UNIVERSITY OF CALIFORNIA, SAN DIEGO

Implications of Environmental Heterogeneity for Community Structure, Colonization, and Trophic Dynamics at Eastern Pacific Methane Seeps

A dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy

in

Oceanography

by

Benjamin Michael Grupe

Committee in Charge:

Professor Lisa A. Levin, Chair
Professor Lihini I. Aluwihare
Professor Victoria J. Orphan
Professor Gregory W. Rouse
Professor Jonathan B. Shurin
Professor Jennifer E. Smith

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The Dissertation of Benjamin Michael Grupe is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2014

DEDICATION

For Megan, whose love, companionship, adventurous personality, and constant faith in me inspired this work and fortified my spirit along the way. Graduate school has defined most of our years together, and I hope to be able to support your future endeavors as well as you have mine for the last decade. Let's keep alive our "inborn sense of wonder," find time to discover the mysteries of the oceans, and get lost in nature!

EPIGRAPH

We can only sense that in the deep and turbulent recesses of the sea are hidden mysteries far greater than any we have solved.

Rachel Carson
"The Sea Around Us"

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VITA

2003	Bachelor of Arts, Environmental Studies and Biology, Gettysburg College. Advisors, John A. Commito & Peter Fong.
2003–2004	Research Assistant, South Slough National Estuarine Research Reserve, Charleston, Oregon.
2004–2006	NSF Graduate Teaching Fellow in K–12 Education, Oregon Institute of Marine Biology, Charleston, Oregon.
2006	Teaching Assistant, Oregon Institute of Marine Biology, University of Oregon.
2006	Master of Science, Biology, Oregon Institute of Marine Biology, University of Oregon. Advisor, Alan L. Shanks.
2007	Biomonitoring Coordinator, South Slough National Estuarine Research Reserve, Charleston, Oregon.
2008–2011	National Defense Science and Engineering Graduate Fellow, Scripps Institution of Oceanography, University of California, San Diego.
2011–2014	Teaching Assistant, Scripps Institution of Oceanography, University of California, San Diego.
2014	Doctor of Philosophy, Oceanography, Scripps Institution of Oceanography, University of California, San Diego. Advisor, Lisa A. Levin.

PUBLICATIONS

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ABSTRACT OF THE DISSERTATION

Implications of Environmental Heterogeneity for Community Structure, Colonization, and Trophic Dynamics at Eastern Pacific Methane Seeps

by

Benjamin Michael Grupe

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Professor Lisa A. Levin, Chair

Methane seeps in the deep sea are chemosynthetic ecosystems that host highly productive communities, are spatially heterogeneous and temporally dynamic, and enhance regional diversity on all continental margins. The goal of this dissertation was to examine the relative influences of chemical, substrate, and spatial heterogeneity on macrofaunal community structure and dynamics within three Pacific Ocean seep ecosystems. The discovery of the Del Mar methane seep, only tens of meters across but with many microhabitats and associated species, highlights the ecosystem services seeps offer humans. These include exporting primary production to support food webs, sequestering inorganic carbon, providing habitat for fishery species, and enhancing

biodiversity through microhabitat heterogeneity. The effects of substrate and seepage heterogeneity on community structure and dynamics were investigated at two methane seeps: Mound 12, Costa Rica and Hydrate Ridge, Oregon. Deployment of substrates representative of chemosynthetic ecosystems [authigenic carbonate (seeps), wood (sunken wood falls), bones (whale falls), and biogenic material (seep ecosystem engineers)] at sites with varying influence of active fluid seepage led to several key findings regarding macrofaunal colonization patterns. After about one year, active seepage best explained the structure of colonizing macrofaunal communities, which had species identities and densities resembling native carbonates and were dominated by seep-endemic gastropods like *Provanna* spp., *Pyropelta* spp., and Neolepetopsidae. At inactive sites, colonization trends were more variable with reduced macrofaunal densities but higher diversity compared to active sites. There was a mixture of regional margin species, some seep endemics, and (at Hydrate Ridge) substrate specialists that bored into wood (*Xylophaga washingtona*) and bone (*Osedax* spp.). As bare substrates were colonized or when carbonates were transplanted between active and inactive sites, the trophic structure as measured by stable isotopes ($\delta^{13}C$, $\delta^{15}N$) better resembled *in situ* carbonate communities at active seeps. These results suggest that niche-based dynamics regulate diversity patterns on hard substrates at active methane seeps, while competition-colonization tradeoffs and successional dynamics become increasingly important for nearby inactive carbonate communities. A metacommunity framework will be useful for those interested in maintaining the integrity of chemosynthetic ecosystems as the human footprint expands in the deep sea.

CHAPTER ONE

INTRODUCTION

Background

Chemosynthetic ecosystems such as hydrothermal vents and methane seeps have revolutionized our understanding of life on earth, and especially the potential for novel types of microbial production to support entire metazoan food webs. Since the discoveries of vents on the Galapagos Rift in 1977 and seeps in the Gulf of Mexico and Northeast Pacific Ocean less than a decade later (Lonsdale 1977; Corliss et al. 1979; Paull et al. 1984; Suess et al. 1985; Paull et al. 1985; Kulm et al. 1986), it has become apparent that such environments are widespread and contribute significantly to primary production in the deep sea, global biodiversity patterns, and marine geochemical cycling (Bennett et al. 2008; Baker et al. 2010). Within chemosynthetic environments, geological and chemical processes lead to high levels of reduced compounds such as hydrogen sulfide (H_2S) and methane (CH_4), which are used as energy sources by carbon-fixing microbes; these form the base of the food web for dense biological communities that have been described as oases in the deep sea (Corliss and Ballard 1977).

The physical and chemical settings of cold seeps

Cold seeps are common on the world's active and passive continental margins. At active margins such as the western margins of North and Central America where this research was conducted, oceanic plates subduct underneath the continents. Downward

slabs are subjected to intense temperature and pressure, organic carbon from millions of years worth of accumulated sediments is converted to methane, pore pressures increase, and fluids are expelled (Boetius and Suess 2004; Saffer and Tobin 2011). These fluids, containing various hydrocarbons such as methane or ethane, rise upward through fissures in the overlying crust, eventually escaping from cold seeps. At seeps, the anaerobic oxidation of methane (AOM) is carried out by consortia of anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) (Boetius et al. 2000; Orphan et al. 2001). The net reaction resulting from this syntrophic relationship is:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$$

AOM is the dominant sink for methane in marine systems, so most of the hydrocarbon fluids making their way upwards at seeps of Hydrate Ridge (Oregon) and Costa Rica are converted into either organic matter or bicarbonate, the production of which ultimately leads to the accretion of carbonate (Ritger et al. 1987; Aloisi et al. 2000). Low $\delta^{13}C$ signatures of organic carbonate (ca. -48‰ at Hydrate Ridge and -46‰ at Costa Rica's Mound 12) are evidence that they are derived from methane (Bohrmann et al. 1998; Teichert et al. 2005). Since AOM involves SRB, it results in the accumulation of H_2S, allowing sulfide-oxidizing bacteria (SOB) such as *Beggiatoa* and *Thioploca* an energy source at seeps, where they form mats and may even cover carbonate rocks.

Mats of SOB are thickest where sulfide levels are highest, while other bacteria live inside megafauna as symbionts, and others live freely on hard surfaces or in the water column. Many of the metazoans at seeps and other chemosynthetic ecosystems are

dependent on chemoautotrophic microbes as their sole source of nutrition, which is reflected in the isotopic ratios of seep animals (Van Dover and Fry 1994; Levin and Michener 2002; Thurber et al. 2010). A range of behaviors, morphologies, and physiological conditions have evolved to allow a host of animals to use these microbial resources (Powell and Somero 1986; MacAvoy et al. 2005; Thurber et al. 2012; Levin et al. 2013), and new microbial-metazoan interactions are frequently described (Thurber et al. 2011).

Substrates and heterogeneity in deep-sea chemosynthetic ecosystems

Chemosynthetic ecosystems are ideal habitats in which to study community structure and diversity patterns as they relate to different types of heterogeneity because of striking physical complexity and steep gradients in chemistry and productivity that create an inherently patchy, heterogeneous environment (Cordes et al. 2010b). While most of the deep sea is covered in muddy sediments, vents and seeps usually contain abundant hard substrate (basalts, sulfides or carbonates) with distinct community compositions.

Seeps are associated with mounds, scarps, and other areas of topographic relief along continental margins such as in the East Pacific (Sahling et al. 2008). These habitats contribute both structural and chemical heterogeneity in the environment, existing as a patchwork of individual seeps that have locally high sulfide and methane concentrations relative to ambient, inactive conditions. Methane seeps are fragmented, patchy habitats that are arranged at a hierarchy of scales, which must be taken into account when

considering community patterns. These would be generally soft-sediment habitats were it not for ANME and SRB consortia and the precipitation of carbonate as a byproduct of AOM. Hence, these microbes are "ecosystem engineers" (sensu Jones et al. 1994), modifying abiotic conditions for the entire community through the creation of a novel habitat. Carbonate pavements, boulders, and cobble provide living sites for epifauna, some of which create additional structures that contribute to heterogeneity and serve various ecosystem functions. Biological structures are important in adding physical structure all along continental margins (Buhl-Mortensen et al. 2010), especially at methane seeps (Cordes et al. 2005; Levin 2005; Cordes et al. 2010b). Mussels and tubeworms attach to carbonates, and these aggregating organisms create a complex microhabitat with many microcrevices and interstices in which smaller macrofauna find food or refuge (Levin 2005; Cordes et al. 2010a).

In recent years, scientists have recognized that sunken organic material, such as whale and wood falls, also harbor chemosynthetic communities that are strikingly similar to vent and seep communities (Turner 1977; Smith et al. 1989; Smith and Baco 2003). As concentrated organic material decays, bacteria deplete local oxygen levels and eventually create high concentrations of sulfide. This chemical and microbial environment favors taxa that are phylogenetically similar to many of the species found at seeps and vents (Smith and Baco 2003; Bernardino et al. 2012), and even the microbiological communities display evolutionary relationships among chemosynthetic ecosystems (Fagervold et al. 2012). The similarities between habitat characteristics and taxa in deep-sea chemosynthetic communities raise the question of how habitat associations have

changed in these groups over time. It is unclear whether wood falls, whale falls, seeps, and vents each have their own associated communities, regulated by substrate attributes, or whether a subset of their species are shared among ecosystems. It appears that over evolutionary time, certain groups such as provannid snails, lepetodrilid limpets, and dovilleid, ampharetid, and vestimentiferan polychaetes have developed the ability to adapt to multiple reducing ecosystems, resulting in the radiation of more species than might otherwise have occurred (e.g. Johnson et al. 2008; 2010; Thornhill et al. 2012).

Wood falls and whale falls are probably common in the regions this research was conducted. Sunken logs and palm fronds were frequently encountered at Costa Rica seeps (B. Grupe, L. Levin personal observations), and are also common on the Oregon margin, where terrestrial watersheds are heavily forested (Voight 2007). Additionally, whales regularly use these as feeding or breeding regions, so species that are capable of living at multiple reducing environments could exhibit population connectivity that unites the metacommunity dynamics of seemingly distinct ecosystems.

Habitat heterogeneity and diversity patterns

At the scale of the continental slope, cold methane seeps are known to contribute to regional biodiversity patterns via provision of a novel geochemical environment and various hard substrates (Levin et al. 2010; Sellanes et al. 2010; Cordes et al. 2010b). The *in situ* primary production of vents and seeps supports higher biomass of consumers at all trophic levels relative to background benthic habitats, whose organisms depend on sinking photosynthetically-derived particulate organic matter (POM) for nutrition

(Sahling et al. 2002). Countless studies have compared habitat heterogeneity to diversity patterns in various environments and for different taxa, including those in the deep sea [bathyal soft-sediments, (Jumars 1975); methane seeps, (Cordes et al. 2010b); biotic structures on continental margins, (Buhl-Mortensen et al. 2010); for nematodes, (Vanreusel et al. 2010)], though observations have tended to focus on soft-sediment habitats that are relatively simple to sample and quantify with different types of cores. These studies often conclude that heterogeneity – which may stem from biological structures and behavior (burrows, polychaete tubes and mudballs, mounds, large organic falls), sediment characteristics, POM flux, or hydrodynamics – plays a role in maintaining the extraordinarily high biodiversity observed in deep-sea sediments (reviewed in Etter and Mullineaux 2001; Buhl-Mortensen et al. 2010).

Diversity and abundance patterns are harder to quantify on hard substrate habitats, which can't be sampled the same way as soft-sediments (primarily via cores). To accurately measure total diversity in these habitats, pieces of substrate must be collected so that the entire associated fauna – whether free-living, epibiotic, or endobiotic – can be identified and counted. Performing careful inventories of the meio- and macrofauna associated with hard substrates may lead to comparative analyses of diversity among the microhabitats occurring at cold seeps and other deep-sea habitats.

Habitat heterogeneity has frequently been observed to correlate with biodiversity, but whether this is a fundamental relationship that is pervasive in all ecological settings remains to be seen (e.g. Simpson 1964; MacArthur and Wilson 1967; Bazzaz 1975; Vivian-Smith 1997). Heterogeneity can have many sources – geological, chemical, and

biological – all of which can occur at a variety of spatial scales. Since species richness increases with spatial area (MacArthur and Wilson 1967; Connor and McCoy 1979), it can be difficult to distinguish the effects of increased area from habitat heterogeneity on species diversity patterns (Kohn and Walsh 1994; Rosenzweig 1995). Moreover, species are sensitive to particular spatial scales, so an environment that appears homogeneous to one individual could be just one part of a heterogeneous environment to an individual of a different species with a larger ambit. The result is that any relationship between heterogeneity and diversity is highly dependent on the spatial scale considered (Tews et al. 2004). By performing manipulative colonization experiments that include different sources of heterogeneity across multiple scales, I have attempted to consider the separate roles of environmental heterogeneity and spatial scale in influencing community structure.

Colonization and succession in chemosynthetic ecosystems

Knowledge of ecological patterns and relationships in chemosynthetic ecosystems are somewhat limited by the logistical and financial constraints involved with work that requires submersibles, ROVs, and long periods of ship-time. Repeated visits to the same site are expensive and infrequent, but several observational and manipulative studies have begun to inform our understanding of community succession and colonization patterns in these habitats (reviewed in Van Dover and Trask 2000; Young 2009). There have been fewer studies of recruitment and colonization studies at seeps compared to vents, especially on hard substrates. In a colonization experiment near vents and seeps but not exposed to their reduced fluids, Gaudron et al. (2010) found that species with chemosynthetic affinities colonized multiple substrates, with mollusks and polychaetes

being most common. Though overall species richness was quite low (2-12 species colonized each treatment), there was a trend of higher recruitment rates of species and individuals on organic substrates (wood and alfalfa), especially near seeps. This could reflect a possible role of sulfide as a settlement cue for colonizing larvae, as suggested by Levin et al. (2006) and Bernardino et al. (2010) for soft-sediment chemosynthetic habitats in the Northeast Pacific. Specialists on organic food falls rapidly colonize deployments of their preferred substrate [the polychaete *Osedax* spp. on bones of whales and other marine animals (Smith and Baco 2003; Braby et al. 2007; Rouse et al. 2011); the bivalves *Xylophaga* spp. on wood (Turner 1977; Voight 2007; Bernardino et al. 2010); the asteroids *Xyloplax* spp. on wood (Voight 2005). These studies point to roles for both chemical activity and substrate in colonization patterns depending on the system studied.

The growing human footprint on deep continental margins – involving fishing, hydrocarbon extraction, mining, and bioprospecting, is creating an accelerated need to understand community structure, successional processes, colonization rates, and the dynamics that maintain diversity within and among margin settings (Levin and Sibuet 2012).

Dissertation Objectives

The overarching objective of this dissertation is to examine how sources of heterogeneity within methane seeps are reflected in community patterns and macrofaunal diversity, and to what extent colonization and trophic dynamics are impacted by environmental heterogeneity. In Chapter 2, I describe the ecological setting of a newly-

discovered methane seep off Del Mar, California. Using samples and imagery collected with remotely-operated vehicles (ROVs), I characterize the macrofaunal structure and diversity of the various microhabitats associated with this seep. Several lines of evidence are used to argue that methane seeps are likely significant contributors to ecosystem services along continental margins, such as relatively high densities of fishery target species that were associated with active areas around the seep. Additionally, the Del Mar seep and other cold seeps are sinks for inorganic carbon, enhance regional diversity and associated biological regulatory activities, and export primary production to support the food webs of surrounding continental margin habitats.

Chapters 3 and 4 describe experiments conducted in order to better understand the factors controlling community structure and trophic patterns on substrates at seeps. We performed approximately yearlong experimental manipulations at Mound 12 off Costa Rica and at Hydrate Ridge off Oregon. At both seeps, we deployed a range of substrates (carbonate, wood, bone, biogenic shells and tubes) in different chemical environments, and replicated these experiments in space. Chapter 3 describes the results of our colonization experiment at Mound 12, where we observed rapid macrofaunal colonization on all substrates, as long as they were placed near active fluid seepage. A suite of gastropod species, which dominate the native carbonates at Costa Rica, recruited in high densities to experimental substrates, while polychaetes were relatively less successful colonizers. Stable isotope data suggest that species with flexible diets tended to be more successful early colonizers, and these included several of the most dominant gastropod species. This chapter provides evidence that one year is insufficient for

recovery of diversity and trophic structure on bare substrates, which has implications for large-scale succession and ecosystem recovery from disturbance.

In Chapter 4, I use stable isotopic data from similar one-year substrate deployments at Hydrate Ridge to explore the relationship between trophic structure and dynamics of macrofaunal colonization. Communities colonizing substrates at active sites had mean $\delta^{13}C$ values from about -25 to -45‰, reflecting a strong influence of chemosynthetic production, while communities colonizing inactive substrates had reduced trophic diversity based on photosynthetic production. Communities on different substrates showed similar isotopic patterns, although some patterns from wood and bone suggested that these substrates might be able to act as chemosynthetic stepping stones that could connect distant populations of seep species.

Finally, in Chapter 5, I consider the dynamics of seep ecosystems in a metacommunity context. I analyze the communities on colonization substrates and native carbonates from Hydrate Ridge using a metacommunity framework, which acknowledges that substrates contain patch communities that are connected to other communities via the individual movement or larval dispersal. Several analyses suggest that the spatial arrangement of substrates contributes little to resulting metacommunity structure, while environmental variables – especially proximity to seeping fluids – explain ecological patterns at local and regional scales. In inactive areas less exposed to reduced fluids, however, less predictable colonization patterns and a different species pool from native carbonates implicated a role for succession and colonization-competition tradeoffs. This

work has implications for protecting biodiversity in deep-sea settings where human impacts may impact chemosynthetic metacommunities.

As the research and taxonomic studies related to these cruises are ongoing, there remain species to be described, and certain names may change or be refined in the future. For this reason, readers are advised to search for up-to-date manuscripts and contact the author before citing information related to specific species or taxonomic groups from this dissertation.

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CHAPTER TWO

METHANE SEEPS ENHANCE CONTINENTAL MARGIN ECOSYSTEM SERVICES: EVIDENCE FROM THE RECENTLY DISCOVERED DEL MAR METHANE SEEP

Abstract

Recent discovery of a methane seep in the San Diego Trough (1020 m) with abundant groundfish off the coast of Del Mar, California raised questions about the role of seeps in margin ecosystems and their services. We used multicorer and ROV grab samples and an ROV survey to characterize macrofaunal structure and diversity of soft sediments and authigenic carbonates, the seep microhabitats and taxa observed, and the abundance and spatial patterns of fishery-relevant species. Biogenic microhabitats near the Del Mar Seep included microbially-precipitated carbonate boulders, bacterial mats, vesicomyid clam beds, frenulate and ampharetid beds, vestimentiferan tubeworm clumps, and fields of *Bathysiphon filiformis* tubes. Macrofaunal densities were enhanced near the edge of the seep relative to background, and mean $\delta^{13}C$ signatures became lighter closer to the seep, suggesting that chemosynthetic production enhanced secondary production and animal densities in close proximity to the seep. Polychaetes dominated sediments, and ampharetids became especially abundant near microbial mats, while gastropods, hydroids, and sponges dominated carbonate rocks. A wide range of stable isotopic signatures reflected the diversity of microhabitats, and methane-derived carbon was the most prevalent source of nutrition for several taxa, especially those associated with carbonates. Megafaunal species living near the seep included longspine thornyhead

(*Sebastolobus altivelis*), Pacific dover sole (*Microstomus pacificus*), and lithodid crabs (*Paralomis verrilli*), which represent targets for demersal fisheries. *Sebastolobus altivelis* was especially abundant (8.6 fish 100 m^{-2}) and appeared to aggregate near the most active seep microhabitats. The Del Mar Methane Seep, like many others along the world's continental margins, likely contributes a range of ecosystem services to humans including a sink for inorganic carbon (through community biomass and the precipitation of authigenic carbonate), enhanced regional diversity and associated biological regulation, export of primary production and food web support, and habitat for fishery species.

Introduction

The deep sea is popularly described as remote, alien, and disconnected from human society, but this vast region covering 64% of the earth's surface contributes many ecosystem services [reviewed in Armstrong (2012)]. In particular, a myriad of human activities acutely affect deep continental margins, which host a diversity of habitats that contribute essential fisheries production, mineral and gas resources, and other ecological and regulating services (Levin and Dayton 2009; Levin and Sibuet 2012).

Ecosystem services offered by unseen deep-sea ecosystems such as methane seeps are easily discounted but have global consequences. Methane seeps play a role in global biogeochemical cycling and elemental transformation of carbon, sulfur, and nitrogen (Hinrichs and Boetius 2002; Dekas et al. 2009; Boetius and Wenzhöfer 2013). The anaerobic oxidation of methane (AOM) and associated precipitation of carbonate at cold seeps constitute a major carbon sink in sediments, introducing a mechanism for benthic biogeochemical processes to influence potential greenhouse gas sources (Ritger et al. 1987; Reeburgh 2007). The seep biota acts as a methane filter that prevents methane stored in gas hydrates and the deep biosphere from freely entering the hydrosphere and atmosphere; as much as 20–80% of methanic carbon may be converted into benthic biomass and carbonate, depending upon fluid flow rates (Boetius and Wenzhöfer 2013).

The microbial biogeochemical processes that depend on reduced compounds (methane, sulfide, and hydrogen) at seeps create chemosynthetic primary production that sustains heterotrophic and symbiont-bearing fauna endemic to cold seeps, as well as background consumers that may aggregate at these productive benthic ecosystems (Levin

2005; Sellanes et al. 2008). At a regional scale, seeps are unique ecosystems that add physical, chemical, and biological habitat heterogeneity to continental margins (Cordes et al. 2010). High beta diversity at seeps and surrounding regions can enhance the overall species richness, ecosystem function, and biological regulation that can occur on continental margins (Levin et al. 2010; Levin and Sibuet 2012). Examples of non-endemic seep fauna utilizing the habitat offered by methane seeps include egg-laying sites and nurseries for benthic octopuses and elasmobranchs (Treude et al. 2011; Drazen et al. 2003), sponge-garden refugia for macrofauna (Thurber et al. 2010), and structural habitat for predatory fish and crabs [Patagonian toothfish, (Sellanes et al. 2008; 2012) sablefish, BMG & LAL personal observations; Lithodid decapods, (Niemann et al. 2006)]. Despite the co-occurrence of commercial fisheries and seeps on continental margins (Sellanes et al. 2008; Bowden et al. 2013), it is unclear to what extent, if at all, energy is transferred from chemosynthetic production into margin-wide secondary production and fished species.

Finally, technological advances may soon increase our utilization of provisioning ecosystem services of methane seeps. Frozen methane hydrates represent a potential vast source of fossil fuel energy (Cyranoski 2013), while the pharmaceutical industry hopes to use bioprospecting to take advantage of the novel adaptations allowing seep microbes and animals to survive chemically stressful environments to bring about breakthroughs in the field of natural products and drug discovery (Thornburg et al. 2010). And while difficult to quantify, underexplored deep-sea environments like methane seeps have intrinsic societal value, as well as aesthetic value that has influenced writers (e.g., Frank Schatzing

and *The Swarm*), filmmakers (e.g. James Cameron and *The Abyss*), and artists (e.g. Lily Simonson, www.cb1gallery.com/artists/simonson.html, *accessed* 26 March 2014).

In this investigation, we explore the ways in which a methane seep recently discovered in southern California might enhance or otherwise influence the ecosystem services provided by the continental margin. The Del Mar Methane Seep occurs at the lower boundary of the oxygen minimum zone (OMZ), and with overlying water already low in oxygen, its fauna and habitats are jointly affected by sharp reducing gradients in sediments. Despite knowledge of several methane seeps off southern California, the macrofaunal biology and community structure has previously been examined only at the San Clemente seeps, which occur much deeper than the OMZ at 1800 m [(Bernardino and Smith 2010); but see references for other northeast Pacific seeps at Monterey (Barry et al. 1996), northern California (Levin et al. 2003; 2010), and Oregon (Kulm et al. 1986; Sahling et al. 2002; Levin et al. 2010).

While the deep sea provides a host of ecosystem services, here we focus specifically on habitat heterogeneity and biodiversity [which sustains biological regulation (Danovaro et al. 2008)], trophic support for the benthic ecosystem, and habitat for demersal fishery species, with the premise that such ecosystem services may be common to both known and undiscovered seeps. As the Del Mar Seep was only discovered in 2012, another of our objectives is to provide an initial characterization of its habitats and taxa. In regards to potential seep ecosystem services, we specifically hypothesized that:

(1) Distinct species assemblages would occur in different biogenic habitats.

(2) Macrofaunal density in sediments would increase with proximity to the seep.

(3) Species diversity would be higher near the seep center than surrounding sediments; since taxa in the OMZ already experience a physiologically-stressful ecosystem, high *in-situ* primary production at the seep could allow more species to coexist and meet their energetic needs (Levin et al. 2010).

(4) Methane-derived carbon (MDC) would be detectable in the benthic food web near sources of seeping fluids, and isotopically-light $\delta^{13}C$ signatures characteristic of chemosynthesis would be evident in sediment macrofauna but would decline with increased distance from the seep.

(5) Densities of fished species (*Sebastolobus altivelis* and *Microstomus pacificus*) would be relatively high at the methane seep compared to the surrounding seafloor. Moreover, we hypothesized these demersal fish would be more likely to occur specifically with three-dimensionally complex seep microhabitats (carbonate outcrops, rubble, clam beds) than over flat sediments.

Study Area

This study was conducted at the Del Mar Methane Seep (32º 54.25'N, 117º 46.94'W) at a depth of 1020 m in the northern portion of the San Diego Trough, approximately 50 km west of the Scripps Institution of Oceanography (San Diego, California). This recently discovered seep is situated on a pop-up structure within a series of strike-slip faults, where a compressional restraining stepover exerts tectonic control to focus upward fluid flux that feeds dense chemosynthetic assemblages (Ryan et al. 2012;

Maloney 2013). A number of hydrocarbon seeps (methane, tar, petroleum, etc.) are known from the southern California bight in the Northeast Pacific, with the closest known methane seeps being in the San Clemente Basin (Torres et al. 2002) and the Santa Monica Mounds (Paull et al. 2008). The OMZ (<0.5 mL O_2 L^{-1}) above the San Diego Trough extends from approximately 500 – 1000 m, and the oxygen concentrations measured at the Del Mar Methane Seep were about 0.4 mL O_2 L^{-1} at a depth of 1000 m in both July and December 2012.

Material and Methods

Field Sampling

Samples were acquired during three cruises in July 2012 (RV *Melville* leg MV1209), December 2012 (RV *Melville* leg MV1217), and May 2013 (RV *Western Flyer*) (Figure 2.1A,B). Three zones around the seep were sampled: the seep center (100% coverage of chemosynthetic bacterial mats and carbonate boulders, Figure 2.1E, F, H); the seep periphery (a mix of carbonates, bacterial mats, clams, and non-chemosynthetic sediments, Figure 2.1D,G); and sediments further from the seep lacking visual indications of seep activity (e.g. no microbial mats or seep endemic fauna), but sometimes containing pieces of clam shells as evidence of past seepage (Figure 2.1C). We deployed a multicorer (tube diameter 9 cm) to collect sediments from sites varying in their proximity to the seep center: we refer to these locations as A_{173} (~173m from seep center, 3 multicorer drops), B_{108} (~108m, 2 drops), and C_{32} (~32m, 2 drops) (Figure 2.1A, Table 2.1). Distances are based on GPS coordinates associated with the ship position corrected to wire position and should be considered approximate. One core per drop was

sectioned, sieved at 300 μm, and sorted shipboard to obtain animals for stable isotope analysis, and usually three cores were sectioned and preserved in 8% formalin or 70% ethanol for subsequent macrofaunal characterization. The second multicorer drop at C_{32} likely contacted subsurface carbonate, and we recovered only enough intact sediment cores to slice and preserve two replicates. Cores were sectioned as follows: 0–1 (including sieved water), 1–2, 2–3, 3–5, and 5–10 cm. Macrofauna, protists, and microbes retained on a 300 μm screen were sorted live, photographed, identified, and sampled for stable isotopes (described below) or preserved by various means (8% formalin, 95% ethanol, -80ºC storage).

In July and December 2012, CTD casts obtained hydrographic data (including temperature, salinity, dissolved oxygen, and pH) for each multicorer drop location. Sediments from one sediment core per location were analyzed for total organic matter, Chlorophyll a, Phaeopigments, and percent sand and silt-clay. In December, sediment porewater from two multicores at C_{32} and one push core (diameter 4.5 cm) in an orange microbial mat were analyzed for $\delta^{13}CH_4$, CH_4 concentration, δD (deuterium), and sulfide concentration (Table 2.A1).

ROV dives were conducted in December 2012 (the Scripps ROV *Triton*) and May 2013 (the MBARI ROV *Doc Ricketts*) to explore the Del Mar Methane Seep, collect imagery, conduct a survey of seep microhabitats and megafauna, and collect carbonate rocks, sediment push cores, and megafauna from different microhabitats (Table 2.1). Since ROV *Triton* does not have a watertight or partitioned biobox, the communities associated with carbonates collected in December could not be quantified. Six carbonate

rocks collected with the ROV *Doc Ricketts* were stored in separated watertight partitions. Rocks 1–3 came from the seep center, while Rocks 4–6 came from the periphery. Upon recovery, macrofauna were picked or allowed to crawl out of rocks, identified, and processed for stable isotopes. We calculated density by normalizing counts to surface area of each rock. Surface area was calculated by covering a rock with a single layer of aluminum foil, which was weighed and compared to the mass of a known surface area. Push cores (diameter 7 cm) and a hydraulic suction were used to collect macrofauna from distinct microhabitats to quantify the sediment communities and sample individuals for stable isotope analysis, respectively (Table 2.1). Push cores were sectioned and preserved as described above for multicores, and macrofauna were quantified in two each from an ampharetid bed (D_{amph}) and an adjacent orange microbial mat (E_{mat}).

During the 19 May 2013 dive, to characterize the extent of the methane seep and the abundance of megafauna and their habitat associations, we performed a 45-minute visual survey of the seep and surrounding seafloor. The ROV traversed eight roughly parallel transects from 50 to 80 meters long. The total area surveyed was 1437 m^2; for analysis this was divided into the seep center (112 m^2), seep periphery (317 m^2), and off seep (1008 m^2) (Figure 2.1B).

Laboratory and stable isotopes analyses

In the laboratory, preserved cores were sieved at 300 μm. All macrofauna were picked using a dissecting microscope and identified to the lowest possible taxonomic resolution (typically genus for gastropods, family for polychaetes, and class or order for other taxa).

