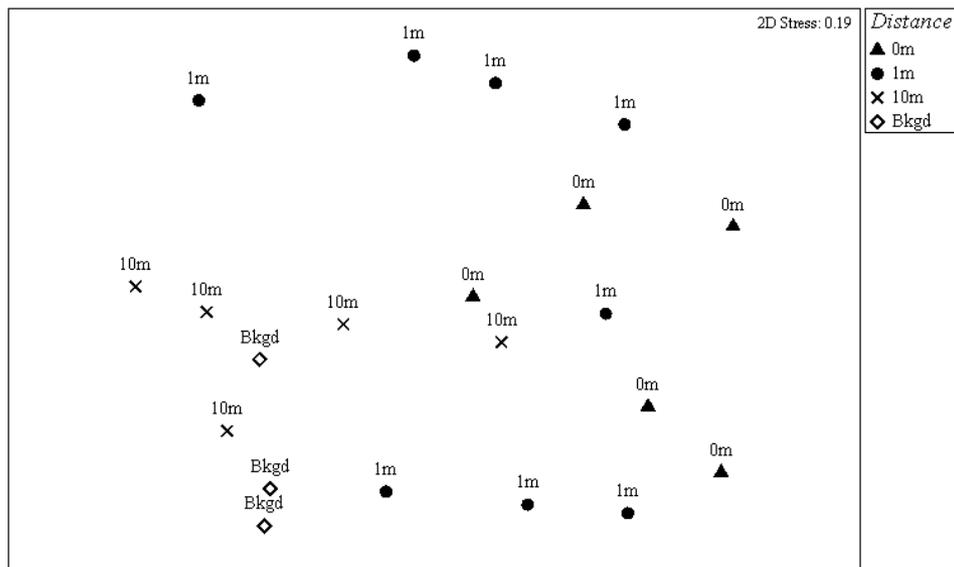


Figure 4.5. Multidimensional scaling plot of San Clemente seep macrofauna (> 300 $\mu$ ).

A total of 74 species were identified at the San Clemente seep macrofauna. Species richness was highly heterogeneous between seep areas, with 34 of 58 species exclusively sampled from 0 to 1 meters. There were a few seep species abundant at 0 and 1m that were not seen or were rare at non-seep areas (i.e. less than 5% relative abundance). These include two top ranked species at seep sites: *Siboglinum* sp. 1, and *Sphaerosyllis* sp. A. At “non-seep” areas, only the top-ranked Tanaid sp. E was abundant at background sediments and not at 0 m sediments.

The number of species overlapping with other reducing habitats at the Californian slope was limited. About 12 % of the macrofaunal species abundant at 0 and 1 m (i.e. “seep” species), were encountered at sediments around wood parcels (Bernardino et al. in prep), and 9% were shared with whale-fall sediments (Smith et al., in prep). The overlapped species are typical heterotrophic organisms (i.e. grazers and predators), with only thyasirid bivalves potentially deriving their nutrition from chemosynthesis (Dando & Spiro 1993). Shared species between seep and wood (n= 4 spp.) included the ampharetid polychaete *Amelina abyssalis*, Thyasirid sp. 2, an

asellote isopod and *Aricidea cf. lopezi*. Overlapped species between whale and seep (n= 3 spp.) included Ampharetid sp. 1 and two paranoid species of the *Aricidea* complex. No species from the seep were observed around kelp parcels, and none of the top-ranked species from San Clemente colonized any organic island.

### *Stable isotope analysis*

Stable isotope analyses were carried on six abundant macrofaunal species from 0 and 1 m (Fig. 4.6). The number of individuals analyzed for each species ranged from N=3 to 13, and carbon and nitrogen isotope values revealed wide nutritional dependence on food sources (Fig. 4.6). Macrofaunal carbon isotope signatures ranged from -57.4‰ to -23‰ and nitrogen values from -7.4‰ to 11.6‰, suggesting both chemosynthetic and heterotrophic nutrition. The average carbon and nitrogen isotope values of the top-ranked species at 0 m and 1 m (-39.7‰ and -0.6‰, respectively), suggests utilization of chemosynthetically fixed organic matter (Fisher 1999, Levin & Michener 2002). Very negative  $\delta^{13}\text{C}$  signatures were evident on two species that host chemosynthetic symbionts. Individuals of *Siboglinum* sp. 1, had the most depleted carbon isotope signature ( $-51.1 \pm 8.3$  ‰), indicating incorporation of methane-derived carbon (Dando et al. 2008). However, the broad carbon and nitrogen isotope range for this species ( $\delta^{13}\text{C} = -44.7$ ‰ to  $-60.4$ ‰ and  $\delta^{15}\text{N} = -6.5$ ‰ to  $-7.4$ ‰) indicates broad individual variability on carbon uptake and suggests either methanotrophic or sulfide-oxidizing symbionts (Fig. 4.6). Specimens of *Thyasirid* sp. 4 had carbon and nitrogen isotope signatures that suggested incorporation of chemosynthetic carbon from sulfide-oxidizing symbionts (Dando & Spiro 1993). Four species exhibited nitrogen isotope values that suggest heterotrophic nutrition (Fig.

4.6). Those were the polychaete *Sphaerosyllis* and the crustacean Tanaid sp. 1, which had carbon isotopic signatures indicative of nutrition on chemosynthetic-derived organic matter. The other two heterotrophs were the paranoid polychaete *Levinsenia oculata* and the dorvilleid *Meiodorvillea*, which likely fed both on photosynthetic organic matter and sulfur bacteria (Fig. 4.6).

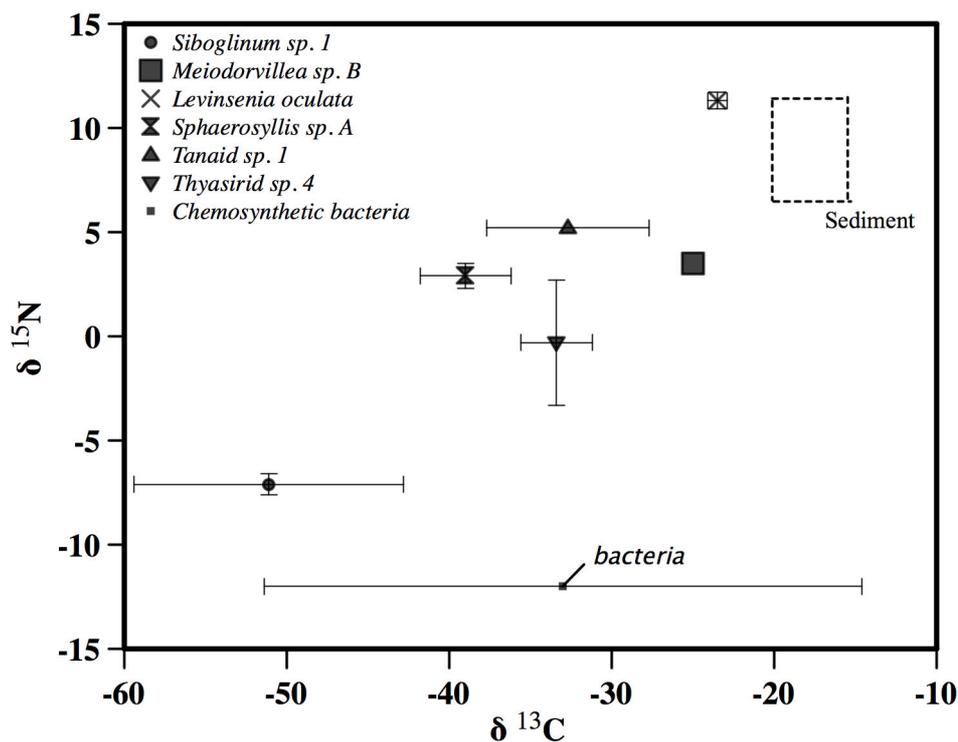


Figure 4.6. Stable isotope values of top-ranked species sampled from 0 to 1 meters at San Clemente cold seep. Data represent average values from individuals ( $N > 1$ ;  $\pm 1$  SD) from single species (see text). Trophic-shift box represents one shift from sediment organic matter sampled in SCr basin (Chapters 2, 3). Bacteria: range in SI values from chemosynthetic bacteria, Ruby et al., 1987.

#### 4.4. Discussion

The seep of San Clemente is a methane seep characterized by extensive deposits of barium sulfate, which are often observed as large blocks on the seafloor and can serve as anchors for large tubeworm thickets (Torres et al. 2002). Tubeworm

thickets and vesicomid clams were commonly observed within dark colored sediments, which were heterogeneously distributed at the seepage area. Sediments covered by bacterial mats were not visually detected at San Clemente suggesting that contrary to several seeps in the Californian slope, this habitat is not prominent at our study site. The presence of vesicomid calms and vestimentiferan tubeworms at San Clemente is compatible with megafaunal biogeographic patterns along seeps in the East and West Pacific continental margins (Barry et al. 1996, Sibuet & Olu 1998). Another prominent group of organisms present at the San Clemente seep were pogonophoran polychaetes (Frenulata: Siboglinidae), which formed conspicuous mats heterogeneity distributed in sediments within 10 meters from tubeworm thickets. Siboglinids (a.k.a pogonophorans) often colonize sediments with lower sulfide or methane levels, contrary to their related vestimentiferans (Southward et al. 2005). At seep areas where siboglinids fields are prominent, they reach high abundances in reducing sediments with dissolved methane or sulfide, allowing them to nurture their bacterial symbionts and yet provide access to oxygenated waters through their anterior end (Schulze & Halaných 2003, Southward et al. 2005, Dando et al. 2008, Sommer et al. 2009). At the San Clemente seep, their higher abundance at sediments nearby tubeworm thickets suggests a heterogeneous spatial distribution on the flux of reduced chemicals, apparently centered at the tubeworm tickets.