For stable isotope analysis, specimens were sorted shipboard, identified to the lowest feasible taxonomic level, left in filtered seawater overnight at 4ºC to clear gut contents, rinsed in milli-Q water, and placed in pre-weighed tin boats using methanol-cleaned forceps. Representatives of most species were photographed live for later comparison with voucher specimens and taxonomic confirmation. Samples were frozen at -80ºC until return to the laboratory, where they were dried to constant weight at 60ºC (~48 hours), weighed, and 0.2–1.4 mg tissue was acidified with 12.5-25 µL 2N PO_4 to remove inorganic carbon. Stable isotope measurements ($\delta^{13}C$, $\delta^{15}N$) were made using a Costech elemental analyzer coupled to a Micromass Isoprime isotope ratio mass spectrometer (EA/IRMS) at Washington State University. Stable isotope values are expressed in the standard δ (delta) notation and reported in units of per mil (‰), where the element X is represented by:

$$\delta X = \frac{R_{sample}}{(R_{standard} - 1)} \times 1000$$

where X is ^{13}C or ^{15}N, and R is the ratio of $\frac{^{13}C}{^{12}C}$ or $\frac{^{15}N}{^{14}N}$. Standards were Pee Dee Belemite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$ (Fry 2006).

Video analysis

Video from the 19 May 2013 ROV survey was used to quantify the number, size, and microhabitat association of the most common megafauna. Scale was determined by measuring objects of known lengths (e.g. aluminum cans, bottles) that appeared at particular points on the viewing screen. Demersal fish and crabs were counted and measured when they passed specific points at mid-screen or at the bottom of the screen,

areas with sufficient lighting to accurately identify and measure most animals. Despite slight variation in the height of the ROV above the seafloor (~1–2 meters, though we lacked altimetry data), the width of the viewing area at mid-screen was fairly consistent and averaged 3 meters. Latitude and longitude data associated with the ROV were used to plot transects and calculate total survey length. A small section of the fourth transect was not used to prevent replicating part of the previous transect. We calculated the total surveyed area of habitat zones (seep center, seep periphery, off-seep) in order to determine whether species were more likely to associate with particular portions of the seep. When an epibenthic species appeared in the frame, we recorded the time, position, location, substrate association (soft sediment, carbonates), and association with biogenic habitat (dead or live clams, bacterial mats, *Bathysiphon filiformis* tubes).

Statistical analyses

One-way analysis of variance (ANOVA) was used to test whether the random factor location had a significant effect on density of total macrofauna in addition to that of the most abundant taxonomic groups. Data were log-transformed when they did not meet the assumptions of normality or equal variance. Species diversity indices (Shannon index H', Pielou's evenness J', ES_{20}, and ES_{100}) were calculated for pooled replicates and compared among core locations and carbonate rocks. Rarefaction curves were created to compare taxonomic richness among microhabitats. The influence of locations on community structure was inspected with multivariate community analyses (Bray-Curtis Similarity measures, nMDS) and ANOSIM was used to test for differences. Error terms are presented as the standard error of the mean unless stated otherwise.

Stable isotope data were inspected with biplots, and one-way ANOVAs were used to separately ask whether taxonomic group or microhabitat influenced $\delta^{13}C$ or $\delta^{15}N$ signatures. Post-hoc comparisons were made with Tukey HSD tests. A single isotope, two-source mixing model (Fry and Sherr 1984) was used to quantify the fraction of MDC making up macrofaunal tissue. Maximum estimated MDC was calculated as:

$$F_m = \frac{(\delta_t - \delta_{POC})}{(\delta_m - \delta_{POC})}$$

where δ_t, δ_m, and δ_{POC} are the $\delta^{13}C$ signatures of tissue, methane, and particulate organic carbon (POC), respectively. An estimate for the minimum MDC was calculated by substituting δ_{SOB} [$\delta^{13}C$ of sulfur-oxidizing bacteria (SOB)] in place of δ_{POC} (after Levin and Michener 2002; Thurber et al. 2010).

The null hypothesis that longspine thornyhead (*Sebastolobus altivelis*) were distributed independent with respect to the habitat zones was tested using a 2x3 Chi-squared test. ANOVA was performed on log-transformed lengths to test whether different sizes of fish associated with different habitat zones. All statistical analyses were performed in JMP 11 (SAS Institute Inc. 2013), except for multivariate community analyses and calculation of diversity indices, which were performed in PRIMER 6.1 (Clarke and Gorley 2006).

Results

Del Mar Methane Seep microhabitats

Visible features of the Del Mar Seep cover only about 1200 m^2, but encompass a variety of substrate types, microbial mats, symbiont-bearing fauna, and macrofaunal assemblages. The center of the seep (Figure 2.1E) has a heterogeneous topography with carbonate boulders (1–3 m boulder size) and pavement, nearly all covered by extensive orange and white bacterial mats, with signs of possible subsurface methane hydrate (meter-scale pits and craters; Figure 2.1H). Red anemones (~3-6 cm diameter) were attached to many carbonates and bacterial mats, which covered most sediments surrounding carbonates. We observed curtains of methane bubbles escaping from the center of orange microbial mats in December 2012 but saw no bubbling in May 2013.

The seep periphery is a halo 10–20 m wide that surrounds the seep center (Figure 2.1B). Clam beds (Vesicomyidae: *Calyptogena pacifica*, *Phreagena "Calyptogena" kilmeri*, and *Archivesica "Vesicomya" gigas*) occurred on all sides of the seep periphery, but were less dense to the northeast, which was characterized by carbonate rocks (< 10 cm to ~1 m) to which vestimentiferan tubeworms (*Escarpia spicata* and *Lamellibrachia barhami*, Figure 2.1G) and predatory sponges (*Asbestopluma rickettsi*) were attached. Substrates in this area were often covered with fine, white, filamentous bacteria, arborescent foraminifera, and folliculinid ciliates. Even further to the northeast beyond the seep periphery, sediments contained many inactive carbonates and dead clam shells. In contrast, the seep periphery to the south and west consisted of soft sediments with dense clam beds, extensive shell hash, patchy microbial mats (orange, yellow, red, and

white; Figure 2.1I), and darker sediments with polychaete tubes (beds of ampharetid polychaetes and the frenulate siboglinid *Siboglinum veleronis*). No carbonates were observed in the sediments southwest of the seep, but tubes of the large, agglutinated foraminiferan *Bathysiphon filiformis* were observed (Figure 2.1C) at every multicorer location, and often were the dominant surface feature.

Sediment macrofaunal assemblage

Proximity to the seep influenced macrofaunal densities, which increased at sites closer to the center of the seep (Figure 2.2; ANOVA, $F_{2,17} = 5.15$, $p = 0.018$). The closest site, C_{32}, had significantly higher faunal density (8888 ± 717 ind. m^{-2}) than A_{173}, furthest from the seep (6113 ± 534; Tukey HSD, $p = 0.017$). The densities of Annelida, Mollusca, and dorvilleid polychaetes were significantly higher at locations closer to the seep (Tukey HSD, $\alpha < 0.05$, Figure 2.2), but several other abundant groups did not vary among locations (Crustacea, Ophiuroidea, and the polychaetes Paraonidae and Ampharetidae; Figure 2.2). Both the highest and lowest faunal densities observed came from locations within centimeters of each other in active seep sediments: mean macrofaunal densities were 909 individuals m^{-2} in the bacterial mat E_{mat}, and 16,240 individuals m^{-2} in an adjacent ampharetid bed (D_{amph}).

Sediments from D_{amph} and the multicores were dominated by Annelida (66% and 78% of all macrofauna, respectively), especially Paraonidae (19–24% of individuals at each Location), Cirratulidae (4–9%), and Ampharetidae (6–8%) (Figure 2.3). Ampharetids made up 65% of the individuals in the push cores in the ampharetid bed,

and trochid gastropods (12%) and cuspidarid bivalves (6%) were also common. However, these taxa did not appear in the adjacent microbial mat push cores, where the macrofauna included juvenile vesicomyid clams and a polynoid and hesionid polychaete.

Carbonate rock assemblage

The density of macrofauna on carbonate rocks, 360 ± 46 individuals m^{-2}, was over an order of magnitude lower than in sediments. Carbonates contained a distinct assemblage that was dominated by gastropods (45%, Figure 2.3). Carbonate gastropods included mainly *Provanna laevis* (137.9 ± 35.2 ind. m^{-2}) and *Pyropelta corymba* (18.2 ± 17 ind. m^{-2}), which typically occurred on the shells of *P. laevis*. Gastropods were significantly more common on Rocks 1–3 from the seep center (255 ± 26 gastropods m^{-2}) than Rocks 4–6 from the seep periphery (66 ± 14 gastropods m^{-2}, $t_4 = 6.15$, $p = 0.003$). Hydroids were common on the carbonates collected from the seep periphery (7339 hydroids m^{-2}) but absent on those from the seep center. Annelids were much less abundant on carbonates than in sediment cores, but several families represented a higher proportion of total macrofauna on carbonates than they did in sediments away from the seep (Dorvilleidae, 5% vs 4%; Polynoidae, 2.7% vs 0.6%; Syllidae 4.7% vs 0.9%).

Diversity among microhabitats

Rarefaction curves indicate that diversity was very similar for multicores at different locations, with ES_{100} = 25.3–26.3 (Figure 2.4, Table 2.3). Rarified diversity was much lower in the ampharetid bed (ES_{100} = 11.3) and the orange mat (ES_{100} = 4), in which only 7 individuals belonging to 4 species were recovered. Similarly, Shannon

diversity (H') and Pielou's evenness were highest for multicorer samples far from the seep (2.88–2.97; 0.81–0.86) and lowest for sediments at the seep center (1.35 & 0.54 for D_{amph}; indices for E_{mat} unreliable based on low sample size) (Table 2.3). For carbonates, rarefaction diversity (ES_{100} = 22.7) was slightly lower than for sediments, but much higher than diversity in the ampharetid bed and bacterial mat sediments (Figure 2.4). Diversity on carbonates was higher further from the seep center. Compared to the carbonates from the seep center, those from the seep periphery had slightly higher H' (2.48 vs. 2.14), J' (0.79 vs. 0.69), and ES_{100} (21.2 vs. 19.3).

Trophic sources and methane-derived carbon

Stable isotope signatures indicate widespread dependence of macrofauna on both chemosynthetic and photosynthetic primary production (Figure 2.5A, Tables 2 & 2.A2). For macrofauna, the range of $\delta^{13}C$ was -15 to -60‰, and the range for $\delta^{15}N$ was -9 to +19‰. Porewater methane from the Del Mar Seep has an average $\delta^{13}C$ of -59.9‰ and δD of -184.8‰ (Table 2.A1), which are indicative of biogenic methanogenesis via microbial CO_2 reduction (Whiticar 1999). Using a two-source mixing model, we estimate that at least ten seep taxa (five polychaetes, four gastropods, and an encrusting sponge) may depend on MDC indirectly for at least half their organic carbon (Table 2.2). The species with the lightest average $\delta^{13}C$ signatures were the patellogastropod limpet *Paralepetopsis* sp. ($\delta^{13}C$ = -53.5‰; MDC = 74–84%; Figure 2.1J), an oligochaete (-47.5‰; 57–69%), a white encrusting sponge (-47.2‰; 50–68%), the gastropods *Pyropelta* spp. (-46.4‰; 47–66%) and *Provanna laevis* (-41.4‰; 29–53%), and the polychaetes *Nereis* sp. (-44.7‰; 41–62%; Figure 2.1J) and *Dorvillea* sp. (-40.7‰; 33–51%).

Faunal stable isotope signatures varied significantly by microhabitat ($\delta^{13}C$: ANOVA, $F_{4,406} = 72.3$, $p < 0.0001$; $\delta^{15}N$: $F_{4,245} = 5.09$, $p = 0.0006$). Mean $\delta^{13}C$ was lightest for macrofauna from carbonates (-35.7 ± 0.6‰) and heaviest (-22.2 ± 0.5‰) for those from sediments at A_{173} (Tukey HSD, $p < 0.05$, Figure 2.5B). Macrofauna on carbonates from different zones of the seep exhibited a disparity in isotopic signatures. Macrofauna from Rocks 1–3 at the seep center had significantly lighter $\delta^{13}C$ (-36.2 ± 1.0‰) and $\delta^{15}N$ (3.43 ± 0.64‰) than those from Rocks 4–6 at the periphery (-27.6 ± 1.1‰, 9.22 ± 0.69‰, respectively), about 5–10 m from the center of the seep ($\delta^{13}C$: $t_{97} = 5.75$; $\delta^{15}N$: $t_{97} = 6.13$; $p < 0.0001$, Figure 2.5B). $\delta^{13}C$ signatures for macrofauna varied among multicorer locations (ANOVA, $F_{2,178} = 3.70$, $p = 0.027$), as A_{173} furthest from the seep had an average $\delta^{13}C$ (-20.1 ± 0.8‰) that was heavier than B_{108} (-22.3 ± 0.4‰; Tukey HSD, $p = 0.03$), but was not significantly different from C_{32} (-22.9 ± 1.1‰; Tukey HSD, $p = 0.09$).

Demersal fish and epibenthic invertebrate densities

Dominant megafauna observed during the ROV survey included the longspine thornyhead *Sebastolobus altivelis*, the Pacific dover sole *Microstomus pacificus*, the lithodid crab *Paralomis verrilli*, and several hagfish *Eptatretus* sp. and zoarcid fish. *Sebastolobus altivelis* was more abundant (8.56 fish 100 m^{-2}) than *M. pacificus* (0.63 fish 100 m^{-2}) or *P. verrilli* (0.56 crabs 100 m^{-2}) (Figure 2.6). Although present in all zones around the seep, *S. altivelis* was not distributed randomly and was more likely to occur in the seep center or periphery than away from the seep (Chi-square, $\chi_{test} = 11.58$, $\chi_{crit} = 5.99$, 2 df). The mean (±1 SD) length of the 122 *S. altivelis* observed and measurable in

ROV footage was 162 ± 61 mm (Figures 6 & S1). The habitat zones did not have an effect on size of *S. altivelis* (ANOVA, $F_{2,119} = 1.73$, $p = 0.18$) although fish in the seep center (190 ± 60 mm, N = 13) were on average larger than those in the seep periphery (156 ± 51 mm, N = 41) and away from the seep (160 ± 66 mm, N = 68) (Figure 2.7). Within the seep center and periphery, thornyheads often occurred in association with orange and white microbial mats (e.g. Figure 2.1E, H), carbonates, clam beds, and dead clam shells.

Discussion

Microhabitats and biogenic structure at the Del Mar Methane Seep

The Del Mar Methane Seep interacts with the background continental margin community to create a biomass hotspot with distinct microhabitats and multiple trophic pathways leading to higher trophic levels. The mix of habitats and taxa present reflect the influence of methane, depth, the OMZ, and bathymetry in a highly productive, upwelling margin. In sediments away from the seep, fields of *Bathysiphon filiformis* and *Siboglinum veleronis* contribute structural heterogeneity and host a relatively diverse suite of soft-sediment macrofauna. Largely typical of the regional OMZ, these are not obligate seep taxa. *Bathysiphon* spp. live in high densities (> 100 m^{-2}) in other bathyal environments with high organic flux, such as the Atlantic coast of North America and submarine canyons in New Zealand (Gooday et al. 1992; De Leo et al. 2010). High surface productivity in the California Current, the location of the Del Mar Seep at the edge of the San Diego Trough, plus seep productivity may combine to create highly organic-rich sediments (15-16% total organic matter, Table 2.A1), leading to high densities of

Bathysiphon filiformis tubes. While we did not quantify *Bathysiphon* tubes across all samples, multiple agglutinated tubes were recovered from all multicores, and ROV observations suggest densities may surpass 200–300 individuals m^{-2} (e.g. Figure 2.1C). Frenulate tubeworms, which require sulfide or (in at least one case) methane for their endosymbionts, are common sediment inhabitants in many chemosynthetic settings (e.g. Sahling et al. 2005; Levin and Mendoza 2007; Hilário and Cunha 2008; Levin et al. 2012), but other settings such as the San Diego Trough contain organic-rich, reducing sediments that can also support frenulates (Hartman 1961; Hilário et al. 2011). While *Siboglinum veleronis* is the dominant symbiont-bearing metazoan away from the seep, its density declines near the seep as sulfide levels increase and seep endemic taxa become more abundant, a pattern also observed at the Håkon Mosby mud volcano in the Arctic (Decker et al. 2012).

Around the edge of the seep, a patchwork of clam beds, microbial mats, and polychaete tubes was associated with chemosynthetic production and typical seep taxa: bacteria resembling *Beggiatoa*, *Thioploca*, and *Thiomargarita*; dorvilleid (*Dorvillea* sp., *Ophryotrocha* sp.), ampharetid, and polynoid (*Bathykurila* n. sp.; Katz & Rouse, In prep) polychaetes; and at least three species of vesicomyid clams *Calyptogena pacifica*, *Phreagena kilmeri*, and *Archivesica gigas*. This is a typical assemblage at other Northeast Pacific seeps (Barry et al. 1996; Sahling et al. 2002; Levin et al. 2003). Researchers have hypothesized that evolutionary radiations in these habitats are related to reliance on high sulfide flux and partitioning of microhabitat (Barry et al. 1997) and microbial diets (Levin et al. 2013). *C. pacifica* and *P. kilmeri* in particular have been observed at many

California and Oregon seeps, where their differing sulfide affinities and growth rates contribute to bulls-eye patterns around bacterial mats that we observed at the Del Mar Seep (Barry et al. 1996; Barry and Kochevar 1998). The nearby Santa Monica mound, however, is dominated by a smaller vesicomyid (*Ectenogena elongata*) that we did not find, perhaps because 1020 m was too deep for this OMZ specialist.

Small patches of dark sediments were common in the seep periphery, and two push cores at D_{amph} confirmed dominance by ampharetids (65% of all macrofauna). Our measured macrofaunal density for this habitat was about 16,000 individuals m^{-2}, which is much less than the > 50,000 macrofauna m^{-2} described by Thurber et al. (2010; 2013) off New Zealand. However, at New Zealand seeps, tube-building ampharetids engineer sediment habitat via bioirrigation and consume aerobic methanotrophic bacteria (Thurber et al. 2013). Their occurrence at the Del Mar Seep and Hydrate Ridge, Oregon (LAL, A Thurber, personal observations) raises the possibility that macrofauna have similar biogeochemical cycling roles at seeps along the Northeast Pacific continental margin.

Carbonates at the center of the seep contain a faunal assemblage distinct from surrounding sediments. Fine-scale chemical gradients may also lead to differentiation within carbonate assemblages, as rocks near the seep center were covered with the gastropods *Provanna laevis* and *Pyropelta corymba* and orange or white bacterial mat (Figure 2.1F), while rocks several meters away from the center of the seep had a finer covering of bacterial filaments, arborescent foraminifera, hydroids, and different species of sponges. The dominance of gastropods on seep carbonate is not surprising, as they are commonly associated with carbonates in active seep settings on which they graze bacteria

(Ritt et al. 2010; Levin et al. 2012). An Elachisinidae gastropod (*Laeviphitus verduini*) and a Mytilidae bivalve made up 75–90% of the macrofauna on carbonates inspected by Ritt *et al.* (2010) at a Mediterranean seep, but mytilids do not appear at the Del Mar Seep. The vestimentiferans *Lamellibrachia barhami* and *Escarpia spicata* are very common at the San Clemente seeps (Bernardino and Smith 2010), but only occurred in small clusters at the Del Mar Seep with their roots penetrating carbonate rocks (Figure 2.1G). We hypothesize both the absence of mytilids and the scarcity of vestimentiferans are related to the oxygen environment, as they are also absent from other Pacific seeps occurring in the OMZ (Levin et al. 2010). The presence of these long-lived ecosystem engineers, however scarce, indicates persistence over decades or longer (Cordes et al. 2005), and the Del Mar Seep could play a role in regional connectivity patterns.

Community structure of macrofauna

Sediments surrounding the Del Mar Seep exhibited an increase in animal densities closer to the seep center, without dramatic change in faunal composition. We found higher densities of polychaetes 32 m from the seep compared to 108 m away, and higher densities of molluscs 32 and 108 m from the seep than 173 m away (Figure 2.2). While proximity to the seep seemed to be associated with increases in the abundance of several macrofaunal groups, it did not contribute to a reduction in abundance of any taxa. These results support a role for the Del Mar Seep in providing trophic subsidies to the surrounding margin ecosystem. The sediments at the center of the seep (e.g. D_{amph} and E_{mat}) are physiologically stressful to all but seep endemics, but at tens to hundreds of

meters away from the seep, macrofauna can benefit from an *in-situ* source of production while avoiding high levels of hydrogen sulfide.

We compared macrofaunal composition and diversity in sediments from the Del Mar Methane Seep to sediment macrofaunal communities from other cold seeps (Bernardino et al. 2012). Bernardino *et al.* (2012) assessed sediment core data from different microhabitats, including background sediments. Our pooled data for the multicorer locations exhibited rarefaction diversity ($ES_{100} = 26$) nearly as high as for any of the seep sediment microhabitats examined in Bernardino *et al.* (2012). The macrofaunal community in background sediments from the Del Mar Seep cluster with those from northern California (Eel River) and Oregon (Hydrate Ridge) at the 65% similarity level, and they cluster with the active microhabitats (clam beds, bacterial mats) from those same seeps at the 55% similarity level. San Clemente seep background sediments, on the other hand, are only 45% similar to the Del Mar Seep background sediments. Despite the geographical proximity between the San Clemente and Del Mar Seeps, depth and oxygen gradients have even stronger influences on these margin communities (Levin et al. 2010). Where the Del Mar Seep has bottom water oxygen of 0.4 mL L^{-1} and is near the lower edge of the OMZ, San Clemente has a depth of 1800 m, much below the OMZ, and is relatively well oxygenated (Bernardino and Smith 2010). This is important for considering biodiversity patterns, since methane seeps along the same margin at multiple depths are likely to have greater beta diversity and potentially different types of ecosystem functions than multiple seeps along a single depth contour.

Chemosynthetic contribution to macrofaunal nutrition

We observed isotopically-light carbon signatures in sediments over 100 m from any visible sign of chemosynthetic activity. The average $\delta^{13}C$ for the macrofaunal community at the location B_{108} was -22.3‰ compared to -20.1‰ at A_{173}. This modest difference in carbon signatures suggests that *in-situ* chemosynthetic production is not only leading to higher biomass within the seep setting, but it is being exported into adjacent sediments, which likely explains the increase in macrofaunal densities close to the seep. Macrofauna at A_{173} generally had stable isotope values reflecting photosynthetic production ($\delta^{13}C$ = -16 to -23‰; except for bacterial filaments (-24.5‰), one dorvilleid (-24.7‰), and an agglutinated foraminiferan (-25.6‰) (sediment organic carbon $\delta^{13}C$ = -21.1‰). Given our $\delta^{13}C$ signatures for potential end members ($\delta^{13}C_{POC}$ = -21.2‰; $\delta^{13}C_{POC}$ = -59.9‰), the community at B_{108} could be receiving 0–2.9% of its carbon via methanotrophy. Certain taxa at B_{108} had $\delta^{13}C$ signatures implicating chemosynthetic sources of carbon. Likely thiotrophs, white bacterial filaments from sediments ($\delta^{13}C$ = — -25.3 to -32.3‰) and symbiont-bearing species such as *Siboglinum veleronis* (-22.7 to -39.3‰) and a vesicomyid clam (-36.0‰) were able to access sulfide, despite living at a site that might ordinarily be considered “background sediments”. Some chemosynthetic fauna typically observed in dense aggregations at seeps may be capable of living at many non-seep sites throughout the OMZ, as long as the sulfide-oxygen interface is shallow enough. Frenulates have been observed at other stations in the San Diego Trough (1000-1200 m, below the OMZ) (Hartman 1961), and we recovered a solemyid bivalve (*Acharax* sp.), which harbors sulfide oxidizers, from other stations within the OMZ. As

hotspots of chemosynthetic productivity, both symbiont-bearing and heterotrophic fauna at methane seeps may represent source populations whose larvae enhance metapopulation connectivity regionally for a broader range of margin ecosystems.

Methane-derived carbon (MDC) is clearly being incorporated into the macrofaunal food web in the areas of the Del Mar Seep that are most active – particularly carbonate rocks covered in microbial mats. Sulfide oxidation often leads to $\delta^{13}C$ signatures between -27 and -37‰, but approximately half the gastropods and one-third the polychaetes sampled have $\delta^{13}C$ signatures between -40 and -60‰ (Figure 2.5A). These isotopic signatures indicate by incorporating MDC into their tissues, seep macrofauna contribute to the biological filter that sequesters inorganic carbon that could otherwise enter the atmosphere. In the future, rate studies with labeled isotopes could help determine how quickly MDC may be taken up by seep heterotrophs.

Enhanced abundances of fishery species at the Del Mar Methane Seep

In addition to macrofauna, demersal fish and invertebrates are also concentrated at the seep. Densities of the longspine thornyhead, Pacific dover sole, and lithodid crabs, species often targeted by bottom fisheries, increased in the seep center and periphery relative to background. The Del Mar Seep had densities of 0.125 *S. altivelis* m^{-2} at its center and periphery combined, equivalent to about 125,000 fish per km^2. These densities of thornyhead greatly surpass those reported by trawl surveys at similar depths on the central California and Oregon slope (3000–6000 fish km^{-2}; Jacobson and Vetter 1996) though a video camera sled study in central Oregon observed densities nearly as high as ours (Lauth et al. 2004). As *S. altivelis* and the shortspine thornyhead *S. alascanus* are

important groundfish in the United States and Canadian Pacific fisheries (Stephens and Taylor 2013), we suggest that methane seeps may provide habitat that enhances the productivity of these species, and thus provide an important ecosystem service. In the United States commercial take for thornyhead has declined from over 6000 metric tons (mt) at its peak in the early 1990s to less than 1000 mt, though most exploitation occurs north of Point Conception and at depths shallower than 800 m (Stephens and Taylor 2013). The finding that seeps might provide ecosystem services via fisheries production has greater relevance since California and Oregon host multiple seeps at depths relative to *S. altivelis* distribution (depth range of 600–1700 m; Jacobson and Vetter 1996), and seeps may be regionally abundant (Paull et al. 2005; 2012).

Sebastolobus altivelis settles at a length of about 55 mm, matures at 20 years around 170–190 mm, and reaches a maximum length of about 300 mm, which encompasses the size range of fish we observed (52–350 mm) (Moser 1974; Jacobson and Vetter 1996). Thus, we can speculate on what advantage a methane seep might lend to individuals of any age. Certainly, the structural presence of carbonate may offer a protective habitat, but since thornyhead were about four times less numerous at a reference site with carbonate 1 km away, it seems some aspect of the active seeping fluids might play a role in enhancing or aggregating *S. altivelis*. This species is carnivorous on ophiuroids and other small benthic invertebrates (COSEWIC 2007), so the seep contains an abundance of potential prey items. Thornyheads are OMZ specialists with low metabolic needs, and at 1000 m, biological maintenance may require a meal of just 5% an individual's body mass every 150 days (Vetter and Lynn 1997). A relatively

small, productive habitat such as the Del Mar Seep could thus support a high density of *S. altivelis*, and perhaps lead to enhanced growth rates compared to food-poor habitats. Another possibility is that the chemical environment of the seep benefits *S. altivelis*, perhaps by reducing parasite loads or providing a refuge from predation.

Conclusions

The continental margins are exposed to ever-increasing human activity, be it industrial (oil drilling, gas and minerals exploration, trawling), commercial (shipping traffic, cable laying), or recreational (fishing, whale-watching), and it is unclear how biodiversity and other ecosystem functions will respond in the long term (Levin and Sibuet 2012). In addition, the effects of climate change, which specifically impact margins through deoxygenation, pH reduction, and altered productivity patterns, are expected to increase through the 21st century, potentially limiting the level of ecosystem services the ocean provides humans (Mora et al. 2013). This study highlights an important gap in continental margin research: namely, the lack of adequate measures or even descriptions of the ecosystem services methane seeps and other deep-sea chemosynthetic ecosystems provide. Given the seeming ubiquity of cold seeps along all margins and the rapid discovery of new sites (Levin et al. 2012; Brothers et al. 2013), we cannot yet estimate global ecosystem services from seeps. However, the mounting evidence from sites such as the Del Mar Seep, just 50 km from a densely-populated urban area, suggests that managers and policymakers implementing ecosystem-based management practices would be wise to account for the value cold seeps provide. Future research that quantifies trophic subsidies from methane seeps, documents relationships

between fishery species and seep ecosystems, clearly defines links between seep diversity and ecosystem function, or explores the role of seeps as sources of larvae to surrounding habitats will contribute to more effective management of our continental margins.

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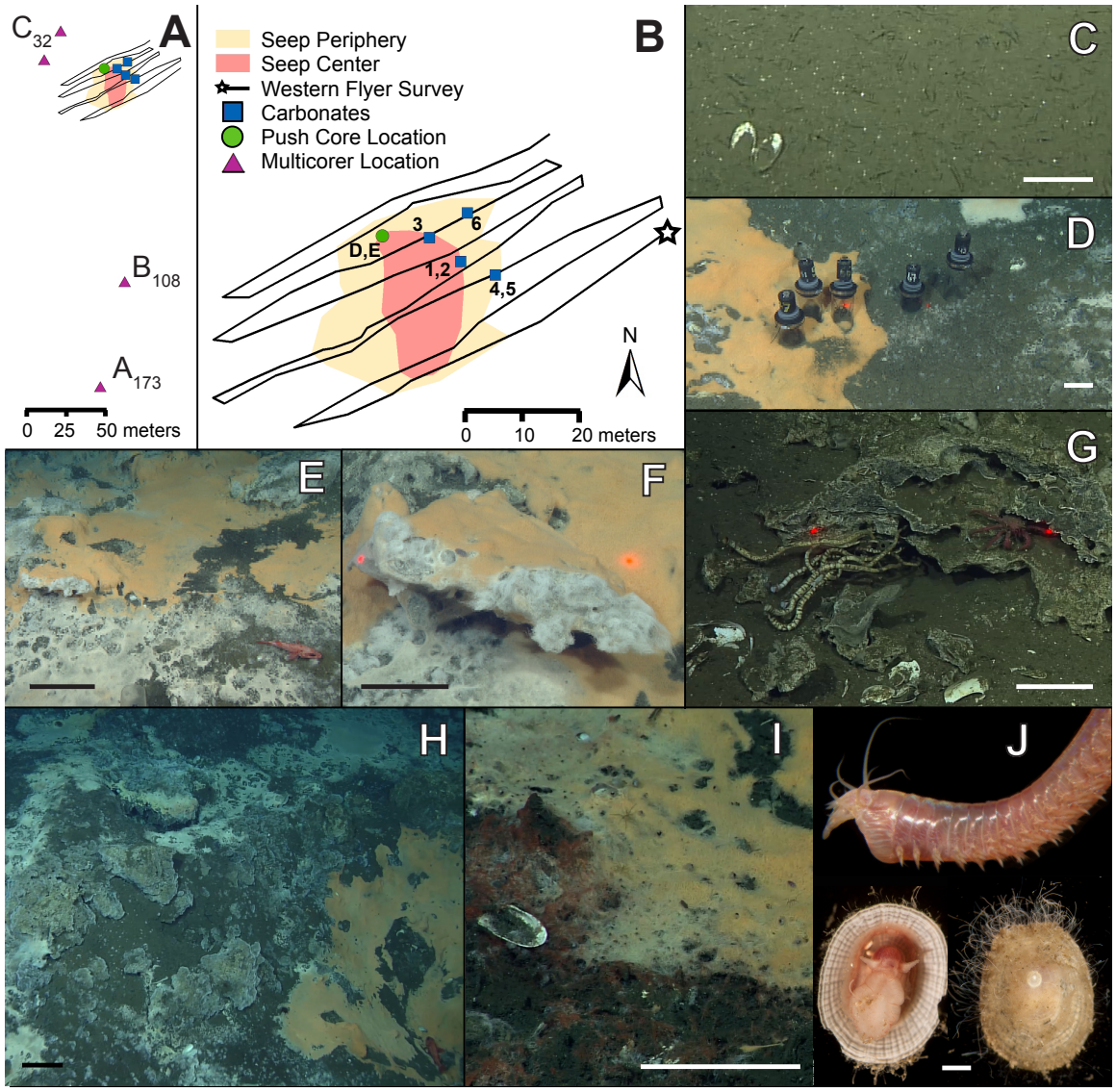

Figure 2.1. A) Multicorer locations (A_{173}, B_{108}, C_{32}) relative to the Del Mar Seep; **B)** ROV *Doc Ricketts* survey line and locations of push cores and carbonates (R1–6) collected; **C)** Sediments away from seep with *Bathysiphon filiformis* tubes; **D)** Push cores in ampharetid bed (D_{amph}) and orange microbial mat (E_{mat}); **E)** Seep center with *Sebastolobus altivelis*; **F)** Carbonate R3, also visible on the left side of panel 1e; **G)** Small clump of *Lamellibrachia* sp. and a juvenile *Paralomis virrilli* found inside an authigenic carbonate after outer piece of rock was removed; **H)** seep center with carbonate boulders and site of possible past hydrate dissociations; **I)** diversity of microhabitats at edge of seep center; **J)** two of the main consumers of methane-derived carbon are *Nereis* sp. (top) and *Paralepetopsis* sp. (bottom). All scale bars are 10cm, except E) and H) are 30 cm, and J) is approximately 1mm. Photo credits: MBARI except for J) (B Grupe).