The polychaete *Siboglinum* was extremely abundant in some areas within the seep and their characteristic chitinous tubes potentially modified the sedimentary structure and influenced the macrofaunal composition within their habitat (Bergquist et al. 2003, Dando et al. 2008). We could not taxonomically distinguish more than one *Siboglinum* species at San Clemente, but siboglinids are commonly found in multiple species associations and is an extremely diverse group (Southward et al.

2005, Hilário & Cunha 2008). Considering their fundamental role as macrofaunal organisms at sediments within 0 to 1 meter of tubeworm thickets, these areas will be further denominated as the “siboglinid habitat”.

Density differences between the siboglinid habitat and background sediments were significant in the San Clemente seep and is consistent with increased local energy input within a food poor deep-sea background (Levin 2005). The higher energetic availability influenced the macrofaunal abundance up to 10 meters from tubeworm clumps, although the number of organisms gradually decreased along the sampled transects. The observed differences on macrofaunal abundance also suggests stronger reliance on chemosynthetic food sources (Levin & Michener 2002), and is consistent with the dominance of chemosynthetic organisms within the siboglinid habitat. The siboglinid habitat exhibited macrofaunal densities ( $6821 \text{ ind. m}^{-2}$ ) comparable to pogonophoran fields at deeper seeps in the Gulf of Alaska ( $\sim 4000\text{m}$ ;  $6625$  to  $9739 \text{ ind. m}^{-2}$ ), but lower densities than similar habitats at shelf to slope depths in the North Sea and Gulf of Cadiz (Dando et al. 1994, Levin 2005, Levin & Mendoza 2007, Sommer et al. 2009). Depth-related effects of food limitation on macrofaunal abundance are masked at seep sites where the input of chemosynthetic carbon can provide the fauna with additional energy sources (Levin 2005). At our study site we observed the local effects of an increased supply of chemosynthetic carbon to the fauna. The higher macrofaunal densities at abyssal seeps in the Gulf of Alaska if compared to San Clemente are probably related to increased organic input (chemosynthetic and photosynthetic) in Alaska, producing the differences on faunal abundance between these areas (Smith & Demopoulos 2003, Rathburn et al. 2009).

Supporting our hypothesis, the macroinfauna composition at San Clemente was markedly distinct between the siboglinid habitat and background sediments.

Multidimensional analysis evidenced the strong spatial heterogeneity in the San Clemente seep sediments, but with a visible separation on macrofauna composition between the siboglinid habitat and background sediments. Species composition within the siboglinid habitat was dominated by *Siboglinum* sp. 1. Siboglinids are abundant at seeps in Pacific and in the Atlantic, notably at the Gulf of Alaska, New Zealand, at mud volcanos and organically enriched sediments in the deep NE Atlantic (Levin & Mendoza 2007, Dando et al. 2008, Hilário & Cunha 2008, Baco et al. 2009). Syllid polychaetes, peracarid crustaceans and thyasirid bivalves were also very abundant at the siboglinid habitat in San Clemente. Thyasirid bivalves have wide trophic strategies, from heterotrophy to chemosynthesis through sulfur-oxidizing symbionts (Dando & Spiro 1993, Dando et al. 2008). Tanaids are also a conspicuous group at pogonophoran fields in the Gulf of Alaska and at clam bed habitats and background sediments on the Californian margin (Levin et al. 2003, Levin & Mendoza 2007). At San Clemente, at least four thyasirid species and two tanaids were successfully exploiting both siboglinid and background habitats, with different species prevailing between those habitats.

Dorvilleid polychaetes were poorly represented at San Clemente but are often a dominant group at methane seeps (Sahling et al. 2002, Levin et al. 2003). Dorvilleids are especially abundant in bacterial mat habitats where high sulphidic sediments might favor their recruitment and increase food availability through bacterial biomass (Levin et al. 2003, Levin 2005, Levin et al. 2006). The absence of bacterial mat habitats at our study site explains the lower abundance of dorvilleids and indicates lower sulfide availability over the sediment surface at the time of sampling. Siboglinids are known to tolerate very low sulfide levels and are able to utilize enormous quantities of sulfide or methane before concentrations peak at surface

sediments (Schulze & Halanych 2003, Dando et al. 2008, Sommer et al. 2009). The high densities attained by *Siboglinum* at 0 to 1 meter sediments could well result in efficient sulfide utilization in San Clemente sediments, and inhibit the growth of bacterial carpets and its associated fauna. We need a better geochemical data to fully address the sulfide and methane levels within San Clemente sediments and to test if there is any biological control in the development of optimal habitats for dorvilleids.