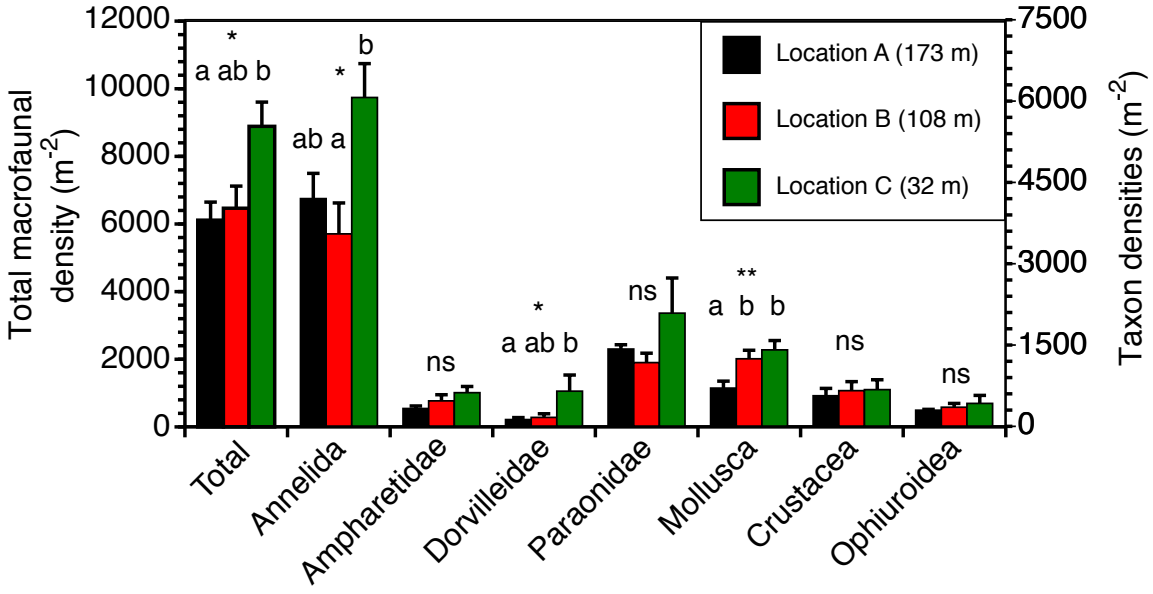

Figure 2.2. Macrofaunal densities vary with proximity to the seep. Note y-axis at left applies to total macrofauna, while y-axis at right applies to individual taxa. Shared letters indicate a lack of significance (Tukey HSD, * $\alpha < 0.05$, ** $\alpha < 0.001$). Annelida, Dorvilleidae, Paraonidae, and Ophiuroidea were $\log_{(e)}$-transformed to conform to assumptions of normality or equal variances.

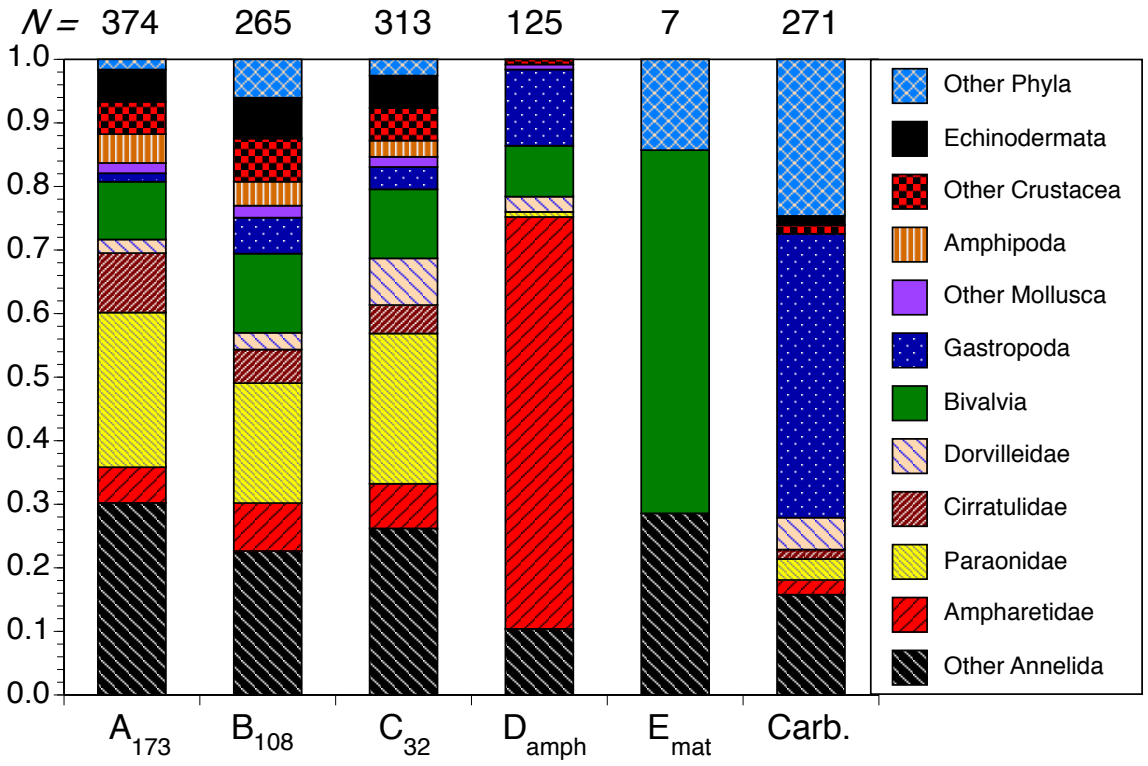

Figure 2.3. Faunal composition for sediments and carbonates. Number of total individuals displayed above each bar.

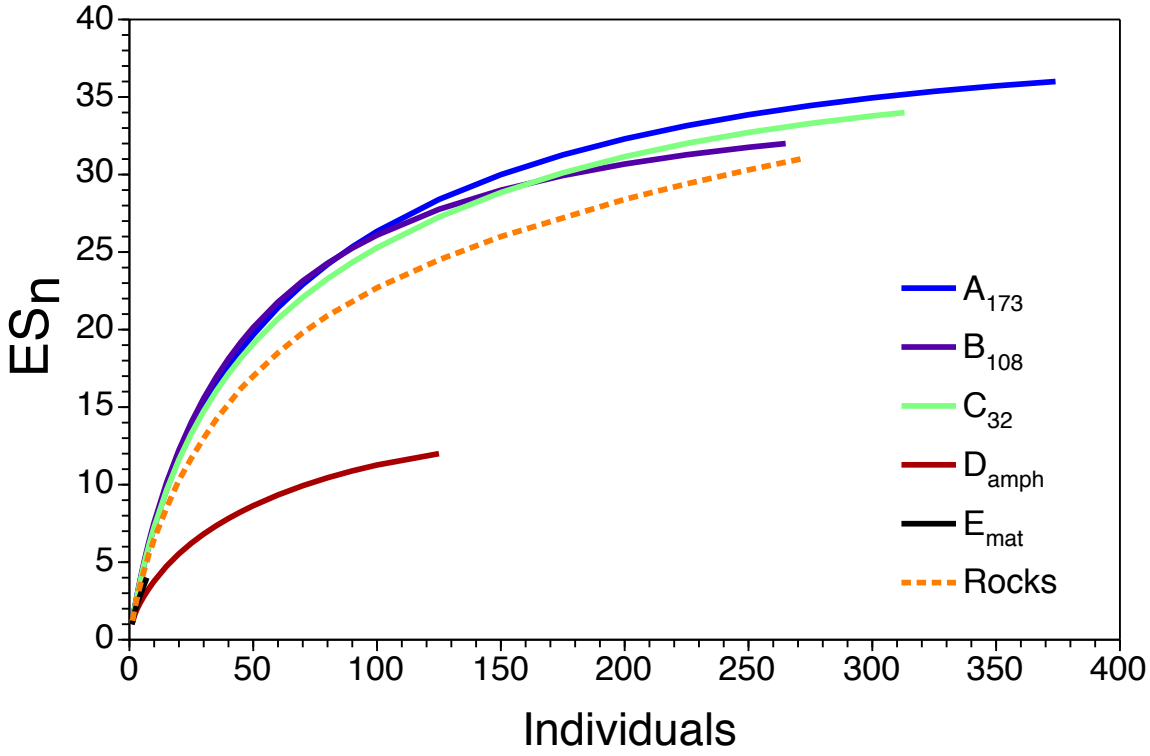

Figure 2.4. Influence of microhabitat and proximity to seep on rarefaction of taxonomic richness. Macrofauna from multicores and push cores are pooled by location, and all carbonate macrofauna are included in rocks. Note E_{mat} is particularly short as we found only 7 individuals in 2 cores.

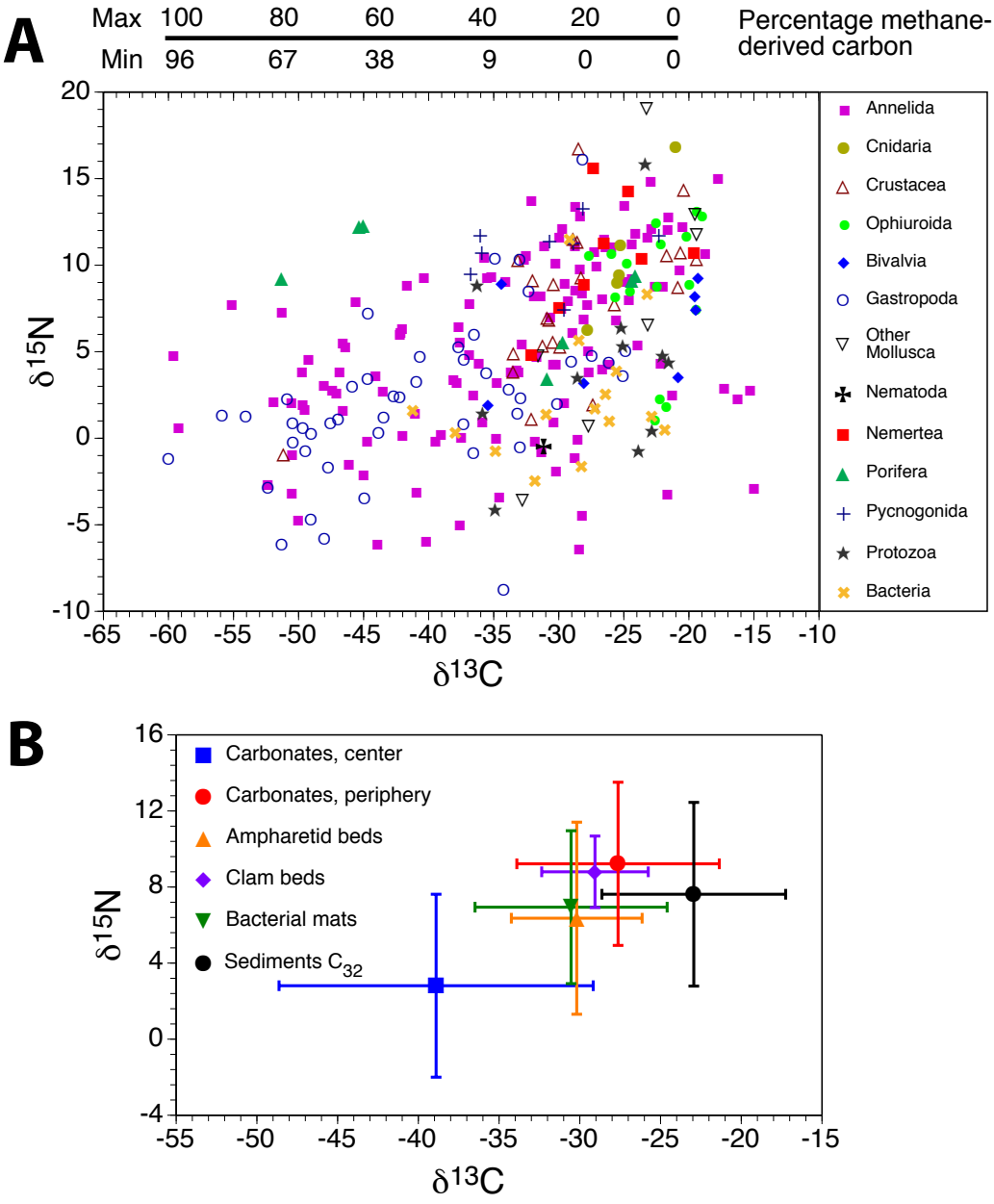

Figure 2.5. **A)** Stable isotope signatures of macrofauna, protozoa, and bacteria at the Del Mar Seep from all microhabitats. Minimum and maximum estimates of methane-derived carbon are shown above the x-axis, where $\delta^{13}CH_4$ and $\delta^{13}C_{POC}$ are the end points for the maximum MDC, and $\delta^{13}CH_4$ and $\delta^{13}C_{SOB}$ are the end points for minimum MDC; **B)** Means by microhabitat for data in a). "Carbonates, center" are Rocks 1–3, while "Carbonates, periphery" include Rocks 4–6. For sediments away from the seep, only C_{32} is shown because $\delta^{15}N$ data were analyzed improperly for samples from A_{173} and B_{108}. The mean $\delta^{13}C$ signature of macrofauna at A_{173} (-20.1 ± 2.5) is greater than that at B_{108} (-22.2 ± 5.1) (statistics in text), but neither are significantly different from C_{32}.

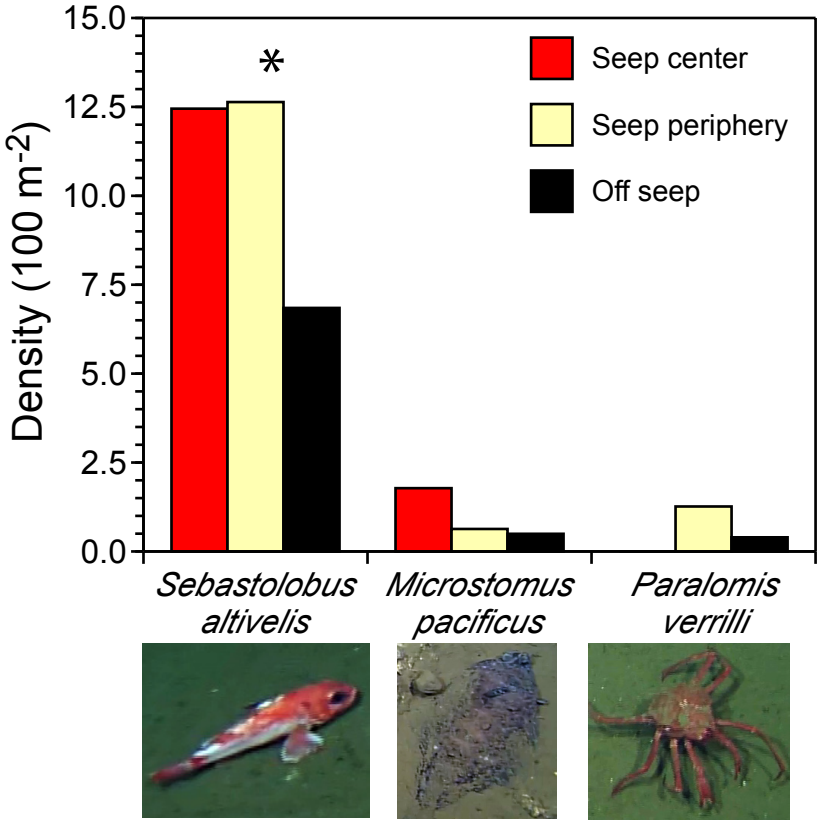

Figure 2.6. Densities of groundfish and crabs observed in different habitat zones of the Del Mar Seep: Seep center (112 m^2 surveyed), seep periphery (317 m^2 surveyed), and off seep (1008 m^2 surveyed). * represents non-randomly distributed individuals with respect to proportions of habitat zones ($p < 0.05$). Photo credits: MBARI.

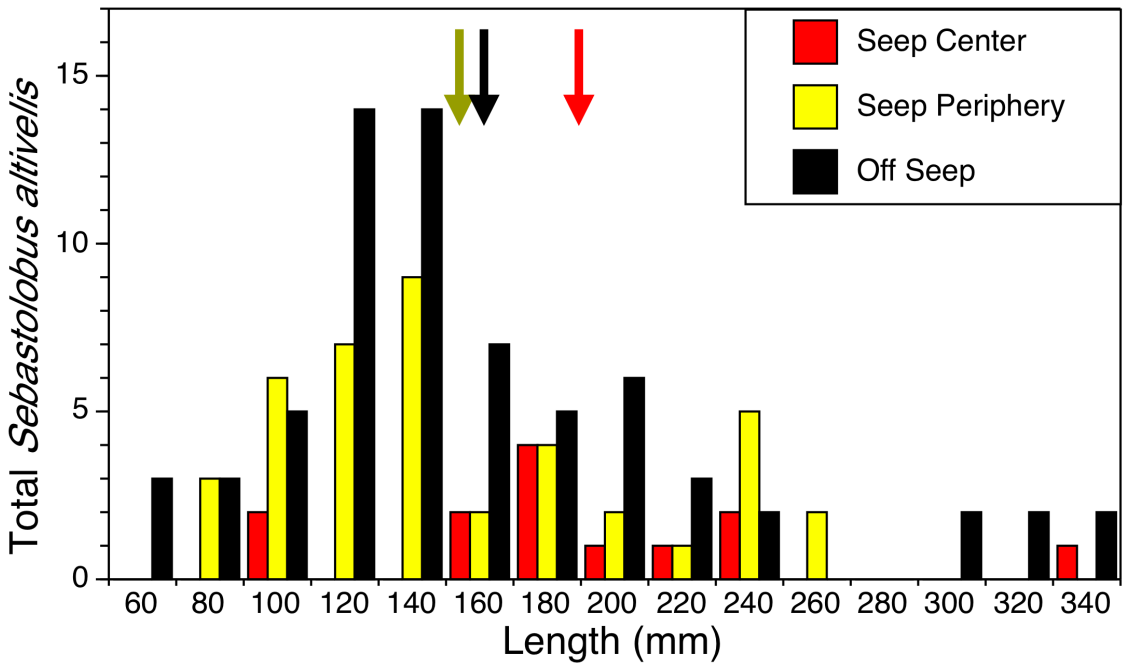

Figure 2.7. Size structure of *Sebastolobus altivelis* from different habitat zones of the Del Mar Seep: Seep center (112 m^2 surveyed), seep periphery (317 m^2 surveyed), and off seep (1008 m^2 surveyed). Overall mean (± SD) fish length was 162 ± 61 mm, and arrows indicate the approximate mean lengths for each habitat.

Table 2.1. Sampling locations and habitats at the Del Mar Methane Seep

Date (dd/mm/yr)	Sampling Gear	Depth (m)	Distance (m) to seep (and Location)	Habitat sampled	Latitude (N)	Longitude (W)	Stable isotope data?	Macrofaunal density
R/V *Melville*								
08/07/12	Multicorer	1038[a]	173 m (A)	*Bathysiphon* tubes	32°54.154'	117°46.945'	Yes	Yes - 3 cores
10/07/12	Multicorer	1038[a]	173 m (A)	*Bathysiphon* tubes	"	"		Yes - 3 cores
10/07/12	Multicorer	1038[a]	173 m (A)	*Bathysiphon* tubes	"	"		Yes - 3 cores
10/07/12	Multicorer	1030[a]	108 m (B)	*Bathysiphon* tubes, Frenulates	32°54.189'	117°46.937'	Yes	Yes - 3 cores
10/07/12	Multicorer	1030[a]	108 m (B)	*Bathysiphon* tubes, Frenulates	"	"	Yes	Yes - 3 cores
R/V *Melville* with Scripps Institution of Oceanography ROV *Triton*								
12/12/12	Multicorer	1026[a]	32 m (C)	*Bathysiphon* tubes, polychaete tubes	32°54.274'	117°46.959'	Yes	Yes - 3 cores
12/12/12	Multicorer	1026[a]	32 m (C)	Polychaete tubes	32°54.264'	117°46.964'	Yes	Yes - 2 cores
11/12/12	Grab	1020	0 m	Carbonate rocks (3)	[b]		Yes	
11/12/12	Grab	1020	0 m	Vesicomyid clams	[b]		Yes	
13/12/12	Grab	1020	0 m	Carbonate rocks (6)	[b]		Yes	
13/12/12	Grab	1020	0 m	Vesicomyid clams	[b]		Yes	
13/12/12	Push Core	1020	0 m	Orange microbial mat	[b]		Yes	
R/V *Western Flyer* with MBARI ROV *Doc Ricketts*								
19/05/13	Push Core	1020	0 m (D)	Ampharetid bed adjacent to microbial mat	32°54.261'	117°46.944'		Yes; 2 cores
19/05/13	Suction	1020	0 m	Ampharetid bed adjacent to microbial mat	32°54.263'	117°46.936'	Yes	
19/05/13	Push Core	1020	0 m (E)	Orange microbial mat	32°54.261'	117°46.944'		Yes; 2 cores
19/05/13	Suction	1020	0 m	Orange microbial mat	"	"	Yes	
19/05/13	Push Core	1020	3 m	Muddy tube field	32°54.256'	117°46.946'		Yes
19/05/13	Suction	1020	3 m	Muddy tube field	"	"	Yes	
19/05/13	Suction	1020	0 m	White microbial mat	32°54.261'	117°46.944'	Yes	
19/05/13	Suction	1021	11 m	*Bathysiphon* tubes	32°54.242'	117°46.942'	Yes	
19/05/13	Grab R1	1020	0 m	Carbonate covered in white bacterial mat	32°54.259'	117°46.937'	Yes	Yes
19/05/13	Grab R2	1020	0 m	"	"	"	Yes	Yes
19/05/13	Grab R3	1020	0 m	Carbonate covered in orange bacterial mat	32°54.261'	117°46.940'	Yes	Yes
19/05/13	Grab R4	1020	5 m	Carbonate with white, microbial filaments and arborescent foraminifera	32°54.257'	117°46.934'	Yes	Yes
19/05/13	Grab R5	1020	5 m	"	"	"	Yes	Yes
19/05/13	Grab R6	1020	6 m	"	"	"	Yes	Yes
19/05/13	Grab	1020	2 m	Carbonate covered in red bacterial mat	32°54.253'	117°46.935'	Yes	
19/05/13	Grab	1020	3 m	Tubeworms	32°54.259'	117°46.934'	Yes	
19/05/13	Scoop	1020	14 m	Clams	32°54.267'	117°46.934'	Yes	

[a] Depth measurements at multicorer Locations were less precise than depth data associated with ROVs and likely were several meters shallower;

[b] GPS location associated with ROV *Triton* was only accurate within ~25 meters, so we cannot provide a precise location for these collections; rocks were all collected from the seep center or periphery, orange microbial mat core was taken from the seep center, and clams were collected in the seep periphery.

Table 2.2. Stable isotope signatures of macrofauna from all Del Mar Seep microhabitats. Mean and standard deviation (SD) of $\delta^{13}C$ and $\delta^{15}N$ per taxa, minimum and maximum proportion of methane-derived carbon (MDC), and total individuals (N). Macrofauna from sediments away from the seep center and periphery are not included except for *Siboglinum veleronis*. For comparison with other chemosynthetic taxa. *Note: Table 2.A2 contains similar data except macrofauna collected with multicorer in sediments away from the seep are included.*

	$\delta^{13}C$		$\delta^{15}N$		MDC Percentage		
Taxonomic Group / Species	Mean	SD	Mean	SD	Min	Max	N
Annelida	-34.5	10.0	5.0	5.1	17%	35%	124
Ampharetidae	-34.6	5.5	4.1	3.9	9%	35%	19
Capitellidae	-24.5		9.2		0%	9%	1
Cirratulidae	-31.7	8.1	7.6	3.2	9%	27%	6
Dorvilleidae	-34.6	8.5	-0.5	4.2	15%	35%	19
Dorvillea sp.	-40.7	10.8	-2.2	3.6	33%	51%	6
Ophryotrocha sp.	-32.4	5.4	-0.4	3.6	6%	29%	9
Lacydoniidae	-24.8		14.2		0%	9%	1
Lumbrineridae	-28.4	6.6	9.2	4.1	4%	19%	4
Maldanidae	-37.5	12.7	7.5	3.2	24%	43%	2
Nephtyidae	-34.2		9.0		1%	34%	1
Nereidae	-44.7	7.5	2.5	4.1	41%	62%	16
Oligochaeta	-47.5	12.6	4.1	2.5	57%	69%	5
Orbiniidae	-32.6		10.5		0%	30%	1
Paraonidae	-23.7	2.0	10.5	1.6	0%	7%	6
Phyllodocidae	-21.7		12.0		0%	1%	1
Polynoidae	-29.6	3.9	8.6	3.7	1%	22%	8
Siboglinidae	-16.1	1.0	1.2	2.8	0%	0%	4
Escarpia sp.	-15.1		-3.0		0%	0%	1
Lamellibrachia sp.	-16.4	1.0	2.6	0.3	0%	0%	3
Siboglinum sp.*	-31.9	4.0	6.8	5.2	5%	29%	14*
Sphaerodoridae	-29.1	0.4	10.6	3.8	0%	21%	2
Spionidae	-38.8	11.1	6.6	3.7	28%	46%	5
Syllidae	-29.6	7.1	8.7	4.5	5%	22%	9
Terebellidae	-40.0	6.8	4.4	3.9	26%	49%	11
Trichobranchidae	-50.6		-1.0		63%	77%	1
Chelicerata	-31.5	4.8	10.7	1.7	3%	27%	9
Cnidaria	-25.0	2.5	10.5	3.9	0%	10%	5
Anthozoa	-21.0		16.8		0%	0%	1
Hydrozoa	-26.0	1.2	9.0	2.0	0%	13%	4
Crustacea	-29.6	6.8	7.7	4.0	3%	22%	20
Amphipoda	-31.8	6.2	6.7	3.7	4%	28%	15
Decapoda	-25.7		7.7		0%	12%	1
Euphausiida	-20.5	1.6	10.5	0.2	0%	1%	2
Tanaidacea	-24.6	5.4	12.7	5.7	0%	9%	2
Ophiuroida	-23.8	2.8	10.0	1.7	0%	7%	9
Mollusca	-39.5	9.8	1.8	4.0	28%	48%	52
Aplacophoran	-30.6	2.7	0.6	4.2	0%	25%	3
Bivalvia	-28.1	7.3	4.2	2.8	3%	19%	5
Protobranchia	-22.8	4.6	5.0	2.8	0%	6%	3
Vesicomyidae	-35.5	5.4	1.1	5.0	8%	39%	2
Gastropoda	-41.3	9.1	1.7	4.1	32%	52%	45
Astyris permodesta	-32.3		8.5		0%	29%	1
Cataegis sp.	-42.2		2.3		31%	55%	1
Hyalogyrina sp.	-53.5	7.9	2.4	4.3	74%	84%	3
Paralepetopsis sp.	-27.2	2.0	5.8	4.7	0%	16%	7
Provanna laevis	-41.4	7.4	1.5	3.4	29%	53%	15
Provanna lomana	-37.9	5.3	1.4	0.8	16%	44%	3
Pyropelta sp.	-46.4	4.7	-0.7	3.4	47%	66%	15
Nematoda	-31.1		-0.5		0%	26%	1
Nemertea	-26.5	3.9	10.4	3.5	0%	14%	8
Porifera	-35.8	11.1	8.8	3.3	21%	38%	7
Asbestopluma rickettsi	-27.2	3.5	6.9	2.9	0%	16%	4
Sponge, encrusting	-47.2	3.5	11.3	1.8	50%	68%	3
Sipuncula	-26.9		5.5		0%	15%	1
Protozoa	-28.1	6.1	5.1	5.4	2%	18%	9
Agglutinated foraminifera	-23.4	2.6	5.4	1.4	0%	6%	2
Sediment foraminifera	-23.5	1.5	8.6	6.2	0%	6%	3
Folliculinid ciliates	-33.9	3.6	2.4	5.3	5%	33%	4
Filamentous Bacteria	-29.6	5.4	2.4	3.8	3%	22%	14
Bacteria on carbonates	-32.4	5.3	1.2	2.4	6%	29%	8
Bacteria on sediments	-27.3	1.7	3.7	5.7	0%	16%	4

Table 2.3. Diversity indices for macrofauna at all sampling locations at the Del Mar Seep. Rarefied number of species (ES_{20}) is averaged across samples (cores or rocks), and all indices are presented for pooled samples (species richness, ES_{20}, ES_{100}, H', and J')

	Sediments					Carbonates		
Sampling Location	Away from Seep			Ampharetid bed	Microbial mat	Seep center	Seep periphery	Total
	A_{173}	B_{108}	C_{32}	D_{amph}	E_{mat}	Rocks 1–3	Rocks 4–6	Rocks 1–6
Replicates averaged								
Mean ES_{20}	11.25	11.83	11.61	5.64	2.50	5.78	9.89	8.54
SD	0.68	0.84	1.28	0.39	0.71	3.67	2.16	3.76
SE	0.23	0.34	0.57	0.28	0.50	2.12	1.25	1.53
Replicates	9 multicores	6 multicores	5 multicores	2 push cores	2 push cores	3 rocks	3 rocks	6 rocks
Replicates pooled								
Species	36	32	34	12	4	22	23	31
Individuals	374	265	313	125	7	143	129	271
ES_{20}	11.72	12.23	11.62	5.54	4	8.50	9.90	10.26
ES_{100}	26.34	26.10	25.28	11.27	4	19.32	21.19	22.70
$H'(\log_e)$	2.92	2.97	2.88	1.35	1.15	2.14	2.48	2.59
J'	0.81	0.86	0.82	0.54	0.83	0.69	0.79	0.75

Table 2.A1. Hydrographic and sediment physical and chemical parameters at Del Mar methane seep. Hydrographic data were acquired with a CTD from ~10 meters above the seafloor. Sediment data were analyzed from multicores at off-seep Locations and a push core in an orange microbial mat.