Stable isotope analysis indicated a strong reliance on chemosynthetic nutrition for the dominant species associated with the siboglinid habitat. The siboglinid worms derive their nutrition from sulphur-oxidizing symbiotic bacteria living within their trophosome, with only one species (*Siboglinum poseidoni*) so far known to host methanotrophic symbionts (Southward et al. 2005). At the San Clemente seep, broad isotopic signatures within *Siboglinum* specimens indicate incorporation of chemosynthetic carbon through either methane- or sulfur-oxidizing bacteria and suggest association of multiple congeneric species. Similar variability on mean  $\delta^{13}\text{C}$  signatures (-36.1‰ to -66.6‰) were encountered in siboglinids from New Zealand seeps, where multiple species with varied trophic associations coexist (Thurber et al. 2009). However, although *Siboglinum* from San Clemente suggests incorporation of methane carbon, further molecular analysis are required to confirm methanotrophy or to address if multiple species coexist at this seep site (Dando et al. 2008). *Thyasirid* sp. 4 was another species that relied on sulphur chemosynthetic nutrition from symbionts, confirming their typical thiotrophic associations (Dando & Spiro 1993). The average  $\delta^{13}\text{C}$  values of the siboglinid habitat macrofauna at San Clemente (-36.1‰) is heavier than observed at similar habitats in the Gulf of Alaska (-46.4‰) where the percentage of methane derived carbon utilized by the fauna averages 44% (Levin & Michener 2002). The  $\delta^{13}\text{C}$  values at the siboglinid habitat in San Clemente

indicates strong reliance of chemosynthetic carbon at San Clemente but with increased utilization of other food sources if compared to the deeper Alaskan seeps (Levin & Michener 2002). However, direct comparisons on the contribution of methane-derived carbon and other food sources between our study and other seeps are not possible until source-mixing calculations are carried at the San Clemente macrofauna.

The San Clemente macrofauna seems to host intermediate species richness and diversity when compared to other cold seep areas. A total of 73 species were sampled at seep and non-seep habitats at San Clemente, with 58 species directly associated with the siboglinid habitat. In the Gulf of Mexico, a similar number of species colonize seep vestimentiferan communities, indicating these are habitats hosting a species rich community (Bergquist et al. 2003). Shallower Californian seeps that are characterized by the presence of clam-bed and microbial mat habitats host more species than San Clemente (Levin et al. 2003), although this could be a result of depth and habitat differences. Macrofauna diversity at San Clemente was lower in sediments nearby tubeworm thickets, but peaked at 10 meters due to the presence of seep and background species. The similar diversity levels between the siboglinid habitat and background areas indicates that the San Clemente seep does not host a species-rich community if compared to background sediments. Comparisons among seep sites revealed that pogonophoran fields at the Gulf of Alaska have slightly lower diversity than at San Clemente [Table 4.4; (Levin & Mendoza 2007)]. The San Clemente siboglinid habitat has similar diversity levels to the species rich *Acharax* beds at Hydrate Ridge [Table 4.4; (Sahling et al. 2002)]. The high diversity found at the siboglinid habitat is probably related to lower levels of pore water sulfides (Sahling et al. 2002). This relationship is clear at habitats dominated by *Beggiatoa*

mats and clam beds, which are typically highly sulphidic and frequently exhibit lower diversity and higher faunal endemism [e.g. symbiont bearing organisms; (Tryon & Brown 2001, Sahling et al. 2002, Levin et al. 2003, Levin 2005)].

Table 4.4. Comparison of macrofaunal diversity indexes at deep-sea seep sites grouped by habitat type with published macrofaunal data. Depth in meters.

	Site comparisons per habitat						Seep location (depth)	Reference
	S	J'	H' log2	H' log10	ES(50)	ES(100)		
Siboglinid habitat	58	0.7	4.1	1.2	20.1	30.3	S. Clemente, NE Pacific (1800)	This study (0 and 1m)
				0.7			Kodiak margin, Alaska (4420)	Levin & Mendoza, 2007
				0.9			Unimark margin, Alaska (3280)	Levin & Mendoza, 2007
<i>Acharax</i> beds						29	Hydrate ridge (700)	Sahling et.al. 2002
<i>Beggiatoa</i> mats				0.3		8	Hydrate ridge (700)	Sahling et.al. 2002
							Florida Escarpment (3290)	Levin & Mendoza, 2007
			2.5			18	Eel river, N California (500)	Levin, 2005
<i>Caloptogena</i> beds						14	Hydrate ridge (700)	Sahling et.al. 2002
				4.99		36	Eel river, N California (500)	Levin, 2005