Water column measurements										
Location	Month	Temperature (°C)	Salinity	pH_T	Dissolved oxygen ($mL \cdot L^{-1}$)					
A_{173}	July	4.03	34.48	7.58	0.41					
C_{32}	December	4.17	34.48	7.58	0.44					
Sediment chemistry										
Location	Month	Chlorophyll a ($\mu g \cdot g^{-1}$)	Phaeopigments ($\mu g \cdot g^{-1}$)	Chl a / Phaeo	Sand %	Silt-Clay %	$\delta^{13}CH_4$ ‰	Porewater CH_4 (mM) (SD)	δD ‰	Sulfide (mM)
A_{173}	July	13.59	20.3	0.67	2.04	97.96				
B_{108}	July	11.26	14.83	0.76	8.1	91.9				
C_{32}, drop 1	December	10.39	31.67	0.33			-59.41	22(0.2) – 36(0.8)	-185	NA [b]
C_{32}, drop 2	December							18(1.4) – 36(0.8)		0.02
Orange Mat [a]	December						-60.43	72.8 (3.1)		0.63

[a] This push core was collected from a similar orange microbial mat as the Location E samples, but cores were collected from different mats on different cruises. Seawater may have mixed into this core while still on the ROV, so CH_4 and sulfide concentrations should be interpreted as minimum values for the microbial mat.

[b] Sulfide concentrations were indistinguishable from 0 at all sediment horizons in this multicore.

Table 2.A2. Stable isotope signatures (mean and SD of $\delta^{13}C$ and $\delta^{15}N$) of macrofauna, protozoans, and bacteria by taxa, minimum and maximum methane-derived carbon (MDC), and total samples analyzed (Replicates). Samples are pooled across habitats for all multicores and ROV collections. Fewer replicates exist for $\delta^{15}N$ due to analysis errors for A_{173} and B_{108} samples.

	$\delta^{13}C$		$\delta^{15}N$		% MDC		Replicates	
Taxonomic Group / Species	Mean	SD	Mean	SD	Min	Max	$\delta^{13}C$	$\delta^{15}N$
Annelida	-29.81	10.53	5.25	5.15	10.6	24.4	200	133
Ampharetidae	-29.17	8.92	4.72	4.16	6.0	23.0	29	21
Capitellidae	-19.73	4.47	9.15		0.0	3.7	6	1
Chaetopteridae	-18.91		na		0.0	0.0	1	0
Chrysopetalidae	-18.19		na		0.0	0.0	1	0
Cirratulidae	-23.22	7.12	7.64	3.18	2.6	8.4	20	6
Cossuridae	-19.12	3.36	na		0.0	0.4	2	0
Dorvilleidae	-32.83	9.11	-0.47	4.19	12.5	30.7	22	19
Dorvillea sp.	-40.68	10.83	-2.19	3.63	33.0	51.0	6	6
Ophryotrocha sp.	-32.44	5.40	-0.44	3.58	6.0	29.4	9	9
Hesionidae	-23.24	7.00	6.84		0.0	9.1	2	1
Lacydoniidae	-24.82		14.21		0.0	9.5	1	1
Lumbrineridae	-28.42	6.56	9.21	4.14	3.6	18.9	4	4
Maldanidae	-28.42	12.82	7.48	3.15	11.9	21.3	4	2
Nephtyidae	-26.04	11.55	9.01		0.4	17.0	2	1
Nereidae	-44.74	7.47	2.50	4.12	41.2	61.6	16	16
Oligochaeta	-47.52	12.58	4.08	2.54	56.7	68.9	5	5
Orbiniidae	-32.63		10.48		0.0	29.9	1	1
Paraonidae	-21.79	2.93	11.13	2.26	0.0	3.6	12	7
Phyllodocidae	-21.72		11.98		0.0	1.4	1	1
Polynoidae	-28.92	4.77	9.14	3.88	1.0	20.6	10	9
Siboglinidae	-28.40	9.01	2.14	2.62	3.8	22.4	18	6
Escarpia sp.	-15.10		-2.97		0.0	0.0	1	1
Lamellibrachia sp.	-16.39	1.00	2.58	0.32	0.0	0.0	3	3
Siboglinum sp.	-31.93	6.76	4.03	5.18	4.9	28.8	14	2
Sphaerodoridae	-25.13	3.73	8.49	4.56	0.0	10.3	5	3
Spionidae	-28.61	11.99	6.62	3.71	12.8	20.9	11	5
Sternaspidae	-20.91	3.02	14.80		0.0	2.4	2	1
Syllidae	-27.70	7.63	8.69	4.49	4.4	18.1	11	9
Terebellidae	-39.99	6.82	4.40	3.88	25.9	49.2	11	11
Trichobranchidae	-50.60		-1.01		62.9	77.0	1	1
Chelicerata	-31.48	4.79	10.67	1.72	3.1	26.9	9	9
Cnidaria	-24.21	2.97	10.52	3.92	0.0	8.4	6	5
Anthozoa	-21.05		16.81		0.0	0.0	1	1
Hydrozoa	-24.84	2.83	8.95	2.02	0.0	10.1	5	4
Crustacea	-26.87	6.91	7.85	4.16	2.1	16.1	31	23
Amphipoda	-28.97	7.42	7.20	4.04	3.2	21.4	20	16
Cumacea	-22.47	4.37	1.97		0.0	5.4	3	1
Decapoda	-22.50	4.51	7.71		0.0	5.9	2	1
Euphausiida	-20.52	1.63	10.46	0.15	0.0	0.6	2	2
Isopoda	-28.24		9.29		0.0	18.4	1	1
Ostracoda	-22.61		na		0.0	3.7	1	0
Tanaidacea	-24.62	5.40	12.72	5.66	0.0	9.5	2	2

Table 2.A2. (continued)

Taxonomic Group / Species	$\delta^{13}C$ Mean	$\delta^{13}C$ SD	$\delta^{15}N$ Mean	$\delta^{15}N$ SD	% MDC Min	% MDC Max	Replicates $\delta^{13}C$	Replicates $\delta^{15}N$
Echinodermata	-21.71	3.05	8.67	3.87	0.0	4.0	22	16
Echinoidea	-23.06		na		0.0	4.9	1	0
Ophiuroida	-21.65	3.11	8.67	3.87	0.0	4.0	21	16
Mollusca	-34.39	11.58	3.11	5.09	19.2	35.6	76	61
Aplacophoran	-25.24	5.48	6.71	9.19	0.0	11.9	7	5
Bivalvia	-24.19	7.37	6.07	3.09	1.4	11.2	12	7
Protobranchia	-20.82	3.27	6.32	2.80	0.0	2.7	8	5
Vesicomyidae	-35.48	1.05	5.44	4.96	5.6	37.4	3	2
Gastropoda	-38.58	10.60	2.03	4.41	26.6	46.0	54	47
Astyris permodesta	-26.91	7.12	9.40	1.31	0.0	17.0	6	2
Cataegis sp.	-42.19		2.33		31.0	54.9	1	1
Hyalogyrina sp.	-27.24	2.00	5.75	4.67	0.0	15.8	7	7
Neolepetopsis sp.	-53.48	7.93	2.43	4.30	73.8	84.0	3	3
Provanna laevis	-41.44	7.38	1.50	3.42	28.7	53.0	15	15
Provanna lomana	-37.87	5.28	1.43	0.76	16.1	43.6	3	3
Pyropelta sp.	-46.39	4.65	-0.71	3.44	46.9	65.9	15	15
Scissurellidae	-18.49	1.41	na		0.0	0.0	2	0
Scaphopoda	-21.12	1.88	9.08	3.70	0.0	1.6	3	2
Nematoda	-23.03	4.95	-0.53		0.0	7.6	6	1
Nemertea	-24.42	4.26	10.43	3.47	0.0	9.7	13	8
Porifera	-35.79	11.14	8.76	3.26	21.4	38.2	7	7
Asbestopluma sp.	-27.24	3.53	6.89	2.87	0.0	15.8	4	4
Sponge, encrusting	-47.19	3.53	11.27	1.76	50.0	68.0	3	3
Sipuncula	-26.93		5.47		0.0	15.0	1	1
Protozoa	-23.50	5.17	4.14	5.28	0.6	8.2	33	11
Agglutinated foram	-23.40	3.45	5.35	1.43	0.0	7.0	9	2
arbor forams	-23.33	1.36	5.17	7.54	0.0	5.6	5	4
bathsiphon tubes	-21.45	2.78	5.35	1.43	0.0	2.9	4	2
Carbonate foram	-23.41	6.04	na		0.0	9.8	3	0
Folliculinid Ciliate	-30.50	8.26	2.37	5.35	3.9	26.6	5	4
Sediment foram	-21.39	2.57	5.08	6.54	0.0	2.7	16	5
Filamentous Bacteria	-24.82	5.28	2.31	3.72	1.1	10.6	43	15
From active carbonates	-32.40	5.28	1.24	2.38	5.8	29.3	8	8
From active mats	-27.40	2.54	7.68	5.38	0.0	16.2	2	2
From off-seep sediments	-22.47	3.56	0.48		0.0	5.2	26	1

CHAPTER THREE

DYNAMICS AND TROPHIC PATTERNS OF MACROFAUNA COLONIZING HARD SUBSTRATES AT A COSTA RICA METHANE SEEP

Abstract

While observational studies have shown continental margin habitat diversity increases regional and global biodiversity, few empirical studies provide a means to identify the colonization and successional processes that assemble communities and shape local patterns of diversity. Methane seeps contain physical, geochemical, and biogenic heterogeneity, which is often reflected in the distribution and abundance patterns of endemic and background taxa. At Mound 12 off Costa Rica, we deployed substrates representative of deep-sea chemosynthetic ecosystems for 10.5 months in order to quantify the effects of three types of heterogeneity: fluid flow (active or inactive), substrate identity (authigenic carbonate, wood, or biogenic tubes and shells), and site (three active seeps and paired inactive sites). We compared macrofaunal assemblages among experimental treatments and to those on native, background authigenic carbonates at the methane seep, and we also analyzed trophic diversity patterns.

Macrofauna colonized substrates in active fluid flow at higher densities than in inactive areas. Seep-endemic gastropods (especially *Provanna laevis*, *Pyropelta* spp., *Lepetodrilus guaymensis*, *Neolepetopsis* sp., *Paralepetopsis* sp.) dominated most treatments, although polychaetes were also relatively abundant on inactive compared to active substrates. Active flow was most important in structuring the community during

colonization, with spatial effects playing a secondary role. Colonizing assemblages were similar on carbonate and wood, with reduced species richness and diversity indices compared to background carbonates. The native carbonate community pattern of higher species diversity at active than inactive areas was reversed on colonization substrates, which always had higher rarefied diversity and species richness at inactive areas. Trophic diversity (measured by standard elliptical areas (SEA_C) in $\delta^{13}C$ x $\delta^{15}N$ isotopic space) was greater for most species on native carbonates than colonization carbonates. However, three limpets that were successful colonizers exhibited greater trophic diversity (SEA_C) on colonization carbonates, suggesting that flexibility in diet may explain early successional patterns of dominance. Thus, over one year small habitat patches at methane seeps can regain the densities, but not diversity of macrofauna common on surrounding hard substrates. Colonization rates for hard substrates have not previously been measured at methane seeps and provide insight into mechanisms that maintain diversity and promote ecosystem resilience.

Introduction

Methane seep heterogeneity

Methane seeps are deep-sea ecosystems in which physical, geochemical, and biological processes interact to form dynamic and heterogeneous landscapes at multiple spatial scales. At the scale of the continental slope, cold methane seeps are known to contribute to regional biodiversity patterns via provision of a novel geochemical environment and various hard substrates (Levin et al. 2010; Sellanes et al. 2010; Cordes et al. 2010). Seeps are associated with mounds, scarps, and other areas of topographic relief along continental margins such as in the East Pacific (Sahling et al. 2008). The flow of methane allows for chemosynthetic primary production at seeps and high consumer biomass relative to surrounding benthic habitats that depend on sinking photosynthetically-derived carbon (Sahling et al. 2002). These habitats contribute both structural and chemical heterogeneity in the environment, existing as a patchwork of individual seeps that have locally high sulfide and methane concentrations relative to ambient, inactive sites.

Habitat heterogeneity is known to have a positive impact on biodiversity in terrestrial and marine settings (Tilman 1999; Stachowicz et al. 2008), and while this has been an area of active research in the deep-sea, quantitative analyses of hard substrate communities remain lacking. In the deep sea, countless studies have linked habitat heterogeneity to diversity patterns in various environments and for different taxa (e.g. bathyal soft-sediments (Jumars 1975; Levin et al. 2001); methane seeps and vents (Govenar and Fisher 2007; Cordes et al. 2010); biotic structures on continental margins

(Buhl-Mortensen et al. 2010); for nematodes (Vanreusel et al. 2010)). These studies often conclude that heterogeneity plays a role in maintaining the extraordinarily high biodiversity observed in deep-sea sediments. Heterogeneity due to biological structures and behavior (burrows, polychaete tubes and mudballs, mounds, large organic falls), sediment characteristics, particulate organic matter flux, and hydrodynamics all contribute to diversity patterns (reviewed in (Etter and Mullineaux 2001; Levin and Sibuet 2012)). Sediment cores are ineffective on hard substrates, so video surveys are frequently used to quantify benthic assemblages of seamounts, canyons, and vents, revealing abundance and diversity patterns only for visible megafauna (Vetter et al. 2010). To accurately measure diversity of macrofauna and smaller taxa in these habitats, pieces of substrate must be collected so that the entire associated fauna – whether free-living, epibiotic, or endobiotic – can be identified and counted. Only after careful inventories of macrofauna will it be possible to compare diversity patterns on hard substrates to those in soft sediments at seeps and vents and the surrounding deep sea.

Methane seeps are fragmented with patchy habitats and exist at a hierarchy of scales, which must be taken into account when considering community patterns. Spatial variation in the physical and chemical environment is due to (a) geological features at km scales, (b) varying contribution of several methane pools to local carbon flux within sites, and (c) complex patterns of subsurface fluid flow related to sediment permeability, overlying crusts, and physical processes controlling fluid flow rates and directions (Tryon et al. 2002; Orphan et al. 2004). Observed patterns in animal communities are often correlated with the extent and level of fluid seepage, especially in symbiont-bearing

megafauna whose microbes require sulfide or methane (Sibuet and Olu-Le Roy 2002; Levin et al. 2003; Levin 2005). The availability of methane also leads to the microbial precipitation of hard substrates (Aloisi et al. 2002). Cold seeps would be generally soft-sediment habitats were it not for anaerobic methane oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) consortia and the precipitation of carbonate as a byproduct of the anaerobic oxidation of methane (AOM) (Orphan et al. 2001). Hence, these microbes are ecosystem engineers (*sensu* (Jones et al. 1994)), modifying abiotic conditions for the entire community through the creation of a novel habitat.

Carbonate pavements, boulders, and cobble provide living sites for mobile and sessile epifauna as well as endolithic taxa that live inside authigenic carbonates. Symbiont-hosting megafauna that attach to carbonate at seeps create additional structure that contributes to heterogeneity and key ecosystem functions. Such biological structures are important in adding physical structure all along continental margins (Buhl-Mortensen et al. 2010), especially at methane seeps (Cordes et al. 2005; Levin 2005; Cordes et al. 2010). Mussels and tubeworms depend on carbonates for attachment, and these aggregating organisms create a complex microhabitat with many microcrevices and interstices in which smaller macrofauna find food or refuge (Levin 2005; Cordes et al. 2010). These types of taxa perform similar functions at hydrothermal vents (Van Dover and Trask 2000; Govenar and Fisher 2007; Govenar 2010) as well as in shallow subtidal and intertidal habitats.

Wood falls are common on continental margins near forested continents, and sunken logs and palm fronds are frequently encountered around the Costa Rica seeps

(authors' observations, 2009-2010). Along other margins, whales, kelp, or other organic matter may also provide relatively large quantities of carbon to the seafloor (Smith and Baco 2003; Bernardino et al. 2010; Drazen et al. 2012). Decomposition, biological respiration, and microbial processes contribute to reducing conditions at these organic food falls, and their faunal assemblages share evolutionary affinities with those at seeps and other chemosynthetic habitats (Tunnicliffe et al. 2003; Bernardino et al. 2012). Since different chemosynthetic ecosystems are characterized by unique hard substrates (carbonate, biogenic shell, wood, bone, etc), species that have evolved in these systems may exhibit adaptations to particular substrates. For these reasons, we might expect community succession at seeps to be partially dependent on the availability and type of hard substrate that can be colonized.

Succession in chemosynthetic environments

Our knowledge of succession in chemosynthetic ecosystems is mainly informed by observational studies. Fortuitous volcanic eruptions creating new habitat have led to descriptions of community succession at hydrothermal vents. At vents, after a period of rapid chemical and microbial changes in the environment, early animal colonizers can arrive within a year, and populations can begin to turnover within two years (Tunnicliffe et al. 1997; Shank et al. 1998). Growth of vestimentiferan tubeworms is also very rapid at vents relative to most deep-sea organisms (Lutz et al. 1994). Within three years after an eruption, assemblages of tubeworms and associated organisms may resemble pre-eruption communities (Marcus et al. 2009). Methane seeps usually have longer temporal persistence than vent ecosystems, foundation species are longer-lived, and larval

recruitment rates may be lower, at least for certain taxa (Tunnicliffe et al. 2003; Metaxas and Kelly 2010). Therefore, successional processes likely contribute to changing community structure over decades and centuries, even after a seep has become inactive (Cordes et al. 2005). However, in the absence of eruptive processes that create new habitat such as at vents, experimental approaches may be required to discern the dynamics at play during early succession and faunal colonization at seeps.

Though settlement processes are poorly studied, experiments from both hydrothermal vents and cold seeps suggest that multiple processes contribute to recruitment patterns. Kelly and Metaxas (2008) found small-scale heterogeneity, such as the three-dimensional complexity of available hard substrates, structures macrofaunal colonization at the Juan de Fuca vents, as species richness, diversity, and evenness were higher on sponge substrates than basalt substrates. Similar analyses of complexity have not been conducted for colonists at seeps, although tests of multiple substrate types have yielded marked variation in colonizer composition (Gaudron et al. 2010), and successional stages have been inferred for certain communities (Cordes et al. 2009; Lessard-Pilon et al. 2010).

Proximity to flow plays a strong role in magnitude of colonization (Metaxas and Kelly 2010). The implication of fluid flow varying over days, weeks, and years at seeps (Tryon et al. 2002; Levin 2005) is a constantly changing chemical environment to which successful species must be able to adapt. In sediments, sulfide is thought to regulate communities, as some taxa actively avoid sulfidic sediments while others recruit more heavily to habitats with high sulfide (Levin et al. 2006; Bernardino et al. 2012; Levin et al.

2013). However, recruitment rate can vary by orders of magnitude between faunal groups and across sites and habitats, and it may also differ between soft and hard substrates.

Large and small-scale processes may both reduce the likelihood of larval recruitment. Retention of larvae may be very high on chemosynthetic communities associated with isolated mounds or seamounts (Metaxas 2011), such as seeps on the Costa Rican margin, but larvae from other seeps are known to disperse far in surface currents before sinking and recruiting (Arellano et al. 2014). Once larvae near a settlement site, they may be filtered out of the water by mussels or other filter feeders (Lenihan et al. 2008), and interference competition from mobile invertebrate grazers could also be a source of mortality for successful settlers. In short, variation at large scales (greater than meters) is probably somewhat determined by the larval pool, dispersal, and spatial factors, while post-settlement processes, habitat selection, and organism movement are more important at finer scales (Arellano and Young 2010).

There is a need to better understand colonization processes at seeps, including a focus on what aspects of this dynamic environment are important in structuring biological communities. Spatial structure is likely to influence isolated communities that are linked through larval dispersal (Mouquet and Loreau 2003), such as seeps, which are usually patchy and do not experience much immigration and emigration of adults. At the same time, environmental heterogeneity seems to be reflected in natural seep communities, where bacterial mats, clam beds, or mussel clumps are associated with specific chemical microhabitats. Different seeps may contain different hydrodynamic, geochemical, sediment, and structural characteristics, and therefore may not be identical habitat patches

supporting the same niches. By studying colonization patterns in methane seeps, we can gain insight into which processes structure newly developing assemblages and whether they vary with environmental features such as active fluid flow, substrate type, or site.

Objectives & Hypotheses

The main goal of this research was to examine the influences of environmental heterogeneity on colonization dynamics and trophic patterns of macrofauna at methane seeps. Experiments focused on heterogeneity induced by seepage activity, substrate type, and site. Carbonate, wood, and biogenic substrates ("experimental" or "colonization" substrates) were deployed at three paired active and inactive sites for 10.5 months at Mound 12, Costa Rica. These substrates represent isolated, defaunated patches of habitat to which individuals could recruit or migrate, thus simulating community succession. Since stable isotope patterns of consumers reflect diets and sources of production, isotopic differences among treatments or species could provide insight to succession dynamics that may be related to food resource availability, niche breadth, or resource partitioning as a type of species sorting [*sensu* (Leibold et al. 2004)]. In particular, we address to what extent seepage activity, substrate type, and site influence abundance, composition, diversity, and trophic patterns of colonizing macrofauna, and how such patterns compare or contrast with later successional communities on *in situ* carbonates from the same sites ("native" carbonates). We tend to focus on the gastropod assemblage, due to its diversity and overall dominance on hard substrata. We hypothesized that:

(1) Substrata placed near active seepage should attain greater densities and diversity of colonizing macrofauna compared to inactive sites, due to a greater potential for microbial chemoautotrophic production and breadth of dietary niches.

(2) Community structure will be shaped primarily by activity, and secondarily by substrate, which will be particularly important to just a handful of specialists (e.g. *Xylophaga* spp.) and not most macrofauna.

(3) Diversity will be greater on carbonates than wood, since it is created authigenically at seeps and thus can be considered a more "realistic" substrate type for this community. (i.e. species may preferentially occupy carbonate since it is the substrate most likely to be found at methane seeps.)

(4) After 10.5 months colonizer diversity will lag that of native authigenic carbonates.

(5) Activity will have a greater effect on community trophic structure than substrate type, and species occurring on both experimental and native carbonates will have similar trophic niches, as measured using stable isotopes.

Material and Methods

Study Site

The Pacific margin of Costa Rica is an erosional subduction zone hosting over sixty mounds and seamounts associated with over 100 areas of fluid seepage (Sahling et al. 2008). One of these, Mound 12 (8°56'N, 84°19'W, Figure 3.1A), rises 30 m to a depth of 1000 m, and hosts authigenic carbonates and chemosynthetic communities including

beds of mytilid mussels, clumps of tube worms, bacterial mats, and vesicomyid clam beds (Mau et al. 2006). These assemblages are associated with multiple sites of active seeping fluids near the top of the mound (Figure 3.1B). We visited Mound 12 in 2009 (22–24 February & 5 March) and 2010 (7–10 January) aboard the RV *Atlantis* and DSV *Alvin*.

Field deployments

In 2009, we initiated a colonization experiment by deploying three types of bare, defaunated substrates on Mound 12: authigenic carbonate, wood, and biogenic tubes and shells (Figure 3.1B, Table 3.1). Each was selected as representative of hard substrate that might be present at a reducing ecosystem on the Costa Rica margin (methane seeps, sunken wood falls, and seep-specific tubeworms, mussels, or clams). Carbonate rocks were either from methane seeps at Hydrate Ridge, Oregon (44°40'N, 125°6'W) or were collected from Mound 12, defaunated, dried for at least one week, and then deployed.

To determine surface area, carbonate rocks were covered with a single layer of aluminum foil, which was later weighed. Carbonate surface area was calculated given the known mass of a 5 x 5 cm square of foil (range of experimental rocks: 561–1371 cm^2). Pieces of wood were untreated Douglas fir (*Pseudotsuga menziesii*) cut into blocks approximately 9 x 9 x 24.6 cm (1047.6 cm^2). Biogenic materials (vestimentiferan tubes or bivalve shells from seeps) were arranged into clumps and rubber bands held pairs of shells together. The surface areas of biogenic substrates were calculated using the measured dimensions of tubes or shells and the formulas for common geometric shapes (range: 358–585 cm^2 for shells; 1914–2033 cm^2 for tubes). We surrounded each substrate

with 1.6 cm polypropylene mesh (Easy Gardner BirdBlock Protective Mesh Covering) and attached a floating polypropylene loop to aid handling. Lead weights, enclosed in duct tape, were attached to the wood to ensure they were negatively buoyant.

Scientists diving in *Alvin* inspected Mound 12 for sites with visual indications of active seepage, such as gas bubbling, bacterial mats, or chemosynthetic communities. We selected three seeps where methane and sulfide clearly influenced the biological community or geochemical environment (i.e. "Active" sites), along with three sites without any signs of fluid flow or chemosynthetic fauna (i.e. "Inactive" sites). In two cases, paired sites were separated by approximately 5 m ("Lamelli Lane" and "Yetisburg"), while in one case they were separated by 50 m ("Mussel Beach") (Figure 3.1B). Twenty-five substrates were deployed 22-23 February and 5 March 2009, and all but one were collected 7-10 January 2010 (average 317-day deployment). Two carbonate rocks were collected from each site, except only one was collected from the inactive site at Mussel Beach. Two wood blocks were collected from each Lamelli Lane and Yetisburg site. Biogenic materials were only deployed at Mussel Beach, with tubes and clam shells being collected from the active and inactive sites, and mussel shells collected at the active site only.

Experiment recovery & native carbonates collection

Colonization substrates were retrieved with *Alvin's* manipulator and were placed into an insulated biobox with Plexiglas compartments that maintained separation of fauna from different substrates. Shipboard, substrates were immediately moved to a cold room (4°C) until processing. Each substrate was photographed from various angles in and out

of water, and visible macrofauna were picked from the substrate. Macrofauna associated with the polypropylene mesh, line and weights were separated and not included in counts and analyses. A 300μm sieve was used to remove macrofauna from water in the compartments. The substrate was then allowed to soak in seawater at room temperature for 24 hours, which resulted in most macrofauna evacuating holes and crevices. This water was sieved as before, and macrofauna were sorted under dissecting microscopes at sea and identified to the lowest possible taxonomic group. Specimens of each identified taxon were preserved or fixed by different means (frozen, 8% formalin, 95% ethanol), and remaining samples were bulk preserved in 70% ethanol.

Additionally, natural seep carbonate rocks (13 active and 7 inactive) were collected from Mound 12 in February and March 2009, and their macrofaunal communities and surface areas were quantified using the same methods as the colonization substrates. These were considered representative of the native seep community, and hypothetically represent a later successional stage than our 10.5-month experimental substrates. As described previously, $\delta^{13}C$ and $\delta^{15}N$ signatures were analyzed for at least one and often several individuals of each taxon recovered from background carbonates. Stable isotopic data and trophic patterns for the natural (i.e. late successional) hard substrate community were used for comparison to colonization (early successional) substrates, and specifically carbonates. Mussels removed from carbonate rocks were treated as another type of substrate, and fauna associated with mussels were quantified as being on a biogenic substrate rather than carbonate. Surface areas for these biogenic treatments were calculated as described previously for mussel shells.

Stable isotopes processing

To assess nutritional sources of colonists, up to three individuals per species per substrate, filamentous bacteria, and substrate samples were also analyzed for stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$). Specimens were allowed to evacuate their guts in 4°C seawater, rinsed in Milli-Q water, and placed in pre-weighed tin boats using methanol-cleaned forceps, prior to being frozen at -80°C. In the lab, specimens were oven dried at 60°C to constant weight (~24 hours), weighed, and acidified using 1% $PtCl_2$ in 1M HCl. Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) were measured from 0.2-0.8 mg of dry tissue on a Eurovector elemental analyzer with Micromass Isoprime isotope ratio mass spectrometer at Washington State University. Samples of carbonate and wood were frozen and dried as above. Carbonate was ground to a fine powder and wood was grated into small splinters prior to stable isotope analysis. Carbonates were acidified for $\delta^{13}C_{organic}$ analysis and received hydrogen peroxide for $\delta^{13}C_{inorganic}$ analysis.

Community statistics

We used counts and substrate surface areas to calculate densities of macrofauna (standardized to 200 cm^2). Two-Way Analysis of Variance (ANOVA) was used to test whether activity or substrate had an effect on the density of colonizing macrofauna (JMP v.11). Diversity patterns were examined with rarefaction curves and the calculation of ES(100), Shannon index (H'), Simpson Index (1-λ), Margalef species richness (d), and Pielou evenness (J') (PRIMER v.6). To investigate effects of activity, substrate, and site on community structure, a Bray Curtis resemblance matrix was created with square root-transformed density data (Primer v.6). We then performed non-metric multidimensional

scaling (MDS), ANOSIM, and SIMPER to examine similarities among communities on experimental substrates and native seep carbonates, test the significance of the treatment groups in driving community patterns, and identify those taxa which drove the patterns, respectively. Additionally, we used linear regression analysis to ask whether taxonomic ranked abundance is correlated among active and inactive, or experimental and native carbonates.

Stable isotopes analyses

We used stable isotopes analysis to test whether community trophic patterns differed among all substrate treatments. The SIAR package for R (v3.0.2, R Core Team), introduced by (Jackson et al. 2011), quantifies stable isotope metrics originally developed by (Layman et al. 2007). Staple isotope values ($\delta^{13}C$ and $\delta^{15}N$) from all species occurring on colonization substrates were represented in two-dimensional space for these analyses, resulting in metrics corresponding to overall variation in diet. Mean distance to centroid (CD), the Euclidean distance of each species to the centroid for that community, is a measure of community trophic diversity, as it increases as species values cover more space in an isotope biplot. Carbon range (dCr) and nitrogen range (dNr) provide an indication of the total range of carbon and nitrogen utilized by a community. Mean nearest neighbor distance (MNND) is a measure of species packing within a community, while the standard deviation of nearest neighbor distance (SDNND) provides an indication of the evenness of this packing.

We also used stable isotope metrics to test whether individuals colonizing deployed substrates had different diets than individuals collected from natural substrates.

Most of our stable isotope data represent carbonate-associated fauna, so for this analysis we focused on species that occurred on both natural and colonization carbonates. The Bayesian approach developed by (Jackson et al. 2011) uses multivariate ellipses-based metrics instead of convex hull areas (Layman et al. 2007), since the latter is extremely sensitive to sample size. Corrected standard elliptical areas (SEA_C) adjust for sample size and provide a metric that can be used to compare total niche space that is occupied by the individuals of a particular group. For fourteen species for we had enough samples (4 to 15) to make this comparison. We used the SIBER routine in the SIAR package for R, which calculates SEA, SEA_C, total area of the convex hull (all based on frequentist methods), Bayesian ellipses (based on 10,000 simulations), and overlap between ellipses (Jackson et al. 2011).

Results

Macrofaunal abundance and density

Macrofauna colonized active substrates at rates resulting in densities 7.5 times greater than on inactive substrates (2-Way ANOVA, Activity: $F_{1,2} = 12.2$, $p = 0.005$; Figure 3.2A). This pattern was observed on all substrates, for gastropods the most abundant group, and crustaceans. The density of polychaetes, the second most abundant group after gastropods, did not differ between activity levels. Taxa occurring in higher densities on inactive substrates included ophiuroids, nemerteans, and cnidarians. Substrate type did not affect overall colonizer density at inactive sites, but at active seeps, carbonates contained significantly higher densities of colonizing macrofauna than did wood (2-Way ANOVA, Activity: $F_{1,16} = 8.29$, $p = 0.004$, Figure 3.2A). Biogenic

substrates (clam shells, mussel shells, and tubes of *Lamellibrachia* sp.) contained the highest observed macrofaunal densities at an active seep (237.4 ± 34.8), though they were deployed at a single pair of active and inactive sites ("Mussel Beach"), and these data were not included in further analyses. Colonization rates varied among sites, as macrofaunal densities were highest on substrates near Yetisburg, intermediate at Mussel Beach, and lowest near Lamelli Lane (2-Way ANOVA, Site: $F=6.53$, $df=2,2$, $p=0.012$).

Gastropods were the most abundant group of colonizers. Different species had higher proportional abundances on different substrate-activity combinations. *Provanna laevis* was the most dominant species on carbonates (~55% of all gastropods on Active, ~52% on Inactive), but only made up ~20-30% of the gastropods on wood and biogenic substrates (Figure 3.3A). In contrast, *Lepetodrilus guaymensis* was proportionally more abundant on wood (~20%) than other substrates. *Pyropelta corymba*, a limpet that typically occurs on *Provanna* spp. shells, was common on active substrates (~15-25%) but almost completely absent at inactive areas (~5% or less). With the gastropods, the clade of Patellogastropoda (true limpets) including *Neolepetopsis* sp. and *Paralepetopsis* sp. consistently had higher relative abundance on inactive than active substrates (Figure 3.3B). Vetigastropoda, on the other hand, responded to substrate type, with highest relative abundance on biogenic material and lowest on carbonate.

For active substrates, there was generally a positive relationship between the rank abundance of species on experimental and native carbonates ($R^2 = 0.30$, $p < 0.0001$, Figure 3.4A). There was no similar relationship for inactive substrates ($p = 0.67$, Figure 3.4B), though several species of gastropods that were most abundant on inactive

colonization carbonates were also relatively common on background carbonates. For experimental carbonates, taxonomic rank abundance on active carbonates was positively correlated with that of inactive carbonates ($R^2 = 0.39$, $p < 0.0001$, Figure 3.4C). For native carbonates, rank abundances were not correlated for taxa occurring at both active and inactive sites ($p = 0.29$, Figure 3.4D).

Seep activity affected whole community colonizer composition (Two-way ANOSIM, Global R = 0.229, $p = 0.035$), while substrate type had no effect (Global R = 0.016, $p = 0.476$). The species contributing to dissimilarity between colonizers of active and inactive substrates were more abundant near active fluid seepage, and they were mainly gastropods, especially *Provanna laevis* (9.51%), *Pyropelta corymba* (6.26%), and *Pyropelta musaica* (5.04%) (SIMPER, average dissimilarity = 73.63) (Table 3.A1). In addition to activity, multidimensional scaling suggests that seep site may play a greater role than substrate type in distinguishing colonizing communities (Figure 3.5A). Active substrates at Yetisburg and Mussel Beach clustered as a group, while Lamelli Lane active substrates were just as similar to this group as they were to inactive substrates. Carbonate and wood colonizing communities were both significantly different from those on background carbonates (Figure 3.5B, Two-way ANOSIM, Stage: Global R = 0.274, $p = 0.003$; Activity: Global R = 0.203, $p = 0.002$).

Diversity patterns

In regards to colonization substrates, though total species richness was similar between active (59 species) and inactive areas (72 species), rarefaction diversity was greater on inactive substrates (ES_{100}=36.2 for inactive, 16.3 for active) due to higher

species evenness on the inactive substrates (Figure 3.6A, Table 3.2). Diversity of colonizers on wood and carbonate substrates were similar, but biogenic substrates at the active Mussel Beach seep, which were dominated by just a few species of gastropods, had notably lower diversities as measured by rarefaction (Figure 3.6B). Different seep sites generally contained similar diversity for colonization substrates (Figure 3.6C). However, substrates placed next to tubeworm clusters at Lamelli Lane, the least active site, contained a more diverse assemblage than at other active sites, and were in fact much more similar to those at inactive sites than to substrates at the active Mussel Beach and Yetisburg seeps (Figure 3.6D).

Community recovery

Seep activity dramatically altered the patterns of diversity on natural and colonization carbonates (Figure 3.6C, Table 3.2). On natural carbonates, the background community contained higher diversity and species richness in areas of active seepage (ES_{100} = 30.4) compared to inactive areas (ES_{100} = 25.4). However, on experimental carbonates, colonizers were much more diverse on inactive carbonates (ES_{100} = 33.4). Active colonization carbonates had much lower diversity (ES_{100} = 16.2) than other treatments due to a high degree of dominance by several gastropods (*Provanna laevis* and *Pyropelta* spp.).

Stable isotope analysis

Mean (± SE) bulk isotopic community signatures differed between active and inactive substrates for both $\delta^{13}C$ (Two-way ANOVA, $F_{1,3}$ = 8.293, p = 0.004, Figure 3.7,

Table 3.A2) and $\delta^{15}N$ ($F_{1,3} = 7.611$, $p = 0.006$, Table 3.A2). The community colonizing active substrates had slightly lighter signatures ($\delta^{13}C = -37.01 \pm 1.01‰$; $\delta^{15}N = 3.30 \pm 0.28‰$) compared to those on inactive substrates ($\delta^{13}C = -34.37 \pm 0.84‰$; $\delta^{15}N = 4.64 \pm 0.34‰$, Figure 3.7, Table 3.A3). Post-hoc Tukey HSD contrasts revealed the significant effects of activity on $\delta^{13}C$ were restricted to biogenic substrates ($\delta^{13}C_{\text{Active}} = -43.94‰$; $\delta^{13}C_{\text{Inactive}} = -30.11‰$, $p < 0.001$, Table 3.A2), as pairwise contrasts were not significant for other substrates. However, the lower $\delta^{15}N$ for macrofauna at active sites compared to inactive sites was seen across all substrates. Differences relating to activity level were also evident for many individual species (Tables 3.A3, 3.A4).

While carbonate and wood did not have different effects on the mean $\delta^{13}C$ and $\delta^{15}N$ signatures for whole assemblages (Figure 3.7A,B), trophic structural differences were revealed by community isotope metrics (Table 3.2) (Layman et al. 2007). The $\delta^{13}C$ range (dCr, representative of dietary breadth), was greater for active carbonate than other substrates, including native carbonates. Meanwhile, $\delta^{15}N$ range (dNr, representative of food chain length) on active wood (23.1‰) was similar to that of native carbonates (~25‰), while dNr for inactive wood and colonization carbonates was somewhat less (~15–19‰).

Sample size-corrected standard elliptical areas (SEA_C) can be considered a measure of trophic diversity for macrofauna, since consumption of a greater variety of food sources will result in a population with more variable stable isotopic signatures, and hence a greater SEA_C. Of taxa that occurred on both colonization and native carbonates, we analyzed the stable isotope signatures of at least 4 individuals for 14 species from

each treatment. Three showed a greater SEA_C on colonization carbonates, while 11 had a greater SEA_C on native carbonates (Figure 3.8, Table 3.A5). The species with greater trophic diversity on colonization carbonates as reflected in isotope space were the limpets *Paralepetopsis* sp., *Neolepetopsis* sp., and *Lepetodrilus guaymensis*. SEA_C was also calculated for entire assemblages to compare the trophic diversity on different substrates, at different activity levels, and between colonization and native carbonates (Table 3.3). On carbonates and biogenic substrates, macrofauna displayed greater trophic diversity at active seeps than inactive areas ($Prob_{carb} = 0.997$, $Prob_{Bio} = 0.996$; Prob = Bayesian probability a given treatment is larger than that being compared). At both active and inactive sites, the colonizing assemblage had greater trophic diversity on carbonates than wood ($Prob_{Act} = 1.0$, $Prob_{Inact} = 0.801$, Figure 3.9A). Additionally, trophic diversity at inactive areas was greater on native carbonates than colonization carbonates (Prob = 0.991), but the reverse was true at active seeps, where macrofauna had a slightly greater SEA_C on colonization compared to native carbonates (but note low Probability of 0.677, Table 3.4, Figure 3.9B).

Discussion

This manipulative field experiment provides evidence that both fluid flow and successional time may impact community assembly patterns, and thereby diversity patterns at methane seeps along continental margins. We hypothesized that community composition would respond primarily to differences in seepage activity due to the higher chemoautotrophic productivity that increased seepage provides (Levin et al. 2006; 2013). Not only does more active seepage increase the total food available but also sulfide

concentrations, a potentially strong settlement cue. As sulfide inhibits aerobic metabolism, an ability to detoxify sulfide is an adaptation that may result in distinct species and diversity patterns at methane seeps relative to background margin habitats (Somero et al. 1989; Levin et al. 2013).

We found that colonizers exhibit greater densities but reduced species richness at active compared to inactive sites (Figure 3.2, Figure 3.6, Table 3.2). In areas of active seepage, greater microbial production should be associated with more food resources, secondary production, and trophic levels. Animals in active settings experienced greater food source heterogeneity relative to inactive sites. We hypothesized for carbonate and biogenic substrates that substrate type would have a secondary role in shaping community patterns, whereas certain substrate specialists (e.g. wood specialists such as *Xylophaga* spp.) would show sharp differences in densities between substrate types. While we expected higher diversity in active than inactive sites, we hypothesized that compared to natural seep carbonate communities, our colonization experiments would have reduced diversity since many species in the regional pool could need more than one year to recruit to new habitat patches (Kelly et al. 2007; Mullineaux et al. 2010). Species richness was indeed greatly reduced on experimental substrates compared to native carbonates, indicating 10.5 months was an insufficient time period to recover natural biodiversity to a similar successional stage as the native community in this system.

Lastly, we hypothesized that food web structure (mean and range of $\delta^{13}C$ and $\delta^{15}N$) on experimental substrates at active sites would be similar to that on natural carbonates, if proximity to reduced fluids controls the distribution of primary producing

microbes on which consumers feed. We expected to see greater dietary breadth ($\delta^{13}C$ range, SEA_C) in active compared to inactive sites; this was observed for carbonate but not wood substrates. Differences in species-specific SEA_C between native and colonization carbonates do not support the null hypothesis that dietary niches are similar for these treatments.

Abundance & density

The dramatic differences in faunal densities among substrates placed at variable proximity to seepage highlight the importance of fluid flow in structuring methane seep communities. Much higher densities of macrofauna on substrates closer to active fluid seepage could be related to preferential settlement, increased survival due to greater microbial food resources, or reduced mortality in a chemical environment that may be toxic to predators. Substrates deployed in inactive settings contained about one-eighth the number of recruits on substrates in active flow, and their densities lagged native inactive rocks by more than half, highlighting the slow recovery of faunal biomass on substrates not directly influenced by seepage. Despite this, many individuals on inactive substrates had $d^{13}C$ signatures indicating dependence on chemosynthetic resources. The communities' different rates of recovery of biomass and diversity based on chemical environment mean that factors regulating seepage over time such as tectonic activity, faulting patterns (Olu et al. 1996), or other episodic disturbances will exert strong influence on community resilience. Similarly, colonization processes will be spatially heterogeneous with implications for both community recovery following perturbations and also the design of potential deep-sea reserves.

We can use total macrofaunal abundance and length of deployment to calculate average recruitment rates for comparison to other studies. We observed 0.19 individuals dm^{-2} day^{-2} on active substrates and 0.05 individuals dm^{-2} day^{-2} on inactive substrates. A review by Metaxas and Kelly (2010) found an average recruitment rate of 0.3 individuals dm^{-2} day^{-1} in methane seeps, which is similar to our findings, but comes from only two studies, one in sediments (Levin et al. 2006) and the other on mollusk shells (Watanabe et al. 2009). Metaxas and Kelly calculated recruitment for gastropods to be above 0.01 individuals dm^{-2} day^{-2}, whereas gastropods constituted the majority of our recruits (Figure 3.2B), a discrepancy that may be explained by an absence of carbonate substrates in previous studies.

Gastropods may be functionally important as microbial grazers on seep carbonates in Costa Rica, where they are diverse, abundant (Levin et al. in prep), and the most common colonizers among macrofauna. Substrates at inactive sites had recruitment rates of 0.03 individuals dm^{-2} day^{-1}, which is lower than active sites but still greater than typical recruitment rates in the deep sea (Grassle and Morse-Porteous 1987; Smith and Hessler 1987). Gaudron et al. (2010) working in the Northeast Atlantic, deployed substrates near several chemosynthetic ecosystems, but away from active flow (i.e. comparable to our “inactive” sites). They observed lower taxonomic richness on carbonate than we did (1–3 taxa per deployment) and macrofaunal recruitment was comparable. However, at Costa Rica’s Mound 12, wood and carbonate substrates attained macrofauna of relatively similar identities and densities. There was a notable lack of a

wood-boring community in contrast to Gaudron et al. (2010), who reported very high densities of *Xylophaga* spp. on deployments of wood cubes.

In the eastern Pacific, gastropods from clades observed at Mound 12 consistently dominate mobile macrofaunal assemblages at vents and seeps, where microbial communities flourish on hard substrata near seeping fluids (Shank et al. 1998; Govenar et al. 2005; Metaxas and Kelly 2010). Gastropods may consume a significant proportion of *in situ* primary production while simultaneously maintaining open space on hard substrates; both ecosystem functions have been attributed to gastropods in shallow-water marine habitats (Jones 1948; Dayton 1971; Nielsen 2001; Silliman and Zieman 2001). Manipulative experiments in a variety of marine ecosystems have shown negative effects of gastropods on primary producer biomass (Lodge 1948; Lubchenco 1980; Wootton et al. 1996), and simply the presence of these mobile grazers impacts settlement of spores and larvae (Hawkins et al. 1992). Whether these relationships extend to deep-sea systems, and particularly to hard substrates at methane seeps is unknown and could represent a direction for future research. Additionally, manipulations could determine whether competition for space and resources leads to negative interactions among grazers as it does in other ecosystems (Dayton 1971; Branch and Branch 1980).

Of the species contributing the most to colonizer assemblage dissimilarity between active and inactive substrates, only ophiuroids were more abundant on inactive substrates (Table 3.A1). As suspension feeders, they could be utilizing hard substrates as a means to feed above the seafloor accessing higher current speeds (Buhl-Mortensen et al. 2010), a finding supported by their isotopic composition in this study. Substrate-

provisioning represents an ecosystem function for microbially-precipitated carbonates that continues late in community succession, long after active fluid flow may cease at a particular site (Cordes et al. 2005).

Community structure

Proximity to seeping fluids is the primary factor controlling the colonizing macrofaunal assemblage. For some taxa a dependence upon chemoautotrophic microbes likely explains this pattern. Alternatively, larval settlement patterns may be driven by those of seeping fluids, which could act as settlement cues for some larvae. The patellogastropods *Paralepetopsis* sp. and *Neolepetopsis* sp. were often found on inactive substrates, and they almost certainly consume methane-derived carbon based on their light $\delta^{13}C$ signatures [mean (± SD) = -48.29 ± 0.16‰ and -47.93 ± 0.18‰, respectively (Table 3.A4)]. Wood substrates had assemblages resembling those on carbonates, while both were distinct from native rocks. Microbial and animal populations seem to utilize wood and carbonate in similar manners, at least early in community development.

The successional patterns on active and inactive substrates appear to be distinct, highlighting the role of fluid flow on species composition. Species on native and colonization carbonates had more similar rank abundance patterns in active fluid flow than in inactive areas (Figure 3.4A,B). The tight, positive relationship (especially for mobile gastropods) on active carbonates could result from mass effects (Leibold et al. 2004) if frequent movement of nearby individuals or localized dispersal allows the experimental carbonates to quickly approach the background community in terms of relative species abundance. Abundant species on experimental carbonates were always

represented on native carbonates (Figure 3.4A), suggesting that species common locally contribute most to early community development. There were many species that occurred on active background carbonates that did not occur on active colonization carbonates. These species may not have settled on the experimental deployments, they may be poorer competitors, or they may have lacked their required habitat or food. For inactive carbonates, several gastropods were common both in the background community and on the colonization carbonates. However, there were also species that occurred frequently on either the background or colonization carbonates, but not both, indicating the importance of stochasticity and successional processes.

Limpets and snails that often represent important guilds in shallow subtidal and intertidal marine systems may provide a parallel for the abundant gastropods on seep carbonates. Research has shown littorine gastropods and various limpets are capable of exerting density-dependent grazing effects on algal communities (Branch and Branch 1980). Limpets likely act as space clearers, leaving carbonates with little microbial mat; inactive substrates had a covering of fine, white filaments as if grazing were reduced at these sites. A top-down role for grazers has not previously been demonstrated in any chemosynthetic habitat and may represent a promising direction of future research (though the top-down role of predation at vents is discussed by Micheli et al. (2002).

Wood falls develop a unique assemblage in the deep sea and wood borers seem to be nearly ubiquitous (Turner 1973; 1977; Bienhold et al. 2013), so it is notable that our experimental wood substrates developed communities that were structured similarly to those on deployed carbonates and lacked boring fauna. Off the coast of Oregon, high

densities of *Xylophaga washingtona* colonized identical wood blocks over a similar time frame (Chapters 4, 5), so this absence is not likely to be an experimental artifact. Notably, natural sunken wood recovered from several seeps on the Costa Rica margin tended to contain wood-boring bivalves in the Teredidae family and not Xylophagidae, suggesting colonization of wood-borers may be restricted to surface waters (A. Waren, pers. comm.).

The lack of *Xylophaga* colonization may indicate regional differences in wood availability and associated wood specialists, or a deterrent effect of nearby seepage. The species that colonized wood in our experiment represented a small subset of the native community, but the absence of wood specialists or initiation of organic decomposition suggests a delay between the sinking of wood and impacts on local diversity patterns. Bernardino et al. (2010) investigated macrofauna near sunken wood, and found evidence that several years of boring and decomposition may be necessary before wood organic material begins to support sediment macrofauna. Large wood falls may sustain seep- and whale fall-adapted populations via chemoautotrophic processes for a number of years, potentially increasing habitat heterogeneity and providing a stepping stone environment for larvae, though not existing as a permanent chemoautotrophic community (Bienhold et al. 2013).

Diversity

Seepage activity had a greater influence on diversity and species evenness than did site or substrate type. Rarefaction curves suggest much higher species richness on inactive substrates than active substrates, given the same number of individuals. This pattern is largely driven by the extreme dominance of several species of gastropods on

active substrates at the Yetisburg and Mussel Beach sites. Accordingly, the active seep at Lamelli Lane was not dominated by gastropods to the same degree, and thus had diversity patterns that were similar to inactive sites instead of the other active sites. These results reveal the dynamic nature and spatial heterogeneity of diversity drivers at the scales of meters and 10s of meters. Native carbonates actually show a contrasting diversity pattern, as rarified taxa richness is greater in active than inactive sites. Dominant habitat-forming species at Mound 12 include mussels (three new species of *Bathymodiolus*; Rouse et al., In prep) and tubeworms (*Escarpia spicata* and *Lamellibrachia barhami*), but these were largely underrepresented on our colonization experiments. These ecosystem engineers can be positively correlated with local species diversity, so their eventual recruitment, as well as that of rare species in the community, might alter our observed rarefaction patterns (Govenar 2010).

To fully understand how communities are maintained and how diversity responds to disturbance, we need to do manipulations with multiple time points while measuring the community response. In the present study, we have only a single time point at 10.5 months. It is not enough to fully describe succession, which would give us insight into potential trajectories of community recovery following disturbance, but diversity and abundance patterns suggest that substrates in active fluid flow are rapidly colonized by a few species with high recruitment potential and able to consume the present microbial resources. Those microbes, however, may also represent just a subset of the background community. Even if carbonates are quickly populated by ANME groups, the slow growth rates for these consortia [7-month doubling rate; (Nauhaus et al. 2007)] may be

associated with similarly slow succession and community turnover. We would predict that after several years, given additional opportunities for microbial colonization, succession, and additional recruitment by fauna not initially present, diversity and faunal patterns on experimental carbonates would begin to look more similar to native carbonates. At hydrothermal vents, faunal assemblages are tightly linked to physicochemical microhabitats (Luther et al. 2001). We would expect that temporal changes in the microbial and geochemical environments of seep carbonates would also modify macrofaunal communities.

Trophic structure

We observed little differences in the mean isotopes $\delta^{13}C$ and $\delta^{15}N$ for different treatments depending on activity (Figure 3.7). Despite the great difference in community composition and diversity, these data suggest differences in trophic resources between active and inactive sites were not responsible for community differences in this study. However, dietary differences are apparent for individual species living in different microhabitats. Taxa occurring on both native and colonization carbonates allow us to test whether colonists are using different food sources or have different size trophic niches than members of the native community. For some taxa (Polychaetes: Hesionidae sp. 2, Dorvilleidae; Gastropods: *Lepetodrilus guaymensis*, *Neolepetopsis* sp., *Pyropelta wakefieldi*), individuals on colonization carbonates had stable isotope ratios that significantly deviated from those of individuals on native carbonates, signified by little or no overlap in the standard ellipses (Figure 3.8). These colonizers are likely consuming a

different suite of microbes or different sources of carbon than individuals on natural carbonates that have formed at Mound 12.

However, there is no evidence of dietary differences between the treatments for groups that show a high degree of overlap in standard ellipses (Polychaetes: Terebellidae; Gastropods: *Provanna laevis*, *Pyropelta musaica;* Polyplacophora, Figure 3.8). The SEA_C for these species was similar in size between colonization and native carbonates with a high degree of isotopic overlap (Figure 3.8, Table 3.A5). Of the fourteen taxa investigated in this way, eleven had larger SEA_C on native carbonates, while only three (all limpets) had larger SEA_C on experimental carbonates. This observation suggests that most species see their dietary niche reduced on the experimental rocks. Just as ten months was not sufficient to achieve a diversity of animals resembling the background community, ten months is likely not a sufficient period of time for the microbial community to reach its normal diversity or structure.

Although visual observations confirmed high microbial biomass on many of the colonization experiments, the absence of certain types of bacteria or archaea could reduce the potential food sources for consumers, resulting in a narrower range of stable isotopic signatures. Most species exhibited little overlap in SEA_C between native and colonization treatments, suggesting dietary resources change as succession proceeds on seep carbonates (Table 3.A5). Still, the experimental carbonates seem to have developed a more diverse microbial community than the wood blocks, given the wider range of $\delta^{13}C$ signatures in the macrofaunal community (Figure 3.9A). Perhaps the relatively narrow range of potential carbon sources on our experimental wood is related to its lower

colonization rates as well (Figure 3.2A). Longer-term investigations of succession at seeps might focus on the role of microbial colonization in contributing to macrofaunal patterns, or whether the identity of particular grazers may have a top-down microbial structuring role.

Conclusions

We found that colonization may allow defaunated habitat patches at methane seeps to recover some aspects of the ambient community within one year including density, biomass, and dominant species, but it may take several years to develop species richness comparable to that on mature, authigenic carbonates. These data represent the first quantified colonization rates on hard substrates at cold seeps, and suggest that recovery rates may be slower than at hydrothermal vents. Several species of gastropods are numerically dominant on colonization and native substrates. They may serve an important ecosystem function as top-down grazers on hard substrates in active flow, as substrates with high densities of gastropods were rarely covered by microbial mat (pers. obs.). Trophic patterns indicate multiple types of production contribute to nutrition, with the relative influence changing depending on influence of seepage, and possibly the type of substrate.

As the first experimental investigation of colonization processes on hard substrates at methane seeps, this work has implications for the maintenance of diversity at local scales and ecosystem resilience. If bare substrates, which can be thought of as representing small-scale disturbances, require over a year to recover natural levels of biodiversity and ecosystem function, then larger disturbances affecting an entire seep

would certainly require even longer. As human activities such as oil and mineral extractions increase on continental margins, a need exists to not only describe and quantify the habitats and fauna that will be directly impacted, but also to continue to improve our understanding of community dynamics that will dictate the ability of ecosystems to recover from disturbance and regional biodiversity to be maintained (Ramirez-Llodra et al. 2011; Van Dover et al. 2012).

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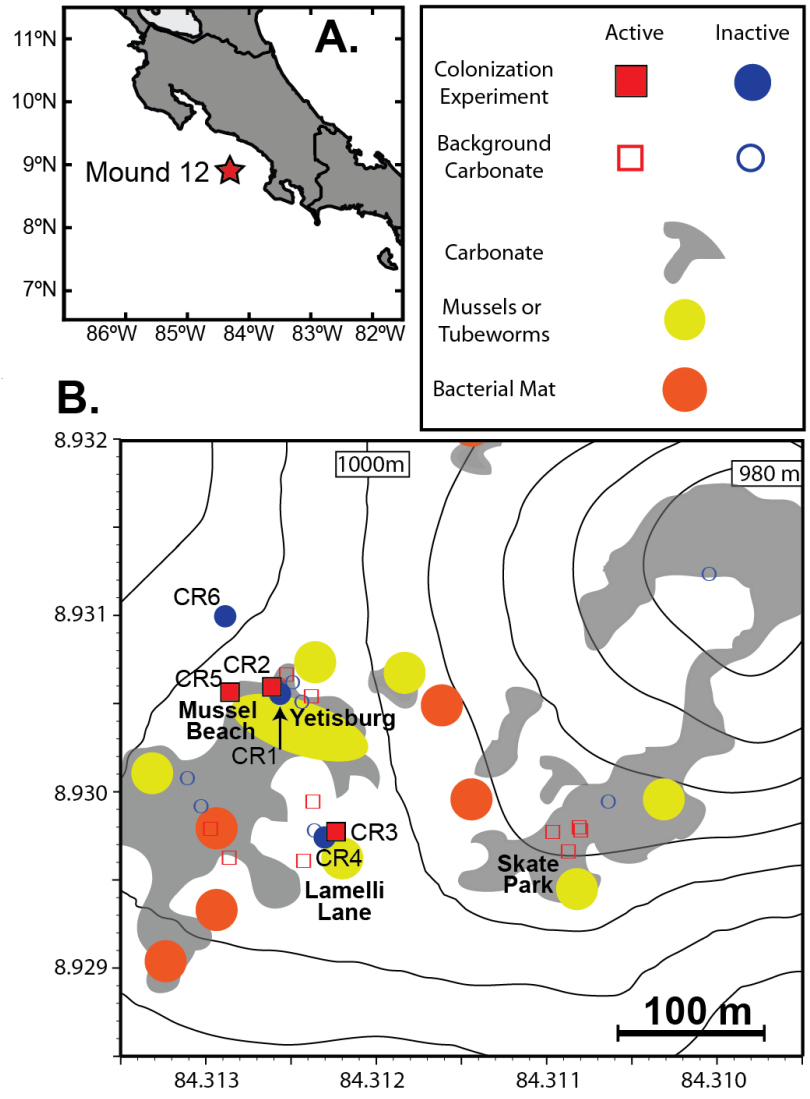


Figure 3.1. A) Location of Mound 12 off Costa Rica; **B)** Locations of experimental deployments (filled symbols) and native carbonates sampled (open symbols) at Mound 12, Costa Rica. Background map adapted from Mau et al. (2006), where grey regions indicate areas of carbonate cover, orange regions indicate bacteria mats, and yellow regions indicate mussel beds or tubeworm clumps. Named sites are Yetisburg (CR1), Mussel Beach (CR5), Lamelli Lane (CR3), and Skate Park (native carbonates collected about 150 m east of Lamelli Lane).

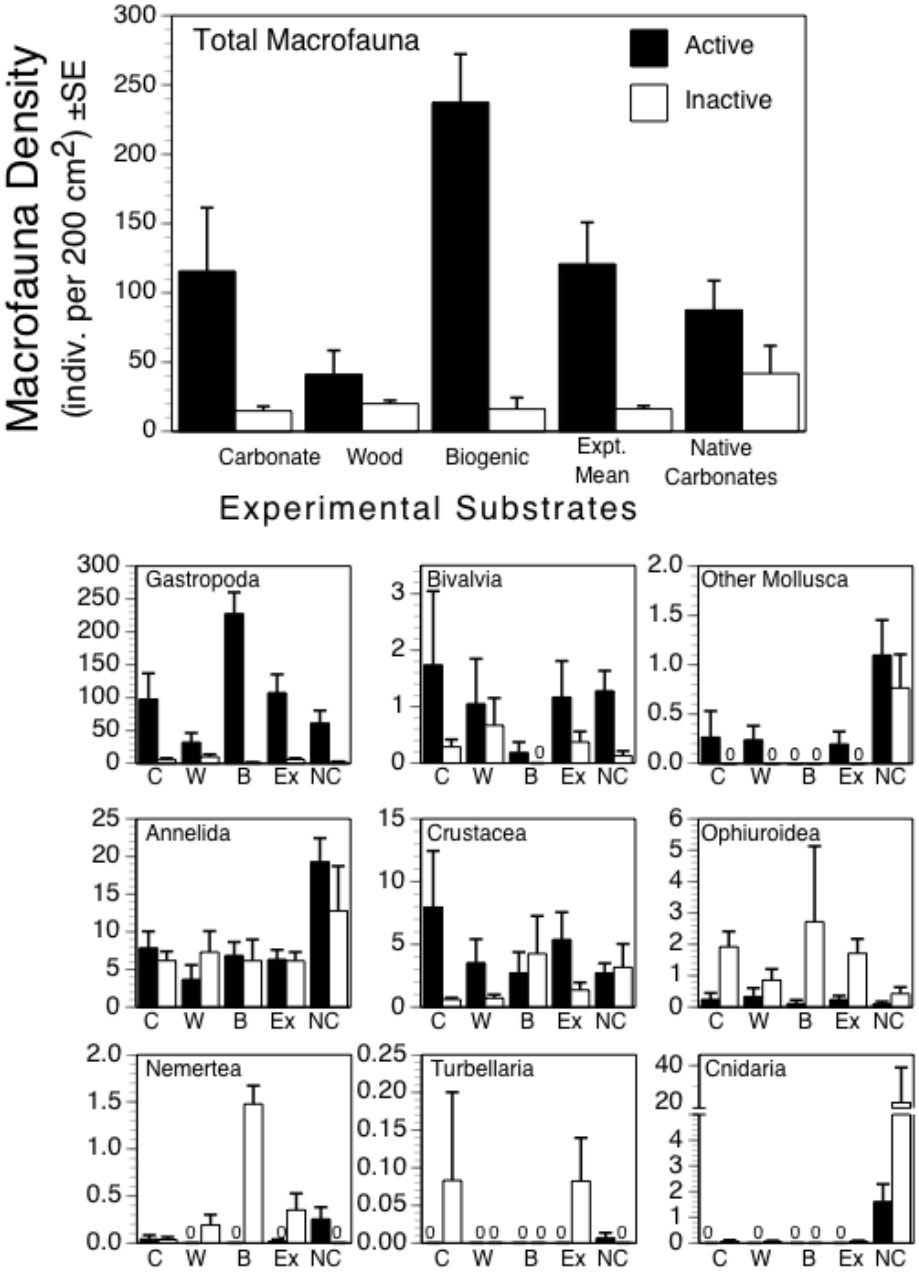
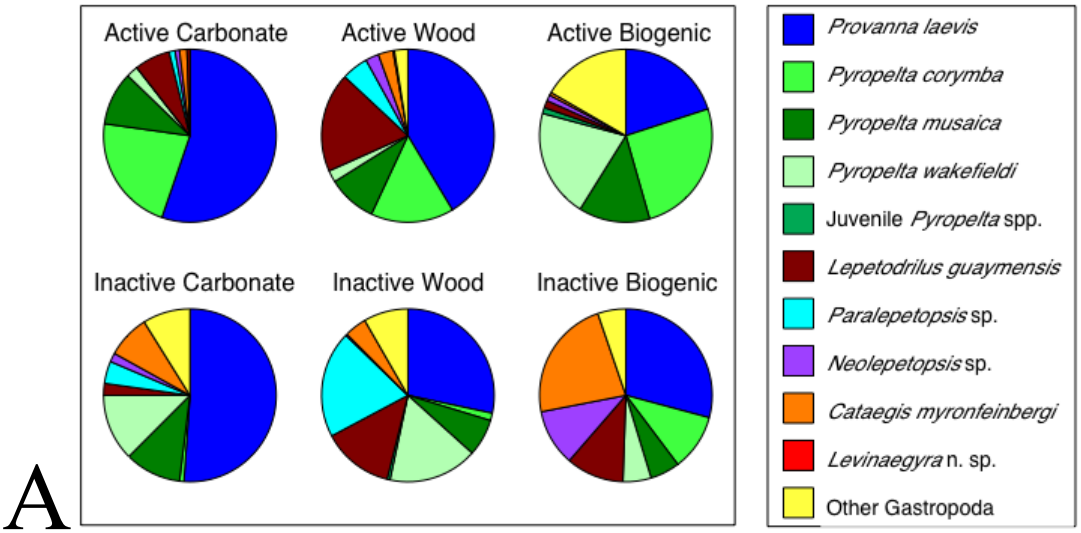
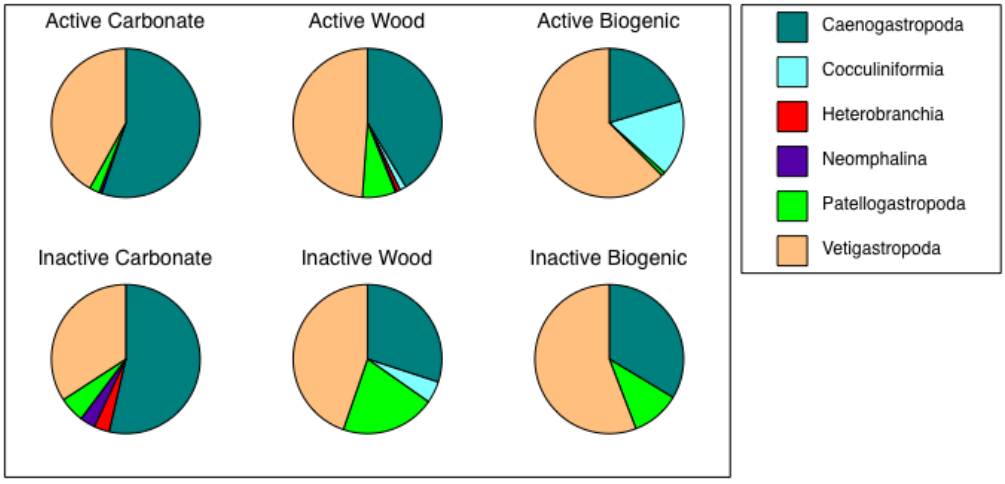


Figure 3.2. Density ± SE of total macrofauna colonizing experimental substrates in actively seeping and inactive environments (top panel), and density of major taxonomic groups colonizing different substrates (carbonate, wood, biogenic material, native carbonates) at active or inactive seep sites. Experimental mean ("Expt. Mean" or "Ex") is the mean of carbonate, wood, and biogenic substrates. Since there were only two inactive biogenic substrates, error bars represent the range of the points around the mean rather than standard error.