The low level of species overlap between the macrofauna from organic falls and the San Clemente seep confirmed our second hypothesis. The San Clemente seep macrofauna is dominated by chemosynthetic-symbiont species, while organic falls seem to host a broad number of enrichment opportunist organisms, sustaining only juvenile stages of chemosynthetic organisms. Sediments around whale, wood and kelp falls are massively colonized by dorvilleids and cumaceans, which are typical enrichment-opportunist respondents to the increased availability of organic sources around these islands. Considering levels sulfide availability and habitat persistence, whale carcasses sustain chemosynthetic conditions most similar to cold seep and vent ecosystems (Treude et al. 2009). The broad number of seep-related taxa encountered at whale-falls give support to the evolutionary stepping stone hypothesis (Smith et al. 1989). However, the San Clemente seep and whale sediment macrofauna did not exhibit high levels of similarity at the species level. The shared species between seep, wood and whale-falls are typically heterotrophic, and represented by deposit-feeding polychaetes such as ampharetids and paranoids. The overlap of the ampharetid *Amellina cf. abyssalis* between wood-falls and the San Clemente seep possibly

suggests broad utilization of reducing habitats by this species. *Amellina* is distributed along the Gulf of California, was highly abundant around wood-falls and has been encountered at deep-sea pockmarks in the Atlantic (Sumida et al. 2004). The low species overlap between seep and non-seep habitats is also influenced by the distinct macrofaunal structure at San Clemente seep, which is dominated by the symbiont-bearing polychaete *Siboglinum* but is colonized by a few dorvilleids, which are abundant at organic falls and other seep sites.

In summary, the San Clemente seep macrofauna sustains a high density of siboglinid polychaetes in sediments within 1 meter from vestimentiferan tubeworm tickets. Within the siboglinid habitat, species richness and diversity were lower than background sediments, partly due to high dominance of a seep endemic fauna. The San Clemente seep macrofauna sustains comparable diversity levels to similar habitats at other seep areas, but is more diverse than highly sulphidic seep habitats such as bacterial mats and clam beds. Stable isotope analysis showed a wide variability in the organic matter sources for the dominant species in the siboglinid habitat, with most macrofaunal groups feeding heterotrophically. Overall, less than 20% of the species overlapped between the San Clemente seep and wood-, whale- and kelp-fall communities at similar ocean depths within the region. This might be partially related to wide differences in sediment geochemistry and species composition between cold seeps and organic falls.

#### 4.5. References

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Appendix 4A. Mean density and relative abundance of sediment macrofauna at San Clemente seep. *SE*- Standard error, *Indet*- Indetermined organism(s).

	0m		1m		10m		Bkgd	
	Density (1SE)	Rel %						
<b>Acrocirridae</b>								
<i>Flabelligella sp. A</i>	25.5 (11.4)	0.3			51 (14)	1.2	42.5 (24.5)	1.3
<b>Ampharetidae</b>			48.4 (12.1)	0.7	25.5 (11.4)	0.6	42.5 (24.5)	1.3
CRS Ampharetid sp. 1	52.0 (23.3)	0.7						
CRS Ampharetid sp. 10					25.5 (11.4)	0.6		
<i>Amelina abyssalis</i>	25.5 (11.4)	0.3						
<b>Capitellidae</b>								
<i>Heteromastus sp. 1</i>			48.4 (12.1)	0.7				
<i>Notomastus sp. 1</i>			48.4 (12.1)	0.7				
CRS Capitellid sp. 9							42.5 (24.5)	1.3
<b>Chaetopteridae</b>								
<i>Phyllochaetopterus limicolus</i>			32.5 (11.5)	0.5				
<b>Cirratulidae (indet.)</b>			453 (36.9)	6.3	359.8 (61.9)	8.6	127.4 (42.5)	3.8
<i>Chaetozone sp. D</i>	25.5 (11.4)	0.3						
CRS Cirratulid sp. 13	25.5 (11.4)	0.3	97.5 (16.8)	1.4	153.9 (33.4)	3.7	84.9 (49)	2.5
<b>Cossuridae (indet.)</b>	25.5 (11.4)	0.3	48.4 (12.1)	0.7				
<i>Cossura cf. rostrata</i>	77.5 (23.2)	1.0	32.5 (11.5)	0.5	180.4 (23.1)	4.3		
<i>Cossura sp.</i>			32.5 (11.5)	0.5				
<b>Dorvilleidae (indet.)</b>	104 (46.5)	1.4						
<i>Pinniphitime sp.</i>					52 (23.3)	1.2		