A



B

Figure 3.3. A) Species composition of gastropods colonizing experimental substrates. Total densities are shown in Figure B; **B)** Composition of colonizing gastropods by taxonomic clade.

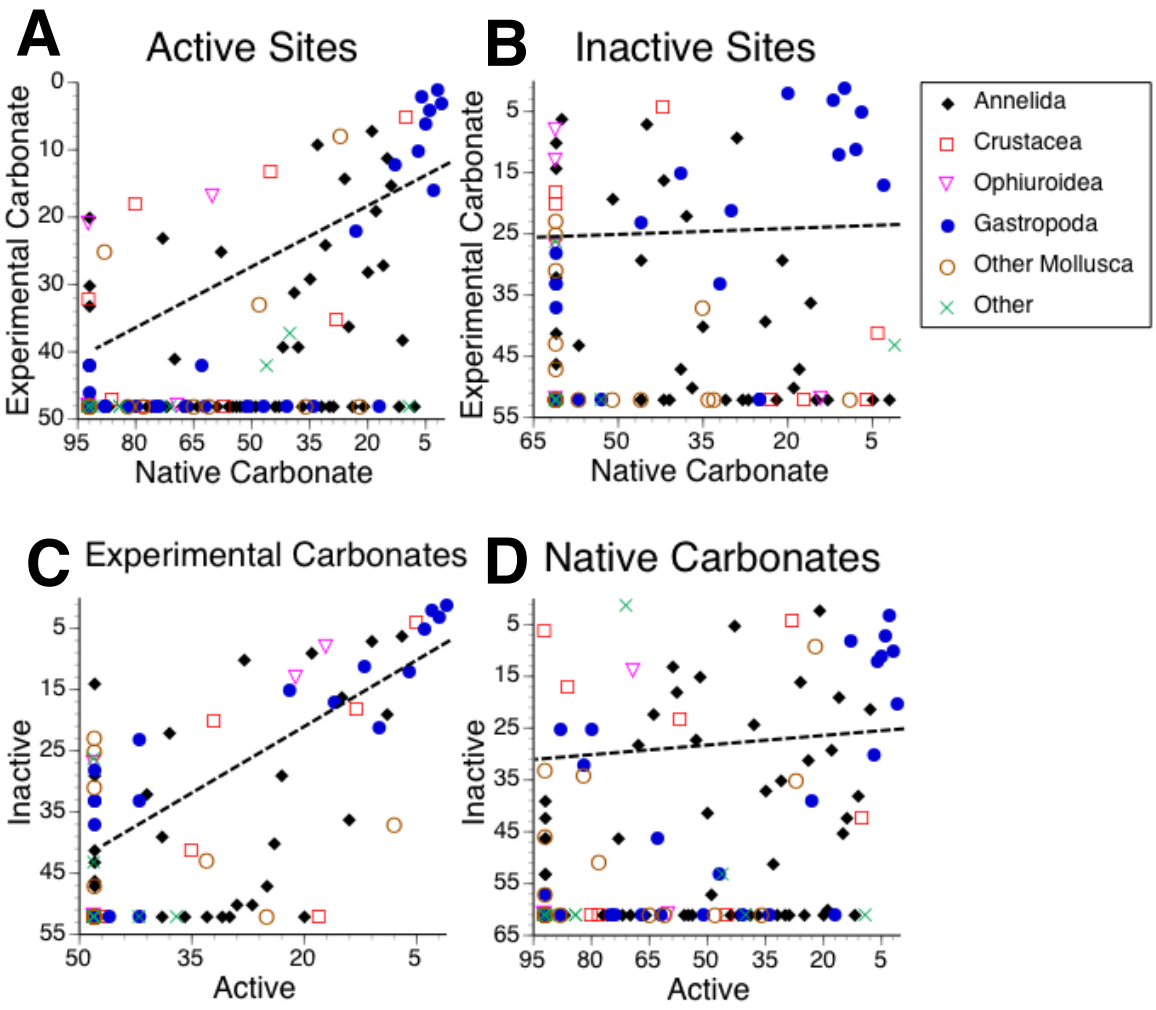


Figure 3.4. Species rank abundance of macrofauna on experimental (colonization) and native carbonates at: **A)** active and **B)** inactive sites. Comparison of macrofaunal species rank abundance at active and inactive settings for: **C)** experimental and **D)** native carbonates. Dashed lines are linear regressions. Axes labels are reversed so that abundant species for both groups will appear at the top right of each plot.

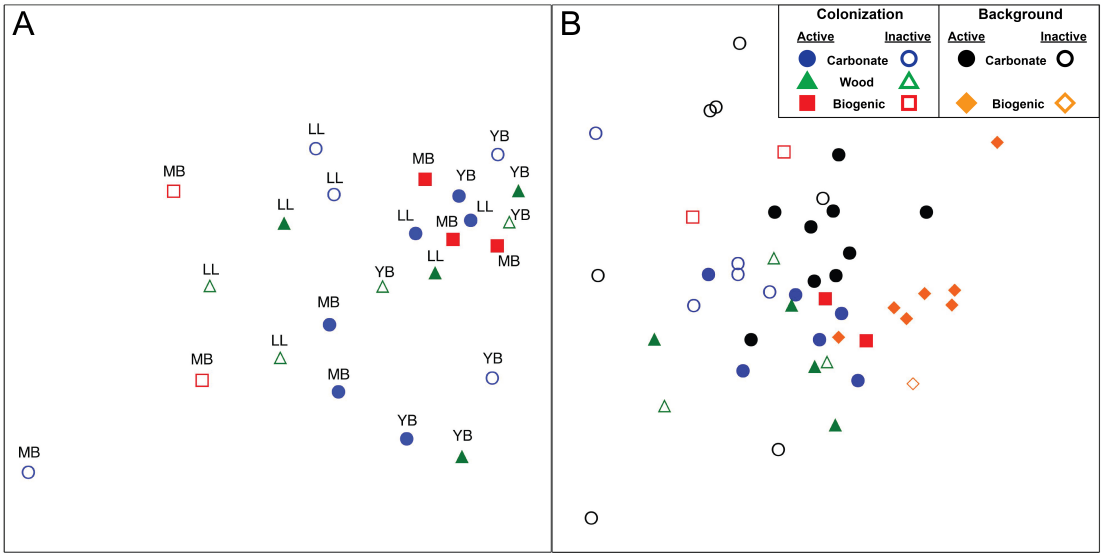


Figure 3.5. A) Similarity of communities colonizing carbonate and wood substrates at active or inactive sites as represented by nonmetric multidimensional scaling. MB="Mussel Beach", YB="Yetisburg", LL="Lamelli Lane", 2D stress = 0.15; **B**) Similarity of macrofaunal communities colonizing different substrates (carbonate, wood, biogenic) and on native carbonates or live mussels in active or inactive settings as represented by nonmetric multidimensional scaling, 2D stress = 0.19. (The inactive biogenic data point represents a clump of mussels that was on a rock that scientists initially deemed "inactive", but probably was in an active area given the presence of symbiont-bearing taxa.)

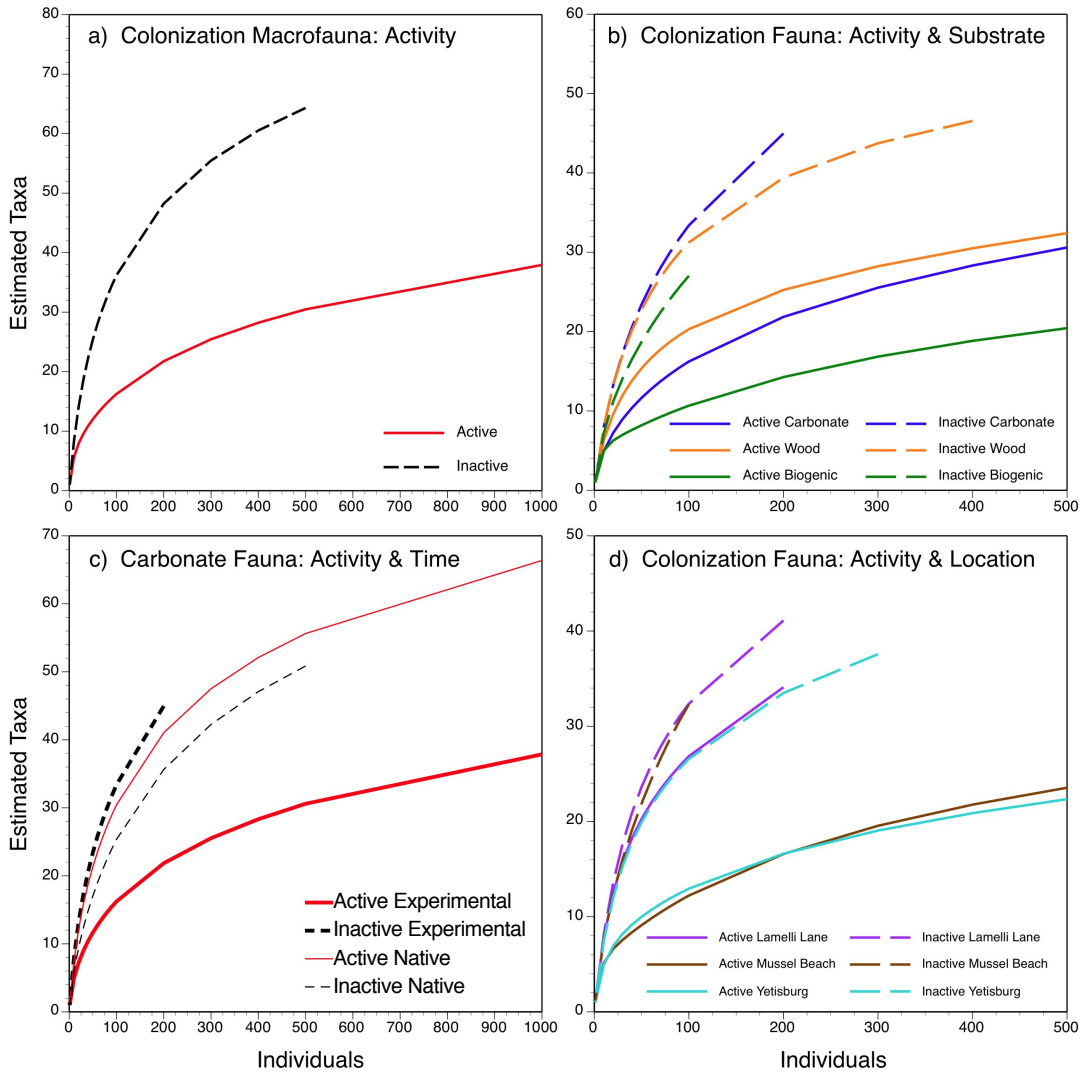


Figure 3.6. Rarefaction curves contrasting diversity for colonizers at: **A**) active and inactive sites; **B**) carbonate, wood, and biogenic material in active and inactive sites; **C**) experimental and native carbonates at active and inactive sites ("time" refers to the longer time of succession for native carbonates relative to the experimental carbonates we deployed); **D**) combinations of site ("location") and activity

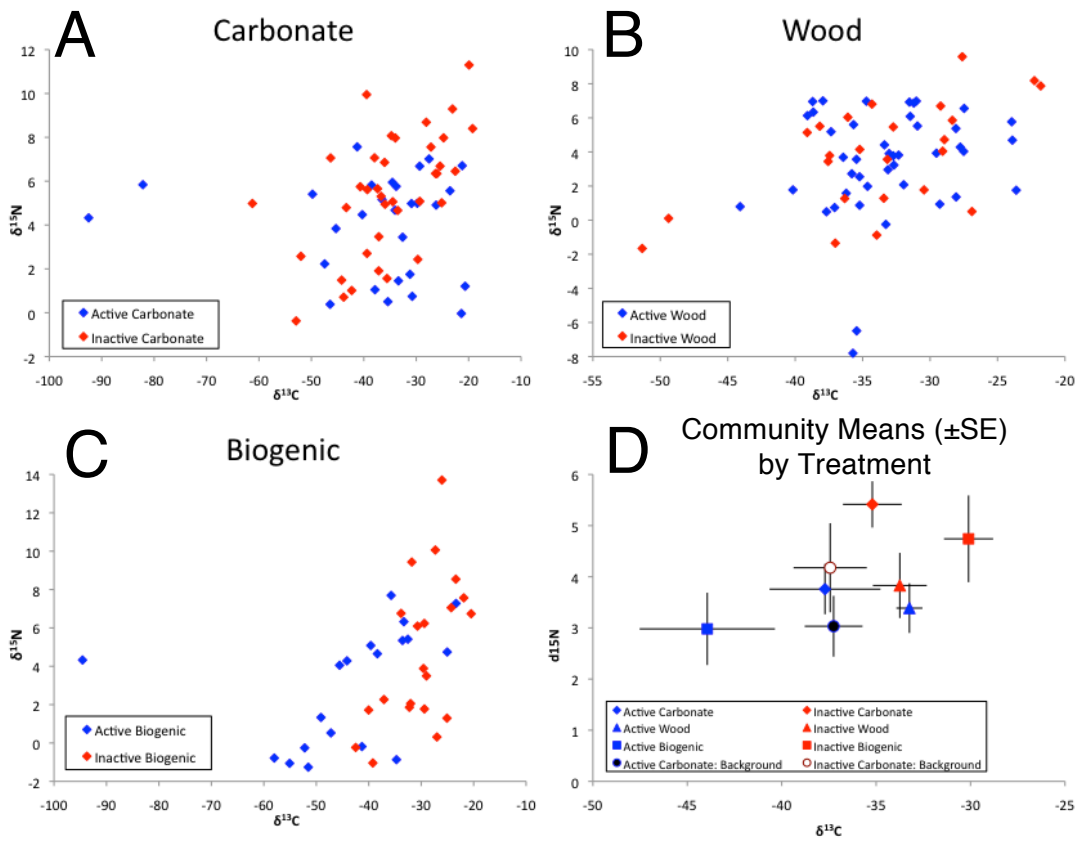


Figure 3.7. Stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$) for colonizing species on: **A**) carbonate, **B**) wood, and **C**) biogenic substrates placed in active and inactive settings. Each point is a distinct species (the average of 1–11 individuals). **D**) Mean stable isotope ratios (± SE) of all taxa for colonization substrates and native carbonates in both active (closed symbols) and inactive settings (open symbols).

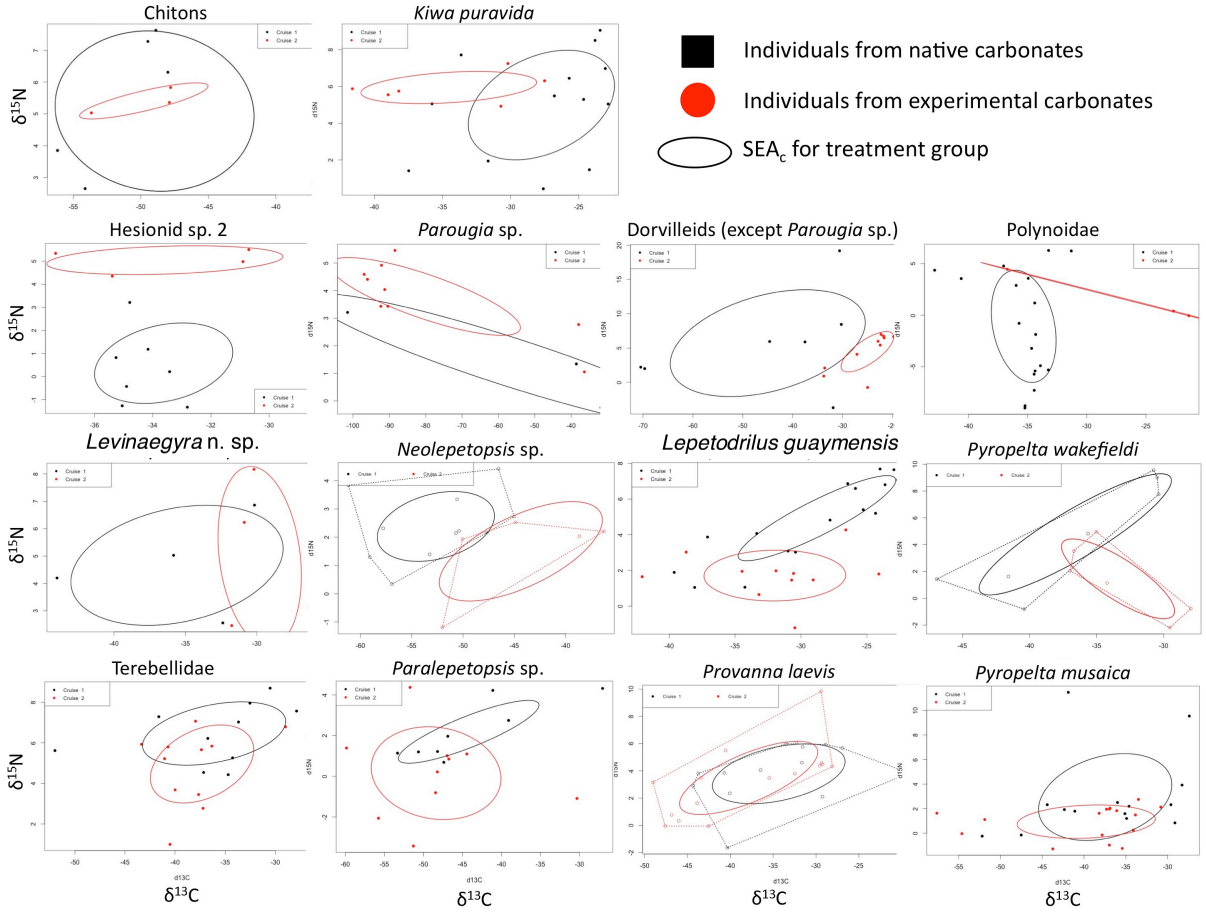


Figure 3.8. Trophic niches for species occurring on native (black squares) and experimental (red circles) carbonates. Points represent stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of individuals. Ellipses are SEA_C and represent the core of a population's trophic niche (see also Table 3.A5). Species shown here include only those with at least four individuals sampled from each treatment group. Table 3.A4 contains isotopic means for species on colonization substrates.

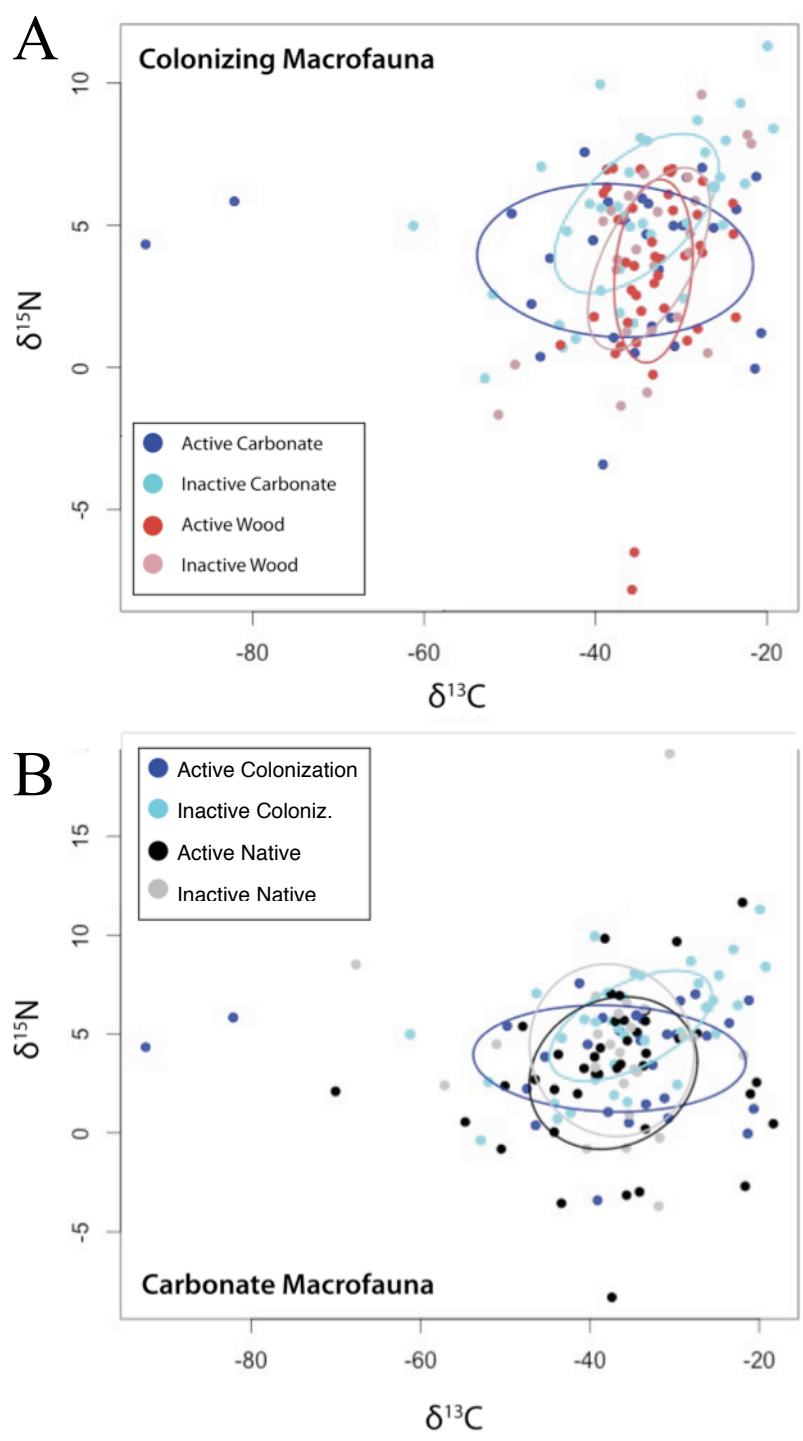


Figure 3.9. The stable isotopic composition of: **A**) species colonizing carbonate and wood in active and inactive sites; and **B**) species on colonization and native carbonates at active and inactive sites. Points represent taxonomic means. Ellipses are SEA_C (after Jackson et al. 2011).

Table 3.1. Sampling design including both experimental substrates (site, substrate type, activity, replicates, recovery and deployment dates) and native carbonates collected *in situ* (site, activity, replicates, collection date, and whether mussels on rock were considered as biogenic substrate).

Experimental Substrates						
Seep Site	Substrate Type	Activity	Replicates	Recovery Date	Deployment Date	Deployment time
Yetisburg	Carbonate	Active	2	7-Jan-2010	22-Feb-2009	319 days
		Inactive	2	7-Jan-2010	22-Feb-2009	319 days
	Wood	Active	2	7-Jan-2010	22-Feb-2009	319 days
		Inactive	2	7-Jan-2010	22-Feb-2009	319 days
Lamelli Lane	Carbonate	Active	2	9-Jan-2010	23-Feb-2009	320 days
		Inactive	2	9-Jan-2010	23-Feb-2009	320 days
	Wood	Active	2	9-Jan-2010	23-Feb-2009	320 days
		Inactive	2	9-Jan-2010	23-Feb-2009	320 days
Mussel Beach	Carbonate	Active	2	10-Jan-2010	5-Mar-2009	311 days
		Inactive	1	8-Jan-2010	5-Mar-2009	309 days
	Biogenic	Active	3	10-Jan-2010	5-Mar-2009	311 days
		Inactive	2	8-Jan-2010	5-Mar-2009	309 days
Native Carbonates						
Seep Site	Substrate	Activity	Replicates	Recovery Date	Mussels (Biogenic)	
Yetisburg	Carbonate	Active	3	22-Feb-2009	on 1 rock	
			1	5-Mar-2009		
		Inactive	1	22-Feb-2009		
Lamelli Lane		Active	2	23-Feb-2009	on 1 rock	
			1	5-Mar-2009		
		Inactive	1	23-Feb-2009		
			1	5-Mar-2009	on 1 rock	
SW Bac. Mats		Active	1	22-Feb-2009		
			1	5-Mar-2009		
		Inactive	1	22-Feb-2009		
			1	23-Feb-2009		
Skate Park		Active	1	23-Feb-2009	on 1 rock	
			2	24-Feb-2009	on 1 rock	
			1	5-Mar-2009		
		Inactive	1	23-Feb-2009		
NE Carbonates		Inactive	1	24-Feb-2009		

Table 3.2. Diversity metrics for colonization substrates on Mound 12 methane seeps, Costa Rica. ES_{100} = estimated number of species given 100 random individuals; S = species richness observed; Ind = number of individuals collected; H' = Shannon diversity; J' = Pielou's evenness; N = number of substrates.

	ES_{100}	S	Ind	H'	J'	N
Activity						
Active	16.3	59	6088	2.27	0.556	13
Inactive	36.2	72	823	3.53	0.825	11
Substrate						
Carbonate	20.7	64	2511	2.31	0.556	11
Wood	28.1	61	1281	3.01	0.732	8
Biogenic	13.6	47	3119	2.11	0.549	5
Substrate & Activity						
Active Carbonate	16.2	45	2241	2.02	0.530	6
Inactive Carbonate	33.4	50	270	3.26	0.834	5
Active Wood	20.3	38	861	2.53	0.695	4
Inactive Wood	31.2	47	420	3.25	0.845	4
Active Biogenic	10.7	33	2986	1.94	0.555	3
Inactive Biogenic	27.0	31	133	2.79	0.812	2
Location						
Lamelli Lane	34.6	62	534	3.44	0.835	8
Mussel Beach	15.1	61	3884	2.19	0.533	8
Yetisburg	18.6	55	2493	2.27	0.566	8
Native Carbonates						
Active Carbonate	30.4	92	6825	3.20	0.709	13
Inactive Carbonate	25.4	58	763	2.56	0.631	7

Table 3.3. A) Stable isotope metrics calculated for species means (one point per species, representing the mean of 1-11 individuals). dNr (range of $\delta^{15}N$ = trophic length), dCr (range of $\delta^{13}C$ = breadth of basal resources), SEA_C (standard elliptical area corrected for sample size = trophic niche), CD (distance to centroid = trophic diversity), MNND (mean nearest neighbor distance = amount of clustering), SDNND (standard deviation of MNND = trophic evenness), TA (convex hull total area = total isotopic niche space). All metrics based off of Layman et al. (2007), except SEA_C is from Jackson et al. (2011)

	dNr	dCr	SEA_C	CD	MNND	SDNND	TA
Active Colonization							
Carbonate	17.5	75.4	136.0	11.39	3.05	3.44	739.4
Wood	23.1	28.5	45.6	5.64	1.67	2.21	361.4
Biogenic	14.8	72.6	148.3	11.95	4.69	8.06	572.6
Inactive Colonization							
Carbonate	18.8	46.2	70.0	8.68	2.29	2.52	490.9
Wood	16.6	33.7	58.1	6.89	2.35	2.24	300.3
Biogenic	18.9	26.0	63.9	6.85	2.53	2.15	261.5
Active Native Carbonate	24.6	54.9	118.8	8.83	2.71	3.23	753.7
Inactive Native Carbonate	25.4	48.0	132.8	8.39	3.60	4.00	662.2

Table 3.4. Comparison of corrected Standard Elliptical Areas (SEA_C, *sensu* Jackson et al. 2011) in stable isotope biplots ($\delta^{13}C$, $\delta^{15}N$, see Figures 3.7, 3.A1) for communities on different substrates and at different activities. SEA_C, an indicator of food resources utilized by the community, is the area of the ellipse for the treatments being compared. "Prob" is to the Bayesian likelihood that SEA_C is larger in "Treatment with Greater SEA_C " (Bold where >99% likelihood). " SEA_C Overlap" is the area of the ellipses shared by both treatment groups. We also calculate the area of the SEA_C that is unique to a particular treatment (="Unique SEA_C "), and the percent of total SEA_C this represents (="Area"). For example, in macrofauna colonizing carbonates, 65% of the community's SEA_C at active sites was not shared by communities at inactive sites. However, only 32% of the inactive SEA_C was unique and not also part of the active community's SEA_C.

Active vs. Inactive Substrates

Colonization Substrate	**SEA_C**		**Treatment with greater SEA_C**	**Prob.**	**SEA_C Overlap**	**Active**		**Inactive**	
	Active	Inactive				Unique SEA_C	Area	Unique SEA_C	Area
Carbonate	136.0	70.0	Active	**0.997**	47.5	88.5	65.1%	22.5	32.2%
Wood	45.6	58.1	Inactive	0.785	38.4	7.2	15.9%	19.7	33.9%
Biogenic	148.3	63.9	Active	**0.996**	27.1	121.2	81.7%	36.7	57.5%

Carbonate vs. Wood

Seep Activity	**SEA_C**		**Treatment with greater SEA_C**	**Prob.**	**SEA_C Overlap**	**Carbonate**		**Wood**	
	Carbonate	Wood				Unique SEA_C	Area	Unique SEA_C	Area
Active	136.0	45.6	Carbonate	**1.000**	40.3	95.8	70.4%	5.4	11.8%
Inactive	70.0	58.1	Carbonate	0.801	32.2	37.8	54.0%	25.9	44.5%

Native vs. Experimental Carbonates

Seep Activity	**SEA_C**		**Treatment with greater SEA_C**	**Prob.**	**SEA_C Overlap**	**Native**		**Experimental**	
	Native	Experimental				Unique SEA_C	Area	Unique SEA_C	Area
Active	118.8	136.0	Experimental	0.677	90.1	28.7	24.2%	45.9	33.8%
Inactive	132.8	70.0	Native	**0.991**	58.5	74.3	56.0%	11.5	16.4%

Table 3.A1. SIMPER percentages identifying taxa responsible for Bray-Curtis dissimilarity among activity and site treatment groups for colonization substrates (carbonate and wood only). Taxa accounting for top 50% of overall dissimilarity are displayed. Treatment with higher density of each taxon is highlighted in bold. Percent contribution is the dissimilarity contributed by a particular species, and cumulative contribution and average dissimilarity calculate dissimilarity up to that point.