CRS Dorv. 23			48.4 (12.1)	0.7	51 (14)	1.2				
<i>Meiodorvillea</i> sp. A			242 (60.3)	3.4						
<i>Meiodorvillea</i> sp. B	101.9 (45.6)	1.4	96.2 (18.7)	1.3						
<b>Fauveliopsidae</b>										
CRS Fauveliopsid sp. C			80.3 (19)	1.1	103 (21.7)	2.5	42.5 (24.5)	1.3		
<b>Flabelligeridae (indet.)</b>							42.5 (24.5)	1.3		
<i>Pherusa</i> sp. A	25.5 (11.4)	0.3								
<b>Goniadidae</b>										
CRS Goniadid sp. 2					52 (23.3)	1.2	42.5 (24.5)	1.3		
<b>Hesionidae (indet.)</b>			80.9 (14.9)	1.1						
Hesionid sp. A	104 (46.5)	1.4								
Hesionid sp. B	25.5 (11.4)	0.3	32.5 (11.5)	0.5						
Hesionid sp. C					51 (14)	1.2	127.4 (73.5)	3.8		
<b>Lumbrineridae</b>										
<i>Ninoe</i> sp. C			15.9 (5.6)	0.2						
Lumbrinerid sp. A	77.5 (23.2)	1.0	15.9 (5.6)	0.2	103 (21.7)	2.5	84.9 (24.5)	2.5		
<b>Maldanidae (indet.)</b>			25.5 (11.4)	0.3	15.9 (5.6)	0.2				
Maldanid sp. A	104 (46.5)	1.4								
<b>Nereidae</b>										
<i>Nereis cf. piscesae</i>			32.5 (11.5)	0.5						
CRS Nereid sp. 5	25.5 (11.4)	0.3	47.8 (16.9)	0.7						
<b>Onuphidae</b>										
CRS Onuphid sp. 1	25.5 (11.4)	0.3								
<b>Opheliidae</b>										
CRS Opheliid sp.2			80.9 (14.9)	1.1	152.9 (41.9)	3.7	127.4 (0)	3.8		
CRS Opheliid sp.3					25.5 (11.4)	0.6				
<b>Orbiniidae</b>										
CRS Orbiniid sp.1					25.5 (11.4)	0.6				
CRS Orbiniid sp.2			65 (23)	0.9						
<b>Paraonidae (indet.)</b>			103 (28.2)	1.4	64.3 (12.2)	0.9	25.5 (11.4)	0.6	42.5 (24.5)	1.3
<i>Levinsenia cf. oculata</i>	101.9 (45.6)	1.4	274.6 (27.7)	3.8	104 (46.5)	2.5	42.5 (24.5)	1.3		
<i>Aricidea cf. rubra</i>	25.5 (11.4)	0.3								
<i>Aricidea cf. symplex</i>	52 (23.3)	0.7								

<i>Aricidea cf. lopezi</i>	52 (23.3)	0.7	15.9 (5.6)	0.2				
<i>Aricidea</i> (Allia) cf. <i>hartleyi</i>			15.9 (5.6)	0.2	25.5 (11.4)	0.6		
<i>Aricidea</i> (Acmira) cf. <i>trilobata</i>			15.9 (5.6)	0.2				
<i>Cirrophorus cf. furcatus</i>	25.5 (11.4)	0.3						
<b>Pholoidae</b>								
<i>Pholoe cf. courtneyae</i>			32.5 (11.5)	0.5	127.4 (25.5)	3.0		
CRS Pholoid sp. 2			15.9 (5.6)	0.2				
<b>Phyllodocidae (indet.)</b>			15.9 (5.6)	0.2				
<b>Pilargiidae</b>								
CRS Pilargid sp.1	52 (23.3)	0.7	32.5 (11.5)	0.5				
<b>Pisionid (indet.)</b>							42.5 (24.5)	1.3
<b>Polynoidae (indet.)</b>							42.5 (24.5)	1.3
CRS Polynoid sp.11			15.9 (5.6)	0.2				
<b>Sabellidae (indet.)</b>	128.4 (36.3)	1.7			25.5 (11.4)	0.6		
<b>Scalibregmidae</b>								
<i>Scalibregma cf. californicum</i>							42.5 (24.5)	1.3
<b>Siboglinidae</b>								
<i>Siboglinum</i> sp.1	2905.5 (606)	38.8	2018.4 (267)	28.1	364 (162.8)	8.7		
<b>Sigalionidae</b>								
CRS Sigalionid sp.1	52 (23.3)	0.7	96.8 (24.1)	1.4	104 (46.5)	2.5	84.9 (24.5)	2.5
<b>Spionidae (indet.)</b>	25.5 (11.4)	0.3	48.4 (12.1)	0.7	127.4 (25.5)	3.0	212.3 (88.4)	6.3
<i>Prionospio</i> sp. A			32.5 (11.5)	0.5				
<i>Aurospio cf. dibranchiata</i>	77.5 (23.2)	1.0	97.5 (16.8)	1.4	255.8 (62.4)	6.1	382.2 (42.5)	11.3
<i>Prionospio</i> sp. C	77.5 (23.2)	1.0	112.8 (16)	1.6	77.5 (23.2)	1.9	254.8 (42.5)	7.5
CRS Spionid sp.11	25.5 (11.4)	0.3	31.8 (11.3)	0.4	153.9 (33.4)	3.7		
<i>Spiophanes cf. anoculata</i>			15.9 (5.6)	0.2	25.5 (11.4)	0.6	42.5 (24.5)	1.3
CRS Spionid sp. A	52 (23.3)	0.7						
<b>Sternaspidae</b>								
<i>Sternaspis cf. fossor</i>							84.9 (24.5)	2.5
<b>Syllidae</b>								

<i>Sphaerosyllis</i> sp. A	415 (94.5)	5.5	321.7 (50.8)	4.5	52 (23.3)	1.2		
<i>Exogone</i> sp. A			241.5 (30.2)	3.4	76.4 (34.2)	1.8		
<b>Terebellidae</b>							42.5 (24.5)	1.3
<b>Trichobranchidae (indet.)</b>	25.5 (11.4)	0.3						
<b>Amphipoda (indet.)</b>	77.5 (23.2)	1.0						
Lysianassid sp. D	52 (23.3)	0.7	96.8 (24.1)	1.4				
<i>Monoculodes</i> sp. 2	52 (23.3)	0.7	31.8 (11.3)	0.4				
<i>Ampelisca</i> sp. 1					51 (22.8)	1.2		
Ampeliscidae sp. 2					25.5 (11.4)	0.6		
Phoxocephalidae sp. 3							42.5 (24.5)	1.3
<b>Cirripedia (indet.)</b>	208 (93)	2.8						
<b>Cumacea (indet.)</b>			32.5 (11.5)	0.5	51 (22.8)	1.2	42.5 (24.5)	1.3
<i>Cumacea</i> sp. E							42.5 (24.5)	1.3
<b>Isopoda (indet.)</b>	25.5 (11.4)	0.3	48.4 (12.1)	0.7	152.9 (68.4)	3.7	297.2 (106.9)	8.8
Asellote Isopod sp. A	104 (46.5)	1.4						
Asellote Isopod sp. H	156 (69.8)	2.1						
<b>Tanaidae (indet.)</b>	52 (23.3)	0.7						
Tanaid sp. 1	988 (442)	13.2	567.1 (118.4)	7.9	154.9 (21.8)	3.7		
Tanaid sp. E			275.3 (52.4)	3.8	204.9 (38.7)	4.9	382.2 (73.5)	11.3
Tanaid sp. J			97.5 (34.5)	1.4				
<b>Aplacophora (indet.)</b>	52 (23.3)	0.7	64.3 (14.9)	0.9			84.9 (24.5)	2.5
<b>Bivalva (indet.)</b>	52 (23.3)	0.7	31.8 (11.3)	0.4			42.5 (24.5)	1.3
Thyasirid sp.					25.5 (11.4)	0.6		
Thyasirid sp. 2	25.5 (11.4)	0.3	194.3 (37.8)	2.7				
Thyasirid sp. 4	362 (46.2)	4.8	144 (39.4)	2.0	180.4 (23.1)	4.3	254.8 (42.5)	7.5
Bivalve sp. R			195 (48.4)	2.7	128.4 (25.7)	3.1		
Bivalve sp. S					25.5 (11.4)	0.6		
Bivalve sp. T					25.5 (11.4)	0.6		
<b>Gastropoda (indet.)</b>			32.5 (11.5)	0.5	51 (22.8)	1.2		
Gastropod sp.3	52 (23.3)	0.7						
<b>Sipunculida (indet.)</b>	52 (23.3)	0.7	65 (15)	0.9	104 (28.5)	2.5	42.5 (24.5)	1.3
<b>Cnidaria</b>	52 (23.3)	0.7						

**Echinodermata**

Holothuroidea	52 (23.3)	0.7	15.9 (5.6)	0.2	25.5 (11.4)	0.6		
Ophiuroidea			65 (15)	0.9	25.5 (11.4)	0.6	42.5 (24.5)	1.3
<b>Total macrofauna</b>	7485 (988)		7170 (421)		4187 (49)		3397 (98)	

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## Chapter 5. Conclusions

The present study focused at patterns of community structure and succession at artificially deployed parcels of wood, kelp and a large whale-fall; and investigated the sediment macrofauna from San Clemente cold seep. The first general hypothesis, that distinct time courses of organic loading on sediments create spatial and temporal differences on macrofaunal colonization and succession, was confirmed. The transfer of organic detritus to the sediments around kelp and wood falls occurred at distinct time scales, creating small to intermediate scale organic disturbances in sediments nearby, and attracting dense numbers of organic opportunist and specialized species. The organic input and bacterial degradation lead to the development of limited sedimentary reducing conditions around kelp and wood parcels, which limits chemoautotrophy to microbes. An end member of high quality organic matter, whale-falls create an intense and long-term organic and sulfide enrichment (>4 years) on sediments nearby. However, the opportunistic successional stage at kelp, wood and whale-falls are remarkably similar in terms of species and trophic succession, albeit different magnitudes of organic disturbance create very distinct temporal scales of succession.