Dissimilarity between Active and Inactive colonization substrates					
Average Dissimiliarity = 73.63%					
	Average Density (200 cm^2)		Percent Contribution	Cumulative Contribution	Average Dissimilarity
Taxon	Active	Inactive			
Provanna laevis	**4.76**	1.26	9.51	9.51	7.00
Pyropelta corymba	**2.86**	0.12	6.26	15.77	4.61
Pyropelta musaica	**1.87**	0.54	5.04	20.81	3.71
Kiwa puravida	**1.44**	0.14	4.14	24.96	3.05
Lepetodrilus guaymensis	**1.63**	0.36	4.01	28.96	2.95
Cataegis myronfeinbergi	**1.01**	0.48	3.54	32.50	2.60
Terebellidae	**0.65**	0.51	3.29	35.79	2.42
Paralepetopsis sp.	**1.01**	0.66	3.29	39.08	2.42
Pyropelta wakefieldi	**0.78**	0.72	3.28	42.36	2.42
Alvinocarididae	**0.90**	0.21	3.20	45.57	2.36
Ophiuroid (unidentified)	0.15	**0.80**	3.08	48.65	2.27
Hesionidae sp. 2	0.50	**0.56**	2.84	51.49	2.09
Dissimilarity among three seep locations					
Yettisburg vs. Lamelli Lane					
Average Dissimiliarity = 75.36%					
	Average Density (200cm-2)		Percent Contribution	Cumulative Contribution	Average Dissimilarity
Taxon	Yetisburg	Lamelli Lane			
Provanna laevis	**4.90**	0.82	12.86	12.86	9.69
Lepetodrilus guaymensis	**2.23**	0.05	7.12	19.98	5.37
Pyropelta musaica	**2.34**	0.41	6.24	26.22	4.70
Pyropelta corymba	**2.05**	0.19	5.44	31.66	4.10
Kiwa puravida	**1.53**	0.11	4.54	36.20	3.42
Pyropelta wakefieldi	**0.79**	0.41	3.28	39.47	2.47
Hesionidae	**0.89**	0.12	3.19	42.67	2.41
Terebellidae	0.48	**0.58**	2.87	45.53	2.16
Paralepetopsis sp.	**0.92**	0.83	2.77	48.31	2.09
Ophryotrocha sp.	**1.04**	0.00	2.74	51.05	2.07
Yettisburg vs. Mussel Beach					
Average Dissimiliarity = 63.95%					
	Average Density (200cm-2)		Percent Contribution	Cumulative Contribution	Average Dissimilarity
Taxon	Yetisburg	Mussel Beach			
Provanna laevis	**4.90**	4.37	8.69	8.69	5.56
Lepetodrilus guaymensis	**2.23**	0.44	6.09	14.78	3.89
Pyropelta musaica	**2.34**	0.50	6.08	20.85	3.89
Pyropelta wakefieldi	0.79	**1.54**	5.23	26.08	3.34
Pyropelta corymba	2.05	**3.92**	4.36	30.44	2.79
Terebellidae	0.48	**0.86**	3.43	33.87	2.19
Ophryotrocha sp.	**1.04**	0.00	3.08	36.95	1.97
Lacydoniidae	0.43	**0.78**	2.98	39.93	1.90
Kiwa puravida	**1.53**	0.84	2.95	42.87	1.88
Parougia or *Dorvillea*	0.37	**0.89**	2.69	45.57	1.72
Hesionidae	**0.89**	0.19	2.68	48.24	1.71
Ophiuroid (white disc sp.)	0.10	**0.73**	2.56	50.80	1.63
Lamelli Lane vs. Mussel Beach					
Average Dissimiliarity = 76.21%					
	Average Density (200cm-2)		Percent Contribution	Cumulative Contribution	Average Dissimilarity
Taxon	Yetisburg	Lamelli Lane			
Provanna laevis	0.82	**4.37**	12.16	12.16	9.27
Pyropelta corymba	0.19	**3.92**	11.21	23.37	8.54
Pyropelta wakefieldi	0.41	**1.54**	4.54	27.91	3.46
Ophiuroid (white disc)	0.14	**0.73**	3.44	31.36	2.62
Amphipod (unidentified)	0.15	**0.36**	3.27	34.62	2.49
Kiwa puravida	0.11	**0.84**	2.88	37.50	2.19
Lacydoniidae	0.38	**0.78**	2.85	40.35	2.17
Parougia or *Dorvillea*	0.00	**0.89**	2.78	43.13	2.12
Hesionidae sp. 2	0.51	**0.56**	2.70	45.83	2.06
Ophiuroid ("fanged" sp.)	0.00	**0.31**	2.29	48.12	1.74
Ophiuroid (unidentified)	**0.51**	0.18	2.17	50.28	1.65

Table 3.A2. Two-way ANOVAs testing the effect of activity and substrate on macrofaunal stable isotopic signatures.

Two-way ANOVA of $\delta^{13}C$ for macrofauna on colonization and native substrates

Source	DF	SS	F ratio	Prob > F
Activity	1	837.2	8.29	**0.004**
Substrate	3	552.1	1.82	0.144
Substrate*Activity	3	1517.8	5.01	**0.002**
Error	230	23219.2		

** Significant interaction due to very light $\delta^{13}C$ signature of macrofauna colonizing tubeworm tubes

Tukey HSD Contrasts: Active vs Inactive Substrates

Least Square Means:	Active	Inactive	*P*
Carbonate (Col)	-37.71	-35.20	0.973
Wood (Col)	-33.24	-33.75	1.000
Biogenic (Col)	-43.94	-30.11	**0.001**
Carbonate (Nat)	-37.25	-37.43	1.000

Two-way ANOVA of $\delta^{15}N$ for macrofauna on colonization and native substrates

Source	DF	SS	F ratio	Prob > F
Activity	1	85.8	7.61	**0.006**
Substrate	3	41.0	1.21	0.306
Substrate*Activity	3	15.4	0.46	0.714
Residual	230	2591.4		

Pairwise Contrast: Active vs. Inactive

	Active	Inactive	*P*
Least Square Means:	3.289	4.452	0.006

Table 3.A3. Mean stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of macrofaunal communities on different colonization substrates (carbonate, wood, biogenic; = "Col") at active and inactive seep settings. Additionally, means are calculated for macrofauna on natural, background carbonates (= "Nat"). Mean is calculated as the average of all species means (minimum of one individual), and N is number of species occurring on that substrate.

	$\delta^{13}C$					$\delta^{15}N$				
	Mean	**SD**	**SE**	**Range**	**N**	**Mean**	**SD**	**SE**	**Range**	**N**
Active										
Carbonate (Col)	-37.71	15.81	2.94	71.8	29	3.76	2.65	0.49	11.0	29
Carbonate (Nat)	-37.25	9.80	1.53	51.7	41	3.03	3.81	0.59	20.0	41
Wood (Col)	-33.24	4.53	0.69	20.5	43	3.39	3.18	0.49	14.8	43
Biogenic (Col)	-43.94	15.62	3.58	71.2	19	2.98	3.08	0.71	9.0	19
Inactive										
Carbonate (Col)	-35.20	9.47	1.56	42.0	37	5.42	2.76	0.45	11.7	37
Carbonate (Nat)	-37.43	9.50	1.94	45.8	24	4.18	4.27	0.87	22.9	24
Wood (Col)	-33.75	6.98	1.43	29.5	24	3.83	3.12	0.64	11.3	24
Biogenic (Col)	-30.11	5.96	1.30	22.0	21	4.74	3.89	0.85	14.8	21
All Active	-37.01	11.60	1.01	76.2	132	3.30	3.25	0.28	20.0	132
All Inactive	-34.37	8.60	0.84	48.4	106	4.64	3.47	0.34	22.9	106
Colonization										
Carbonate	-36.30	12.60	1.55	73.25	66	4.69	2.82	0.35	14.71	66
Wood	-33.42	5.49	0.67	29.54	67	3.55	3.14	0.38	17.40	67
Biogenic	-36.68	13.41	2.12	74.08	40	3.91	3.59	0.57	14.97	40
Native										
Carbonate	-37.32	9.61	1.19	51.72	65	3.46	3.99	0.49	27.49	65

Table 3.A4. Stable isotope means (± SD) ($\delta^{13}C$ and $\delta^{15}N$) for all individuals colonizing experimental substrates in active and inactive locations. Calculations are to the lowest taxonomic level that is still shared by a group of samples. Data are averaged by activity, by combination of substrate type and activity, and as a grand total.

			All Colonization Substrates				Active Colonization Substrates						Inactive Colonization Substrates						Grand total by species	
	Taxon		Active		Inactive		Biogenic		Carbonate		Wood		Biogenic		Carbonate		Wood			
			$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Polychaeta	Ampharetidae	Mean	-30.19	6.08	-36.04	6.86	-38.35	4.65			-27.47	6.55			-36.04	6.86			-32.14	6.34
		SD	8.28	2.42	1.81	0.11	-	-			7.64	2.73			1.81	0.11			7.13	1.92
	Capitellidae	Mean			-25.08	1.30							-25.08	1.30					-25.08	1.30
		SD			6.68	1.03							6.68	1.03					6.68	1.03
	Cirratulidae	Mean	-37.08	0.74	-34.32	6.09					-37.08	0.74	-24.28	7.05	-39.33	5.61			-35.42	3.95
		SD	0.01	2.52	8.90	1.05					0.01	2.52	-	-	2.75	0.90			6.47	3.28
	Dorvilleidae (other)	Mean	-43.65	3.58	-32.54	2.29			-52.80	4.41	-29.52	2.31	-39.22	-1.04	-37.16	1.91	-24.58	4.35	-41.97	3.39
		SD	29.17	3.49	7.51	3.56			34.48	2.13	5.73	4.77	-	-	1.11	1.22	3.27	5.43	27.23	3.48
	Ophryotrocha sp. 4	Mean	-25.04	4.74			-25.04	4.74											-25.04	4.74
		SD	1.09	0.42			1.09	0.42											1.09	0.42
	Parougia sp.	Mean	-94.56	4.33			-94.56	4.33											-94.56	4.33
		SD	-	-			-	-											-	-
	Eulimidae	Mean			-19.92	11.30									-19.92	11.30			-19.92	11.30
		SD			-	-									-	-			-	-
	Euphrosinidae	Mean	-40.27	3.19	-29.32	5.08			-45.32	3.84	-35.23	2.54			-29.32	5.08			-36.62	3.82
		SD	7.13	0.91	-	-			-	-	-	-			-	-			8.09	1.27
	Flabelligeridae	Mean	-34.27	2.44	-35.50	3.94			-39.13	-3.41	-33.06	3.91	-29.39	1.77	-36.99	4.08	-34.09	4.59	-35.06	3.40
		SD	6.12	3.38	4.18	1.57			-	-	6.33	0.96	-	-	2.64	1.64	7.10	0.77	4.76	2.36
	Hesionidae (other)	Mean	-33.30	4.67	-33.62	2.94			-35.29	4.99	-32.02	4.46	-32.02	2.06	-34.24	4.96	-34.80	2.47	-33.43	3.96
		SD	6.44	3.37	4.54	3.48			9.70	4.51	2.82	2.57	6.95	3.61	2.83	0.55	1.93	4.31	5.68	3.48
	Hesionid, black sp.	Mean	-33.31	6.32			-33.31	6.32											-33.31	6.32
		SD	-	-			-	-											-	-
	Hesionid sp. 2	Mean	-32.70	2.53			-33.56	5.34	-31.83	-0.28									-32.70	2.53
		SD	2.57	3.51			3.63	1.86	1.92	1.31									2.57	3.51
	Lacydonidae	Mean	-30.52	6.62	-32.23	7.37	-32.54	5.41	-29.34	6.68	-31.21	6.87	-30.71	6.09	-33.98	7.97	-27.61	9.59	-31.53	7.07
		SD	2.85	1.69	4.47	1.39	-	-	3.60	1.79	2.19	1.95	5.64	1.20	3.12	0.54	-	-	3.91	1.53
	Maldanidae	Mean			-29.40	6.23							-29.40	6.23					-29.40	6.23
		SD			2.68	0.67							2.68	0.67					2.68	0.67
	Nereidae	Mean	-48.40	6.56	-61.25	4.98			-82.15	5.84	-31.53	6.92			-61.25	4.98			-51.62	6.17
		SD	29.28	0.84	-	-			-	-	2.63	0.79			-	-			24.76	1.04
	Paraonidae	Mean	-37.21	7.23	-42.25	6.28	-35.73	7.70			-37.95	7.00			-46.33	7.06	-38.18	5.51	-40.09	6.69
		SD	3.70	0.59	17.19	2.96	-	-			4.91	0.60			27.17	2.33	9.06	4.29	12.64	2.18
	Phyllodocidae	Mean	-20.66	1.21	-21.16	8.85			-20.66	1.21					-21.16	8.85			-20.91	5.03
		SD	0.08	0.51	2.71	0.63			0.08	0.51					2.71	0.63			1.59	4.43
	Polynoidae	Mean	-30.44	-1.14	-32.22	5.25	-34.71	-0.87	-21.38	-0.04	-30.62	-1.70			-32.22	5.25			-31.15	1.42
		SD	7.47	5.48	6.45	3.67	1.78	8.74	-	-	8.87	5.99			6.45	3.67			6.76	5.66
	Sabellidae	Mean			-27.19	7.56									-27.19	7.56			-27.19	7.56
		SD			-	-									-	-			-	-
	Spionidae	Mean			-33.17	3.56											-33.17	3.56	-33.17	3.56
		SD			-	-											-	-	-	-
	Syllidae	Mean			-29.56	3.88							-29.56	3.88					-29.56	3.88
		SD			2.79	2.62							2.79	2.62					2.79	2.62
	Terebellidae	Mean	-35.68	5.16	-37.77	4.52	-39.62	5.08	-36.32	5.52	-27.51	4.03	-40.01	1.72	-38.70	4.17	-34.31	6.81	-36.51	4.91
		SD	7.11	1.74	2.44	2.41	8.13	2.77	6.33	1.30	0.96	2.04	-	-	1.45	2.32	0.13	0.45	5.71	2.00
	Trichobranchidae	Mean	-34.72	6.97	-32.67	4.74					-34.72	6.97	-27.31	10.06	-35.94	4.95	-30.45	1.77	-32.97	5.06
		SD	-	-	4.63	3.82					-	-	-	-	2.72	1.78	4.64	4.54	4.30	3.59

Table 3.A4. Stable isotope means, continued.

			All Colonization Substrates				Active Colonization Substrates						Inactive Colonization Substrates						Grand total by species	
	Taxon		Active		Inactive		Biogenic		Carbonate		Wood		Biogenic		Carbonate		Wood			
			$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Bivalvia (Mollusca)	*Acharax* sp.	Mean	-31.96	2.08	-32.73	5.47					-31.96	2.08					-32.73	5.47	-32.22	3.21
		SD	5.05	0.75	-	-					5.05	0.75					-	-	3.60	2.03
	Bathymodiolus spp.	Mean	-35.45	-6.49							-35.45	-6.49							-35.45	-6.49
		SD	-	-							-	-							-	-
	Nuculanidae	Mean	-35.67	5.60							-35.67	5.60							-35.67	5.60
		SD	3.65	0.45							3.65	0.45							3.65	0.45
	Yoldiella sp.	Mean			-39.72	5.41									-43.34	4.79	-36.10	6.04	-39.72	5.41
		SD			5.12	0.88									-	-	-	-	5.12	0.88
Gastropoda (Mollusca)	*Cataegis myronfeinbergi*	Mean	-40.46	3.99	-38.06	5.03	-45.56	4.05	-40.29	4.47	-35.80	2.72	-33.83	6.76	-40.67	5.75	-37.56	3.44	-39.20	4.54
		SD	5.17	1.62	5.94	1.87	3.51	1.35	5.13	1.86	1.04	1.00	10.50	0.32	6.38	1.65	2.67	1.32	5.57	1.79
	Cocculiniformia	Mean	-44.09	0.79							-44.09	0.79							-44.09	0.79
		SD	-	-							-	-							-	-
	Lepetodrilus guaymensis	Mean	-32.63	2.20	-33.91	1.35	-41.29	-0.19	-31.01	2.33	-27.76	4.28			-35.57	1.56	-33.43	1.29	-33.15	1.85
		SD	6.31	2.42	3.77	1.75	3.13	3.83	4.82	0.98	1.30	1.58			9.17	0.13	1.93	2.01	5.34	2.17
	Neolepetopsis sp.	Mean	-47.04	1.13	-48.33	-0.77	-58.00	-0.78	-47.47	2.23	-35.22	0.87			-42.31	1.01	-51.34	-1.66	-47.93	-0.18
		SD	9.55	1.45	6.73	2.44	-	-	3.66	0.42	-	-			8.50	1.91	3.31	2.28	7.31	2.31
	Levinaegyra n. sp.	Mean	-29.80	4.99					-29.80	4.99									-29.80	4.99
		SD	2.38	2.68					2.38	2.68									2.38	2.68
	Paralepetopsis sp.	Mean	-46.86	0.37	-49.72	-0.06	-55.08	-1.06	-46.43	0.38	-40.17	1.78	-42.49	-0.23	-52.91	-0.38	-49.38	0.10	-48.29	0.16
		SD	8.41	1.53	3.53	2.52	3.16	1.29	8.67	0.98	4.44	2.84	-	-	2.48	4.17	2.19	2.17	6.46	2.05
	Provanna laevis	Mean	-37.37	3.26	-39.78	2.43	-49.10	1.33	-34.04	4.68	-34.64	1.98	-32.25	1.86	-44.23	1.49	-37.45	3.79	-38.35	2.92
		SD	9.27	2.97	6.15	1.90	11.05	3.61	6.68	2.37	4.50	2.55	1.80	2.67	4.93	1.55	4.18	1.48	8.12	2.59
	Pyropelta corymba	Mean	-37.71	0.93	-37.05	-0.44	-47.21	0.53	-35.41	0.51	-32.69	2.88	-37.10	2.27			-37.03	-1.35	-37.58	0.67
		SD	8.34	3.23	2.26	3.00	7.26	1.01	7.04	4.03	3.01	1.38	-	-			2.76	2.94	7.51	3.16
	Pyropelta musaica	Mean	-40.63	0.65	-40.64	0.90	-52.21	-0.26	-37.87	1.05	-38.36	0.55			-47.10	0.35	-36.33	1.27	-40.63	0.73
		SD	7.42	1.43	7.74	1.28	2.69	1.65	6.71	1.34	2.17	1.39			7.53	1.29	4.27	1.25	7.40	1.37
	Pyropelta wakefieldi	Mean	-36.61	1.29	-44.94	2.43	-51.52	-1.26	-33.40	1.45	-31.36	3.34			-44.94	2.43			-38.54	1.55
		SD	8.43	2.55	12.24	0.65	0.49	0.98	3.77	2.66	2.38	0.80			12.24	0.65			9.57	2.28
	Pyropelta juvenile	Mean			-36.89	5.49											-36.89	5.49	-36.89	5.49
		SD			-	-											-	-	-	-
	Turridae	Mean	-38.66	6.33							-38.66	6.33							-38.66	6.33
		SD	-	-							-	-							-	-
Other Mollusca	Monoplacophoran	Mean	-36.22	1.58							-36.22	1.58							-36.22	1.58
		SD	-	-							-	-							-	-
	Polyplacophora	Mean	-46.68	5.35					-49.79	5.41	-37.34	5.19							-46.68	5.35
		SD	6.80	0.35					3.36	0.40	-	-							6.80	0.35
Crustacea	Amphipoda	Mean	-31.02	6.98	-26.58	2.27					-31.02	6.98	-26.99	0.31	-25.54	7.16			-27.14	2.86
		SD	-	-	1.78	5.06					-	-	1.93	4.61	1.08	1.15			2.28	4.97
	Caridea	Mean	-30.34	7.04	-22.53	7.63	-23.36	7.28	-27.55	7.02	-38.70	6.96			-25.45	6.69	-21.80	7.87	-28.17	7.20
		SD	9.77	1.12	2.03	1.92	2.28	1.89	9.58	0.88	7.28	1.51			-	-	1.39	2.14	9.02	1.36
	Galatheidae	Mean	-30.94	5.52	-39.46	9.95					-30.94	5.52			-39.46	9.95			-33.78	7.00
		SD	6.00	0.56	-	-					6.00	0.56			-	-			6.50	2.59
	Isopoda	Mean			-31.79	9.43							-31.79	9.43					-31.79	9.43
		SD			-	-							-	-					-	-
	Kiwa puravida	Mean	-33.59	5.06	-29.22	6.69	-44.15	4.28	-34.54	5.95	-29.53	3.93					-29.22	6.69	-32.66	5.41
		SD	6.09	2.32	1.75	0.82	-	-	5.77	0.78	2.67	3.68					1.75	0.82	5.69	2.18
Echinodermata	Ophiuroid	Mean	-27.20	3.33	-23.51	6.25			-26.20	4.91	-27.95	2.15	-21.60	7.40	-23.98	5.65	-28.35	5.86	-24.63	5.32
		SD	2.50	3.79	4.12	2.20			1.99	1.36	2.85	4.81	2.61	0.84	4.52	2.63	-	-	4.04	3.05
Nematoda	Nematoda	Mean			-26.05	13.71							-26.05	13.71					-26.05	13.71
		SD			-	-							-	-					-	-
Nemertea	Nemertea	Mean	-39.09	6.13	-24.06	7.99					-39.09	6.13	-23.39	8.54	-26.05	6.34			-27.07	7.62
		SD	-	-	3.68	3.81					-	-	4.20	4.46	-	-			7.44	3.40
Cnidaria	Anemone	Mean			-28.65	6.04									-28.08	8.69	-28.94	4.72	-28.65	6.04
		SD			2.57	7.68									-	-	3.57	10.37	2.57	7.68
Total		Mean	-36.92	3.33	-34.63	3.93	-43.88	2.29	-37.91	3.55	-32.92	3.47	-28.96	4.24	-36.31	4.69	-36.43	2.81	-35.93	3.59
		SD	13.26	3.33	9.17	3.63	13.24	3.63	16.32	3.10	5.24	3.45	6.38	4.02	9.88	3.08	8.39	3.75	11.71	3.47

Table 3.A5. Comparison of corrected Standard Elliptical Areas (SEA_C, *sensu* Jackson et al. 2011) in stable isotope biplots ($\delta^{13}C$, $\delta^{15}N$, see Figure 3.8) for species on both native and colonization carbonates. There were fourteen taxa for which a sufficient number of individuals were analyzed for stable isotopes on both native carbonates and colonization carbonates to use Jackson et al.'s SIBER method. SEA_C, an indicator of dietary breadth for each species, is the area of the ellipse for individuals on native or colonization carbonates. "Prob." refers to the Bayesian likelihood that treatment with a larger SEA_C is truly larger. "SEA_C Overlap" is the area of the ellipses shared by both native and colonization groups. We also calculate the area of the SEA_C that is unique to a particular treatment (= "Unique SEA_C"), and the percent of total SEA_C this represents (= "Area")

Major Taxon	Taxon	SEA_C		Treatment with greater SEAc	Prob.	SEA_C Overlap	Native		Colonization	
		Native	Colonization				Unique SEA_C	Area	Unique SEA_C	Area
Molluscs	*Neolepetopsis* sp.	21.1	34.1	Colonization	0.658	0.6	20.4	97.0%	33.5	98.2%
	Paralepetopsis sp.	23.1	59.1	Colonization	**0.963**	10.3	12.8	55.5%	48.8	82.6%
	Lepetodrilus guaymensis	20.4	24.0	Colonization	0.558	1.3	19.1	93.7%	22.7	94.7%
	Pyropelta musaica	84.8	34.9	Native	**0.988**	28.5	56.3	66.4%	6.3	18.2%
	Pyropelta wakefieldi	46.6	23.2	Native	**0.917**	3.3	43.3	92.9%	19.9	85.8%
	Levinaegyra n. sp.	49.2	29.4	Native	0.780	11.9	37.3	75.9%	17.5	59.6%
	Provanna laevis	48.7	48.3	Native	0.524	33.4	15.3	31.4%	14.9	30.8%
	Polyplacophora	58.2	4.9	Native	**0.990**	4.9	53.3	91.6%	0.0	0.0%
Crustaceans	*Kiwa puravida*	47.1	16.8	Native	**0.981**	3.8	43.2	91.8%	12.9	77.1%
Polychaetes	Polynoidae	45.3	1.7	Native	**0.934**	0.1	45.2	99.8%	1.6	95.1%
	Dorvileidae (except *Parougia*)	441.9	33.4	Native	**1.000**	0.3	441.7	99.9%	33.2	99.2%
	Parougia	158.3	69.3	Native	0.679	7.6	150.7	95.2%	61.7	89.0%
	Hesionid sp. 2	12.5	7.7	Native	0.737	0.0	12.5	100.0%	7.7	100.0%
	Terebellidae	32.3	29.0	Native	0.573	13.4	18.9	58.6%	15.6	53.9%

CHAPTER FOUR

SUCCESSIONAL DYNAMICS AND ENVIRONMENTAL HETEROGENEITY SHAPE MACROFAUNAL TROPHIC STRUCTURE ON HARD, CHEMOSYNTHETIC SUBSTRATES

Abstract

Food webs in cold seep ecosystems differ from surrounding margin habitats largely due to chemosynthetic primary production associated with high concentrations of reduced fluids. However, few studies have documented trophic shifts over time, and trophic relationships of communities associated with hard substrates are generally uncharacterized at seeps. At Hydrate Ridge, Oregon (580 – 800 m depth), we used natural abundances of stable isotopes ($\delta^{13}C$, $\delta^{15}N$) whether trophic relationships of species or trophic diversity of whole communities are affected by seepage activity, substrate type, spatial location, or successional stage. We outplanted substrates at two regions, selecting a total of six active seeps and six inactive sites (30–400 m from seeps) to place experimental substrates, which were characteristic of common chemosynthetic habitats along continental margins: carbonate (seeps), wood (wood falls), and bone (whale falls). Native carbonates from each site were collected since their fauna represented a later successional stage than our colonization experiments. We also performed reciprocal transplants of carbonates between active and inactive sites. After one year, we collected colonization substrates and the transplant rocks, measured $\delta^{13}C$ and $\delta^{15}N$ signatures of individual macrofauna, and used community isotope metrics to compare trophic structure among treatments.

Based on stable isotopic signatures and taxonomic affinity, most epifaunal species were found to be heterotrophs dependent on *in situ* primary production or sinking POC. Clear differences in community isotopic patterns were apparent between active and inactive sites, and between carbonates of different successional stages. Substrates colonized at active sites had mean $\delta^{13}C$ signatures reflecting a strong dependence on chemosynthesis, and standard elliptical areas (SEA_C) were more similar to native carbonates than those at inactive areas. If trophic recovery within successional communities were rapid, we would expect to see communities on colonization substrates and transplanted carbonates with stable isotopic signatures resembling those on nearby native carbonates. Colonization substrates did contain communities with similar mean $\delta^{13}C$, $\delta^{15}N$, and SEA_C to native carbonates. However, transplanted carbonates showed more rapid trophic recovery at active seeps than at inactive sites. Different types of substrates showed similar community isotopic patterns, though bones contained communities with higher mean $\delta^{15}N$ and larger ranges of $\delta^{15}N$ than carbonate and wood. Together, these results suggest that during early succession, the assembly of seep food webs at active and inactive sites resembles those of natural communities. However, early successional communities after one year lag behind later successional stages in terms of breadth of resources and trophic diversity. As most seep carbonate epifauna at Hydrate Ridge are heterotrophic, our results suggest that microbial succession might place a constraint on the pace at which the metazoan community can recover from disturbance or colonize new habitats.

Introduction

Methane seeps are unusual in the context of the deep sea because they contain high rates of primary productivity, biomass, and faunal abundance (Levin 2005). Despite being far removed from the photic zone, the prevalence of reduced compounds creates an environment dominated by chemosynthetic microbes. While some metazoans (e.g. clams, tubeworms, mussels) gain nutrition from symbiotic partnerships with associated microbes living inside or on specialized structures, most species at cold seeps are heterotrophs ranging in size from single-celled foraminifera to predatory fish. Feeding strategies for these species involve different ways of consuming microbes (both Bacteria and Archaea), including benthic grazing of filamentous mats or on hard substrates, filter feeding, deposit feeding, and consumption of Archaea.

Stable isotopes have been used to improve our knowledge of trophic structure and basal food web dynamics among macrofauna in chemosynthetic ecosystems (Van Dover 2007). They can provide evidence for the source of carbon fixed by primary producers and supporting higher trophic levels, underscore the importance or absence of predation, quantify the breadth of basal feeding strategies, and highlight the existence of niche partitioning or competition for common resources (Levin and Michener 2002; Thurber et al. 2010; 2012; Becker et al. 2013; Levin et al. 2013). Cordes et al. (2010) used stable isotopes to show that temporal succession of tubeworm bushes at Gulf of Mexico seeps leads to increased predation and a decreased use of chemosynthetic production in lower trophic levels. However, stable isotopes do not lead to perfect maps of food webs. Ambiguity can result when there are more food resources present than can be sampled, or

when fractionation patterns are inconsistent. Previous researchers have observed that nitrogen pools are not well characterized at cold seeps (Becker et al. 2014), and nitrogen dynamics probably do not reflect a 3.4‰ shift in $\delta^{15}N$ per trophic level, as is often assumed in photosynthesis-based food webs (Post 2002; Thurber et al. 2012).

Models of succession in cold seep environments have been developed by linking the observations of assemblages to age estimates of habitat-forming species or measurements of geochemical proxies for fluid flow. Community structure is strongly dependent on flow rates and chemical concentrations in sediments, which in turn is related to the complexities of below-seafloor fluid pathways (Hornbach et al. 2007). A progression of successional stages has been described for seeps in the Gulf of Mexico (Cordes et al. 2009) and New Zealand seeps (Bowden et al. 2013) that are likely relevant to other regions. Generally, the onset of seepage and high flux rates introduces methane-rich fluids to the sediment-water interface. Anaerobic oxidation of methane (AOM) within sediments begins to precipitate carbonate and generate sulfide, which then fuels production in sulfide-oxidizing bacteria (SOB). Continued carbonate precipitation may lead to within-sediment nodules and above-sediment cobble, boulders, and pavements ("chemoherm") that serve as attachment sites for epifauna and can be sites of dense chemoautotrophic production. But as carbonate build-up continues, it can eventually constrict and block the path of fluids moving toward the surface, redirecting them to the periphery of the habitat or to new sites, restarting succession. The chemoherm can be colonized by species not dependent on seeps, especially sessile filter feeders, and

continues to serve as complex habitat supporting regional non-seep species, including predatory fish and crabs targeted by fisheries.

Hard substrates are characteristic of many chemosynthetic ecosystems, adding a type of heterogeneity that is typically absent from the deep sea, >95% of which is covered in sediments (Glover and Smith 2003). In addition to carbonates at cold seeps, hard substrates in reducing ecosystems include sunken wood ("wood falls"), bones from whales and other large tetrapods, and basalt and metal-sulfide chimneys at hydrothermal vents. (Seamounts and mid-ocean ridges are also characterized by rocky substrata, but these are not common along continental margins.) While deep-sea chemosynthetic ecosystems tend to contain species with shared evolutionary lineages, few species have been found in multiple systems (Sibuet and Olu 1998; Sasaki et al. 2010; Stiller et al. 2013). Comparing the ways in which species colonize and use different substrates will allow us to measure relationships between substrate and food web structure in the early stages of succession, and possibly infer how constraints relating to trophic resources might shape evolutionary patterns over time. Better understanding of successional dynamics and food web structure will improve our ability to predict the results of perturbations in the deep sea, whether natural or anthropogenic. It is critical that we use information gained from natural communities to improve our forecasts of impacts related to bottom fishing, mining activities, oil and gas hydrate extraction, or climate change, each of which is likely to create disturbances across many spatial and temporal scales in the coming century (Ramirez-Llodra et al. 2011).