Our second hypothesis was also confirmed by the limited diversity levels observed at sediments around kelp and wood parcels, which were markedly lower at times of intense organic enrichment. Although kelp and wood falls attract several organic-enrichment species not present on ambient deep-sea communities, and create mass effects dynamics for wood-borer specialists, these islands sustain lower levels of

diversity if compared to whale-falls and background sediments. The lower diversity levels at kelp and wood sediments can be related to two main processes: 1) the enhanced dominance of opportunistic species under conditions of organic enrichment, which was ecologically similar to patterns of succession following disturbance in shallow water, and 2) the lower organic quality and scales of microbial degradation at these islands, which is unfavorable to sustain high levels of chemosynthesis by a rich and specialized fauna. The consequences are that kelp and wood parcels, although attracting a sub set of specialized and rare fauna that likely contribute to regional and global levels of deep-sea diversity, and create favorable habitats for the stepping stone dispersal of species adapted to use these parcels in deep-sea areas, host a reduced number of species if compared to whale-falls. Time-scales and the intensity of organic availability contributes to the high levels of diversity supported by whale carcasses in the deep-sea, which provide macrofauna communities with a variety of food sources for organisms adapted to utilize it.

The whale-fall macrofauna was also dominated by organic opportunist species that were not typically observed in background sediments, but sulfophilic species also abounded between 4.5 to 6.8 y. The whale carcass studied was at the end of its organic enrichment stage, as indicated by the wide availability of free sediment sulfides and an absence of broad sedimentary organic enrichment. Therefore, the wide range of organic sources available at later stages of succession at the whale carcass, favored the colonization of a species-rich macrofauna, supporting our third hypothesis that whale-falls increase local (and regional) levels of diversity in the deep-sea. The whale-fall sediments were ecologically analogous to the epifaunal communities that live attached to whale bones, and exhibited one of the highest levels of diversity if compared similar

reducing habitats. The complex range of food sources and trophic associations in organisms that colonize these islands clearly promotes the high diversity levels encountered around whale carcasses. Although similar successional responses to the organic enrichment were observed between whale-, kelp- and wood-falls, there was a limited number of overlapping species between these habitats, also supporting our third hypothesis. The high spatial and temporal variability on species composition at each organic island indicates the extreme diversity that deep-sea communities exhibit at small spatial scales. The specific responses to habitat dynamics and to varied food sources also supports that organic islands have an unique role in maintaining dispersal and habitat suitability for many species not present for wide distances on the typical soft sediment ecosystems in the deep-sea.

Our last hypothesis, that the San Clemente seep macrofauna had a limited number of specialists was partially confirmed, as indicated by the dominance of seep endemics in areas around worm clumps. However, seep sediments did not exhibit higher levels of diversity if compared to background sediments, supporting our fourth hypothesis. The contrast of community composition between seep and non-seep areas was typical of other seep sites. The dominance of a chemosynthetic-symbiont fauna at seep sediments with broad utilization of other organic sources was also a common feature of the San Clemente and other seeps. The presence of a siboglinid bed at seep sediments and the long distance to the other organic islands, reduced levels of species overlap between these habitats.

The colonization and limited overlap of species between organic falls and seeps indicated that organic falls have the potential to sustain chemoautotrophically dependent species, and to create habitat heterogeneity at the typically oligotrophic deep seafloor.

Therefore, there is a great potential for organic falls to increase  $\beta$  (regional) and  $\gamma$  (global) deep-sea diversity, as the frequency of occurrence of such organic islands across deep-sea habitats is likely high. These islands provide food and substrate for generalized opportunists and a variety of specialists, which require sulfide-rich habitat islands to grow and disperse. As these organic islands virtually occur at any location across wide ocean basins, they might favor the dispersal of some species, acting as stepping-stones between long-term chemosynthetic habitats such as cold seeps and vents. Thus, organically enriched sediments around kelp-, wood- and whale-falls, which have received little study, may provide important habitat islands for the persistence and evolution of species at the deep-sea floor. Future studies addressing these organic islands, and their relationship to the biota's of vents, seeps and whale falls need to consider the sediment assemblages residing in the underlying organically enriched sediments.