Objectives

We use stable isotopes as a tool for investigating trophic structure across individual substrates, by means of community stable isotope metrics developed by Layman (2007) and extended by Jackson (2011). These allow us to address specific questions about how macrofaunal trophic patterns respond to environmental heterogeneity. Additionally, by comparing carbonates at different successional stages, we address how location within a seep setting might influence trophic recovery following a disturbance.

We analyzed carbon and nitrogen stable isotopic signatures ($\delta^{13}C$, $\delta^{15}N$) of macrofauna from experimentally manipulated substrates to address the question of whether trophic structure of seep communities is altered by corresponding changes in the environment. In an ecologically resilient system, we might expect that environmental changes or disturbances would not lead to altered trophic structure compared to a stable, natural community. Observations of stable trophic structure across substrates of different types, in different fluid flow environments, or for which macrofaunal communities have experienced varying lengths of successional time would be consistent with the view of seeps being ecologically resilient. Experiments and field sampling were designed to simultaneously compare effects of chemical environments [sites near or far (30–400m) from active fluid seepage; = “Active” and “Inactive” sites, respectively] and substrate types (carbonate, wood, and bone) on stable isotopic signatures of macrofaunal invertebrates. By examining stable isotopic signatures of community members (a) that colonized experimental substrates during a one-year deployment, (b) on carbonates that

were transplanted to new environments, and (c) on native carbonates collected from Hydrate Ridge, we examine the role of successional time in shaping macrofaunal trophic structure. Specifically, we hypothesize that:

(1) Overall trophic structure of macrofauna on hard substrates will differ between active and inactive sites.

(2) Due to differences in successional time, one-year old experimentally outplanted carbonates will have different macrofaunal community isotopic metrics than on native carbonates. Specifically, we expect to observe reduced total trophic diversity (standard elliptical area), utilization of basal food sources (carbon range), number of trophic levels (nitrogen range), and species packing (mean nearest-neighbor distance) for communities on experimental carbonates with less time to undergo succession.

(3) Carbonate rocks that are moved between active and inactive sites will take on the trophic characteristics of their new environment and individuals will have isotopic compositions that appear as a blend of active and inactive food sources. We hypothesize that recovery rates will differ depending on the direction carbonates are moved, and that the community and food web on rocks moved to active sites will more rapidly resemble active controls.

(4) At active sites, where chemosynthetic microbes provide the main source of organic carbon, trophic structure will be similar on experimental carbonate, wood, and bone.

(5) At inactive sites, where chemosynthetic microbes might be less productive, the organic substrates wood and bone will host communities with a different trophic structure than on carbonate (greater trophic diversity and broader basal food sources).

Materials & Methods

Study site

Hydrate Ridge (44°40'N, 125°6'W) contains several seafloor mounds along an accretionary prism formed by subduction of the Juan de Fuca oceanic plate underneath the North American continental plate (Figure 4.1) (Kulm et al. 1986). Tectonic compression and rapid burial of sediments lead to the microbial and thermogenic formation of hydrocarbon-rich fluids that migrate through faults, form sub-surface gas hydrate deposits, and support active methane seepage and dense chemosynthetic communities in several regions along the 25 km-long ridge (Suess et al. 1999; Tryon and Brown 2001). Hydrate Ridge contains subsurface frozen methane hydrate (Tréhu et al. 1999), steep physicochemical gradients (Boetius et al. 2000), high anaerobic oxidation of methane rates (AOM) (Elvert et al. 1999), and biological communities utilizing the high sulfide concentrations that result (Tryon and Brown 2001; Sahling et al. 2002). The seeps on Hydrate Ridge's mounds are impacted by the oxygen minimum zone, which extends from ~650–1100 m on the Oregon margin (Helly and Levin 2004). We measured bottom-water oxygen concentrations between 0.34–0.52 mL L^{-1} at the northern mound (hereafter "HR North", 580 m) and 0.20–0.24 mL L^{-1} at the southern mound ("HR South", 800 m)

in August 2010. Despite our understanding of the geochemistry and microbiology of this seep and its effect on the trophic patterns of soft-sediment communities (Levin and Michener 2002; Levin et al. 2013), the hard substrate macrofaunal assemblage remains enigmatic and unquantified, as is the case for many cold seeps (Levin 2005).

Field methods

Material was collected and experiments were conducted during two cruises to Hydrate Ridge aboard RV *Atlantis* (Figure 4.1). During leg AT15-68 (1 – 8 August 2010) we used DSV *Alvin* to deploy colonization experiments and collect *in situ* carbonate rocks (= "native") to quantify the natural macrofaunal community. About one year later, on leg AT18-10 (31 August – 6 September 2011), we used ROV *Jason II* to retrieve experiments and collect additional rocks. Colonization experiments consisted of defaunated substrates that mimicked hard substrates associated with deep-sea chemosynthetic ecosystems: seep carbonate, wood, and bone (both whale and pig, representing large food falls). Wood was cut blocks of untreated Douglas fir *Pseudotsuga menziesii*, and bark-covered pieces of fir and pine (*Pinus* sp.) native to nearby coastal forests. Each substrate was enclosed by 1.6 cm mesh polypropylene netting that was tied off with a floating polypropylene loop to aid handling. Lead weights, completely wrapped in duct tape, were attached to wood and bone to ensure they were negatively buoyant and not lost during submarine descents.

Colonization experiments and collection of native carbonates were conducted at areas of active seepage and inactive areas, both at HR North (585–620 m) and HR South (774–810 m) (Figure 4.1, Table 4.1). Sites for experiments were chosen by scientists

diving in DSV *Alvin* based on observations of bubbling gases, shimmering water, bacterial mats, bacteria-covered carbonates, and clam beds to indicate seepage of reduced fluids (= "Active"). Sites that contained carbonates but no visible signs of seeping fluids or chemosynthetic activity were judged to be "Inactive" (though subsequent microbiological analyses indicate they are not completely devoid of chemosynthetic activity, V. Orphan & D. Case, personal communication). Clusters of substrates were placed on the seafloor so they did not contact each other, but fell within a 1–2 m radius. Imagery was collected with submersible cameras after deployment and again before recovery. Native carbonates were typically collected from sites after experiments were deployed (Table 4.1).

Additionally, at HR North we conducted a reciprocal transplant experiment, in which native carbonates were picked up with *Alvin*'s manipulator and either transferred to a different chemical environment (Active transferred to Inactive = A-TR; Inactive transferred to Active = I-TR) or placed back in the same place as a control (A-CT and I-CT). Transplants were conducted between two sets of paired active and inactive sites. The misidentification of transplanted carbonates upon retrieval resulted in more control rocks than transplanted rocks (see Table 4.1).

All substrates were collected by individually transferring them with DSV *Alvin* or ROV *Jason II*'s manipulator into a Plexiglas compartment in a subdivided, insulated biobox, so that macrofauna were kept separate and communities could be quantified.

Laboratory methods for stable isotope analysis

Once on board the ship, substrates were immediately moved into a cold room (4°C). After substrates were photographed, macrofauna (>300 μm) were picked or washed from the substrate and sorted live under dissecting microscopes. For stable isotope analysis, specimens were identified to the lowest possible taxonomic level and tissue samples were rinsed in Milli-Q water and placed in pre-weighed tin boats with methanol-cleaned forceps. Larger meiofauna, foraminfera, and Bacteria were also picked for isotope analysis, when feasible. Pieces of carbonate and wood were stored in clean, combusted vials. Samples were kept frozen (-80°C) until return to the lab where they were dried to constant weight (60°C for 24–48 hours).

Known masses of tissue (approximately 0.20–1.0 mg) were acidified with 12.5–25 μL of 1% $PtCl_2$ in 1M HCl and allowed to off-gas and dry, before being compressed inside the tin boat. Several grams of carbonate were ground into a fine powder, and wood was grated into small particles, using methanol-cleaned equipment. Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) of tissue samples and substrates were measured on a Costech elemental analyzer coupled to a Micromass Isoprime isotope ratio mass spectrometer (EA/IRMS) at Washington State University (R. W. Lee). Approximately 1 mg of wood was used to get readings for both $\delta^{13}C$ and $\delta^{15}N$. Carbonate powder was acidified with PO_4 to remove inorganic carbon, and the resulting organic fraction (10–50 mg) was analyzed for $\delta^{13}C$. In total, we generated stable isotope data for 895 individuals from 84 distinct taxa, including macrofauna, meiofauna, protozoans, and bacteria (summary in Appendix 4.1).

Stable isotope values are expressed in the standard δ (delta) notation and reported in unts of per mil (‰), where the element X is represented by:

$$\delta X = \frac{R_{\text{sample}}}{(R_{\text{standard}} - 1)} \times 1000$$

where X is ^{13}C or ^{15}N, and R is the ratio of $\frac{^{13}C}{^{12}C}$ or $\frac{^{15}N}{^{14}N}$ (Fry 2006). Standards were Pee Dee Belemite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$.

To compare faunal isotopic signatures to potential food sources, we also measured the $\delta^{13}C$ and $\delta^{15}N$ of *in situ* particulate organic carbon (POC), large bacterial filaments from substrates, wood ($\delta^{13}C$ only), and the organic fraction of carbonate rocks ($\delta^{13}C$ only). We collected surface and bottom water in Niskin bottles on CTD casts and filtered 2–4 L per sample on combusted glass fiber filters. Concentrated POC was removed from the filter and analyzed for $\delta^{13}C$ and $\delta^{15}N$.

Statistical methods

Since samples were processed live on two different cruises with non-identical personnel, individual records were grouped at a taxonomic level we could ensure would provide consistency between years. For example, many gastropods were identified to at least genus and often to species, but most polychaetes could not be identified to species quickly, and thus are often (but not always) binned at the family level. Taxa from other phyla are often binned at much broader levels (e.g., nemertea, ophiuroidea, etc.).

Two- and three-way analyses of variance were used to test for treatment effects on stable isotope signatures of macrofauna. For carbonates, to determine which factors

influenced macrofaunal isotope signatures, we considered two levels of Activity (active and inactive), two Regions (HR North and HR South), and two successional Stages (colonization carbonate, and native carbonates). For communities on colonization substrates, only activity and substrate type (carbonate, wood, bone) were investigated for effects on mean stable isotope signatures of macrofauna. All factors were considered fixed, and analyses were performed in JMP® v 11.1 (SAS Institute Inc. 2013).

We calculated quantitative trophic metrics of communities for groups of taxa representing different treatment groups (activities, successional stage, or colonization substrate type). These metrics were developed by Layman et al (2007) and placed in a Bayesian framework by Jackson et al (2011). CR and NR respectively represent the range in carbon and nitrogen between species with the highest and lowest $\delta^{13}C$ and $\delta^{15}N$ values, and provide a measure of the breadth of carbon sources and number of trophic levels present in the community. The mean distance to centroid (CD) is a measure of trophic diversity. Mean nearest neighbor distance (MNND) and standard deviation of nearest neighbor distance (SDNND) can be thought to represent degree of species packing, and trophic evenness in the community, respectively. The total convex area (TA) is the two-dimensional area in an isotopic bi-plot encompassed by the members of a community, and is a measure of the total available isotopic niche (Layman et al. 2007). Since TA is especially biased by uneven and incomplete sampling, we created standard elliptical areas corrected for sample size (SEA_C) and also used the mean of 10,000 posterior draws to create Bayesian estimates of the standard elliptical area (SEA_B; Jackson et al. 2011). For colonization substrates, SEA_B (ln-transformed) was examined in a two-way analysis of

variance to test whether activity or substrate affected the isotopic-based trophic diversity of the community. All isotope metrics were calculated using R v.3.0.2 (R Core Team) and the package siar (Parnell and Jackson).

Results

Trophic structure of carbonate-hosted communities

A total of 484 individuals from 68 taxa were recovered from native or colonization carbonate rocks at HR North and HR South. While mean $\delta^{13}C$ of macrofauna was significantly lower at active sites than inactive sites (3-way ANOVA; Activity, $F_{1,531} = 43.2$, $p < 0.0001$; Table 4.2), this difference was diminished at HR South ($\delta^{13}C_{act}$ = -30.7‰; $\delta^{13}C_{inact}$ = -24.4‰) relative to HR North ($\delta^{13}C_{act}$ = -33.3 ± 0.55‰ (SE); $\delta^{13}C_{inact}$ = -21.1 ± 1.36‰; Activity * Region, $F_{1,531} = 4.86$, $p = 0.028$). Mean $\delta^{15}N$ of macrofauna was significantly greater on inactive carbonates (11.0 ± 0.77‰) than on active carbonates (5.60 ± 0.25‰) for all treatments ($F_{1,494} = 44.4$, $p < 0.0001$). Successional stage (whether carbonates were native to Hydrate Ridge or deployed for one year) did not affect overall macrofaunal isotopic signatures (Table 4.2).

The Layman metrics revealed the total range of stable isotopic carbon and nitrogen to be two to four times higher for communities on native carbonates than on colonization carbonates (Table 4.3). Accordingly, total convex area was markedly less on colonization carbonates than native carbonates for both active (263‰ vs. 594‰) and inactive sites (32‰ vs 397‰). These effects of successional stage on the area in isotopic space utilized by each community are clearly illustrated in Figure 4.2, as ellipses (SEA_C)

are larger and are stretched further upward for native than for colonization carbonates. Other Layman metrics did not show strong differences among treatment groups, although mean distance to centroid on inactive colonization carbonates (3.9‰) was less than every other treatment (range: 6.0–7.4‰, Table 4.3).

Comparison between transplanted and control carbonates

Control carbonates, those lifted and set back down, contained communities whose mean $\delta^{13}C$ signature resembled the natural carbonate communities (Figure 4.3). Transplanted carbonates, on the other hand, contained communities with a mean $\delta^{13}C$ signature intermediate to active and inactive control communities. Some taxa on transplanted rocks showed isotopic signatures reflecting the original habitat. In particular, the vetigastropod limpet *Pyropelta corymba* had a mean $\delta^{13}C$ of -38.2‰ (n = 4) on rocks moved to an inactive site 89 m from the nearest seep, which is even lighter than the entire mean assemblage on active control carbonates (Figure 4.3). A sponge and arborescent foraminiferan on rocks moved to the reciprocal active site maintained heavier $\delta^{13}C$ signatures (-23.4 to -21.9‰) reflective of their original inactive sites. Other taxa on transplanted rocks had isotopic signatures more similar to the new location. An individual of *Provanna lomana* on a carbonate transplanted to an active site, and an ophiuroid and polynoid polychaete transplanted to an inactive site all had $\delta^{13}C$ signatures similar to the control assemblages at those new sites. Similar trends were observed in patterns of $\delta^{15}N$, which was lowest on active controls, highest on inactive controls, and intermediate for transplanted assemblages.

Trophic structure of macrofauna colonizing carbonate, wood, and bone

Of macrofauna colonizing experimental substrates, we analyzed isotopic data from 382 individuals in 44 taxa (6–24 taxa, 13–127 individuals per treatment group). The type of wood used in colonization experiments had no effect on mean $\delta^{13}C$ (ANOVA, $F_{2,212} = 0.61$, $p = 0.55$) or $\delta^{15}N$ signature of macrofauna ($F_{2,204} = 0.37$, $p = 0.69$), so data for all wood were combined for the analysis (ANOVA, $\delta^{13}C$: $F_{2,212} = 0.61$, $p = 0.55$; $\delta^{15}N$: $F_{2,204} = 0.37$, $p = 0.69$). Colonizing macrofauna at active sites had significantly lower mean (± SE) $\delta^{13}C$ (-30.38 ± 0.37‰) than at inactive sites (-21.09 ± 0.74‰), while there were no differences among substrate types (ANOVA, $F_{1,2} = 127.1$, $p < 0.0001$; Table 4.4). Macrofauna had higher $\delta^{15}N$ on inactive substrates (11.13 ± 0.59‰) than active substrates (5.28 ± 0.30‰; ANOVA; $F_{1,2} = 100.6$, $p < 0.0001$). Substrate also had a significant effect on macrofaunal $\delta^{15}N$ signature (ANOVA; $F_{2,2} = 4.92$, $p = 0.008$), which on average was about 2‰ higher for individuals on bones than on wood or carbonate (Tukey HSD, $p<0.001$). Three of the five highest $\delta^{15}N$ signatures observed in this study came from individuals on bone, including a nemertean, dorvilleid, and *Osedax* ($\delta^{15}N$ = 40.17, 28.44, and 27.0‰, respectively).

In addition to mean stable isotopic signatures, seepage activity impacted trophic structure measured as SEA_B, which was over twice as large for communities on active substrates (46.5 ± 6.63‰) as those on inactive substrates (22.6 ± 8.4‰; $F_{1,2} = 4.97$, $p = 0.03$; Table 4.4). SEA_B it was not significantly affected by substrate type ($F_{2,2} = 1.75$, $p = 0.19$).

Discussion

The impact of seep activity on trophic recovery

The significant differences in stable isotopic signatures of macrofauna at active and inactive sites was expected (H_1; Figure 4.2), as they reflect geochemical heterogeneity at Hydrate Ridge that has previously been observed to impact sediment-hosted food webs (Levin and Michener 2002; Bowden et al. 2013). Macrofaunal communities at active sites have a wider range of $\delta^{13}C$, most of which is derived from chemosynthesis, while communities associated with inactive carbonates appear to be supported generally by surface POC production. In contrast, the differences between early- and late-successional food webs were more subtle. After one year, macrofauna on colonization carbonates at both active and inactive sites had similar signatures to those on native carbonates. At active sites, the main difference observed for colonization communities was a decrease in $\delta^{15}N$ range (Table 4.3) that might suggest an absence of predators and/or decomposers in early successional stages. At inactive sites, colonization carbonates had a substantially smaller $\delta^{13}C$ and $\delta^{15}N$ range compared to native carbonates, resulting in a much smaller SEA_C and indicating a narrowed set of food sources. Additionally, centroid distance was reduced on inactive colonization carbonates. Taken together, these results suggest a reduction in trophic diversity and complexity at inactive sites early in succession. Slower recovery rates at inactive sites reflect a slower recovery of macrofaunal abundance on the same substrates (Chapter 5; Chapter 3 for Costa Rica). In methane seep ecosystems, just as proximity to reduced fluid flow drives colonization rates, it also has a strong effect on the assembly of food webs.

We expected that a more rapid recovery would also be observed on inactive carbonates transplanted to active sites compared to the reverse, since high productivity rates should be associated with rapid microbial colonization and population growth. However, this was not necessarily the case. Both groups of transplanted carbonates had an assemblage with mean $\delta^{13}C$ that fell in between those of control active and inactive carbonates (Figure 4.3), but we did not obtain stable isotope data from enough individuals to determine relative rates of change in $\delta^{13}C$. Microbial community composition of transplanted rocks supports our hypothesis (H_3) that at least a portion of the community shows more rapid recovery when moved into active sites (D. Case & V. Orphan, unpublished research). Such an effect of higher fluid flow rates on organismal performance has been observed for bathymodiolin mussels subjected to similar experimental transplants between microhabitats at hydrothermal vents (Smith 1985) and hydrocarbon seeps (Dattagupta et al. 2004). We did not expect macrofauna on active rocks transplanted to inactive areas would be successful, but surprisingly, four *Pyropelta corymba* recovered from such rocks maintained relatively light $\delta^{13}C$ signatures, despite being spatially removed (~90 m) from strong influence of reduced fluids. Additionally, this limpet specializes in living on the shells of provannid snails, but no *Provanna* spp. were recovered from these carbonates. We can conclude that despite a different chemical microhabitat and the loss of its typical substrate, *P. corymba* was able to continue to graze on the surface of rocks, and perhaps consumed portions of the carbonate itself to maintain a $\delta^{13}C$ reflective of chemosynthetic production. This observation also reflects the ability of some chemoautotrophs to maintain production in conditions with relatively low concentrations of methane or sulfide.

Ecological resilience in a methane seep community

Community resilience and recovery is partially a function of species' individual abilities to tolerate changes in productivity patterns. Our experimental colonization substrates initially lacked an *in situ* microbial community to provide faunal trophic support. That many members of the ambient community were able to colonize these small habitat patches in one year suggests a level of ecological resilience in response to small-scale disturbances. The similar isotopic patterns among substrates (Figure 4.4 A) show that the microbes upon which heterotrophs depend were able to colonize multiple types of surfaces, organic and inorganic, porous and solid, smooth and rough. If species are able to colonize the carbonate, wood, and bones we deployed and have stable isotope signatures resembling conspecifics on native carbonates, it is an indication they might be able to quickly recover from temporary disturbances.

At inactive sites, trophic recovery on carbonates may be slow, but organic substrates recovered higher densities (unpublished data) and contained several individuals with light $\delta^{13}C$ signatures suggesting chemosynthetic production. At HR South, an inactive bone and inactive wood block each contained three gastropods with such signatures (Bone: *Provanna lomana,* -35.1‰; *Neptunea* sp., -31.2‰; *Pyropelta corymba*, -28.6‰; Wood: *Margarites* sp., -29.3‰ and -29.0‰; *Provanna* sp., -28.8‰). This highlights the possibility that species we think of as seep endemics are able to consume chemoautotrophic production from organic substrates they colonize away from methane seeps. Their light $\delta^{13}C$ signatures compared to most taxa at inactive sites could stem from consuming heterotrophic microbes living off the wood or bone, or

chemoautotrophic microbes utilizing sulfide associated with organic degradation. In either case, the ability of larvae to locate and recruit to such substrates is a necessary assumption of the stepping stone hypothesis addressing evolutionary radiations in chemosynthetic environments (Smith et al. 1989). Previous researchers have suggested that these sorts of small, patchy, reducing habitats may allow vent and seep populations to be connected among distant sites via flexible habitat and food requirements (Smith and Baco 2003; Cunha et al. 2013). Our results give support to this hypothesis by showing that it is possible for several seep species to colonize organic substrates at least 300 m from the nearest dense chemosynthetic community supported by vigorous seepage.

Substrate-specific macrofaunal patterns

Communities on bones had a greater $\delta^{15}N$ range than on wood or carbonate (Figures 4.4 B,C), suggesting they might contain more trophic levels, or perhaps nitrogen is being incorporated from multiple sources including the bone itself [typical bone isotopic signatures for (a) cattle: $\delta^{13}C$ = -21‰, $\delta^{15}N$ = 4‰; baleen whales: $\delta^{13}C$ = -13 to -15‰ and $\delta^{15}N$ = 12–16‰ (Schoeninger and DeNiro 1984)]. Individuals on bones with particularly heavy $\delta^{15}N$ signatures included a nemertean (40.2‰), dorvilleid (18.2‰ and 28.4‰), *Osedax* (21.1‰ and 27.0‰), *Cirriformia* sp. (21.6‰), and *Pyropelta corymba* (17.9‰). One possibility is that bone is a substrate that attracts macrofaunal predators, and thus more trophic levels are observed on these substrates. Another explanation is that nitrogen is being incorporated from multiple sources (via bone consumed by *Osedax*, POC, and sulfide-oxidizing bacteria on the surface of the bone), leading to a more diverse $\delta^{15}N$ landscape. Regardless, these observations illustrate the difficulty that is encountered

trying to interpret $\delta^{15}N$ signatures in chemosynthetic ecosystems, where the inorganic nitrogen pool is not well characterized, and N_2 fixation can be performed by a subset of the microbial community (Dekas et al. 2009).

In some cases, consistency in trophic pattern was accompanied by non-consistent colonization rates across substrates. The polychaete *Amphisamytha fauchaldi* colonized organic substrates at active sites, but was rarely found on carbonates. It's $\delta^{13}C$ signature (-29.2‰ on bone, -31.7‰ on wood) indicates that the nutritional source appears similar across substrates (-32.7‰ on carbonate), so colonization patterns might be indicative of a chemical cue, preference for a certain type of surface rugosity, or microbial biofilm that could develop more quickly on organic substrates. As *A. fauchaldi* is one of the few species that has been confirmed from both vent and seep environments (Stiller et al. 2013), its recruitment to organic substrates suggests it is a habitat generalist likely with the ability to colonize most deep-sea reducing habitats.

One problem encountered using stable isotopes to study community trophic patterns is related to the lack of resolution among carbon sources. At Hydrate Ridge, POC had a mean $\delta^{13}C$ of about -23‰, filamentous sulfide-oxidizing Bacteria (SOB) from hard substrates were about -29‰, SOB from bacterial mats were reported as -27‰ (Levin et al. 2013), and the organic fraction of native and colonization carbonates ranged from about -25 to -70‰, reflecting microbial resources that might be available to carbonate-associated macrofauna (Figure 4.4 A). The potential for generalist heterotrophs makes bulk stable isotope analysis an impractical tool for determining the specific diets of individual species. However, the incorporation of compound-specific isotopic

methods, fatty acid analyses, or tracer studies could be used in conjunction with natural stable isotope sampling to improve our picture of methane seep food webs (e.g. Thurber et al. 2012).

Shipboard experiments with isotopic tracers helped elucidate the diet of *Provanna* spp. on carbonates at Hydrate Ridge. We introduced combinations of ^{13}C-labeled and unlabeled methane and bicarbonate to carbonates with their natural assemblages, so that chemoautotrophs could incorporate labeled carbon and pass it on to heterotrophs. Provannid snails in treatments with labeled methane nearly always had δ^{13}C signatures between -33 and -38‰, similar to controls. However, when in treatments with labeled bicarbonate, provannids usually had much heavier δ^{13}C signatures (-24 to 49‰ in >90% of individuals), suggesting they are consuming microbes such as SOB that fix organic carbon from bicarbonate, and not methane. However, it might be a mistake to think of these snails as specialists, since there was an individual *P. lomana* in a labeled-methane treatment whose δ^{13}C signature was -1.5‰. Such experiments can improve our understanding of seep food webs while also allowing us to test specific hypotheses related to ecosystem function of community members. These might include fundamental ecological questions such as whether changing environmental conditions influence interaction strengths between grazers and producers, or whether grazing rates are dependent on the presence or absence of potential competing species, questions that are currently unknown and untested in most deep-sea settings.

As we increase our demand for deep ocean resources, fishing, mining, and prospecting activities will increasingly impact cold seeps and other margin habitats

(2011; Levin and Sibuet 2012). If we want to be able to gauge anthropogenic effects in these systems, we first need an understanding of the structure and dynamics of unimpacted, natural communities. This study measures the pace of recovery of a food web at a cold seep in the absence of acute anthropogenic impacts. Across two different depths and oxygen environments, sites with close proximity to fluid flow contain hard substrate communities that can be expected to quickly recover chemoautotrophic microbial productivity to support macrofauna. Further from seeps, sites characterized as inactive with respect to fluid flow might take longer to recover the diversity of trophic resources and isotopic niche space compared to the native community. The recovery of the seep food web on multiple substrate types suggests that aspects of the community are resilient to changes in this aspect of the environment, which could have implications if restoration of cold seeps is ever attempted. Finally, our work demonstrates that transplants of *in situ* substrates to new geochemical habitats can be expected to result in a successional transition of the associated faunal diets, suggesting a potential tool for measuring community transitional rates, which could be useful for preparation of environmental impact assessments at sites targeted for future extractive activities.

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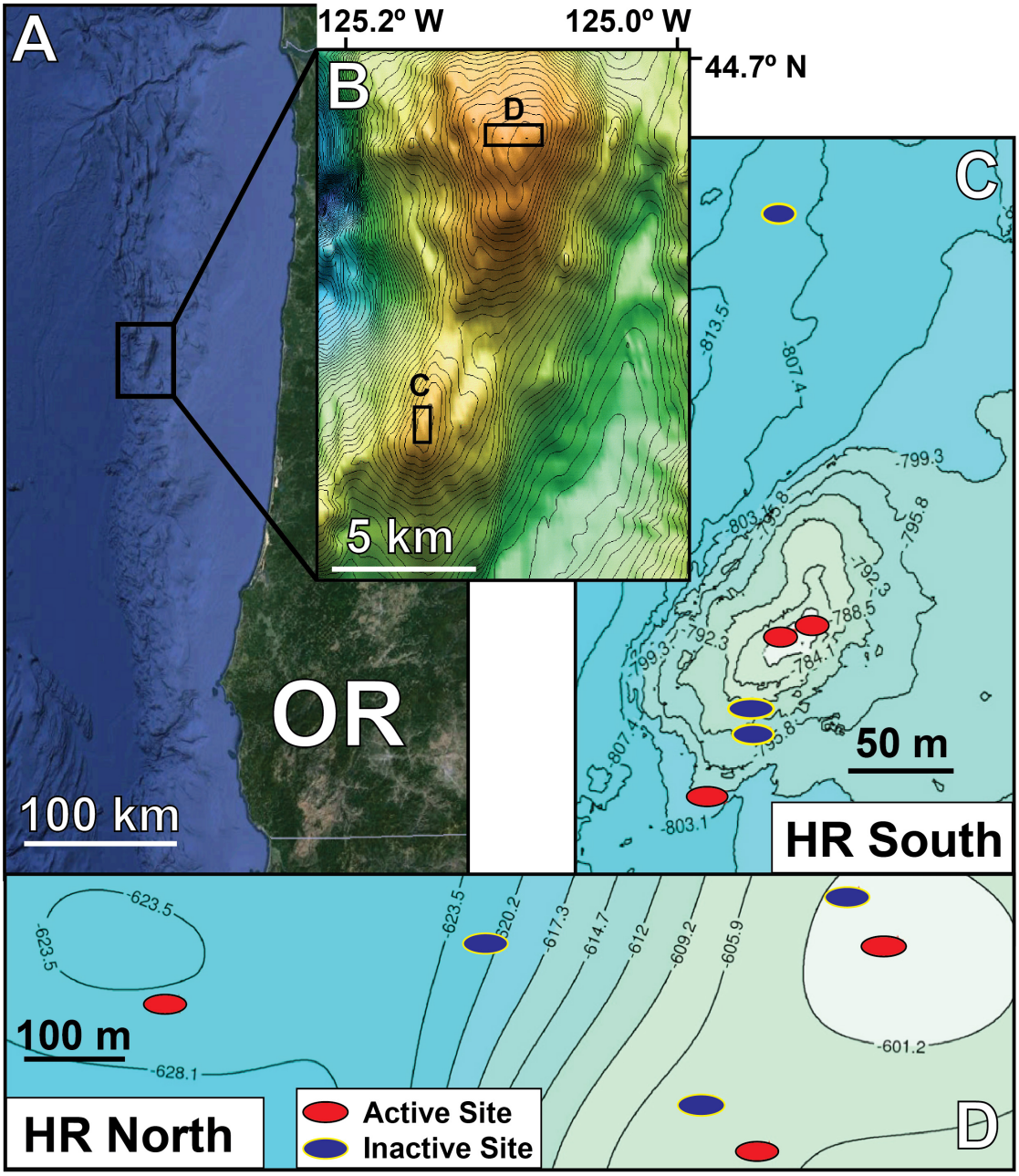

Figure 4.1. A) Map of Hydrate Ridge, Oregon, showing **B)** relative position of the north and south mounds, and locations of substrate experimental deployments at **C)** HR South and **D)** HR North. Red ovals represent active seeps where experimental substrates were deployed, and blue ovals are paired inactive sites, which were spaced 30–400 m from the nearest known seep. Maps courtesy of Google Earth (A) and the Woods Hole Oceanographic Institute (B, C, D).

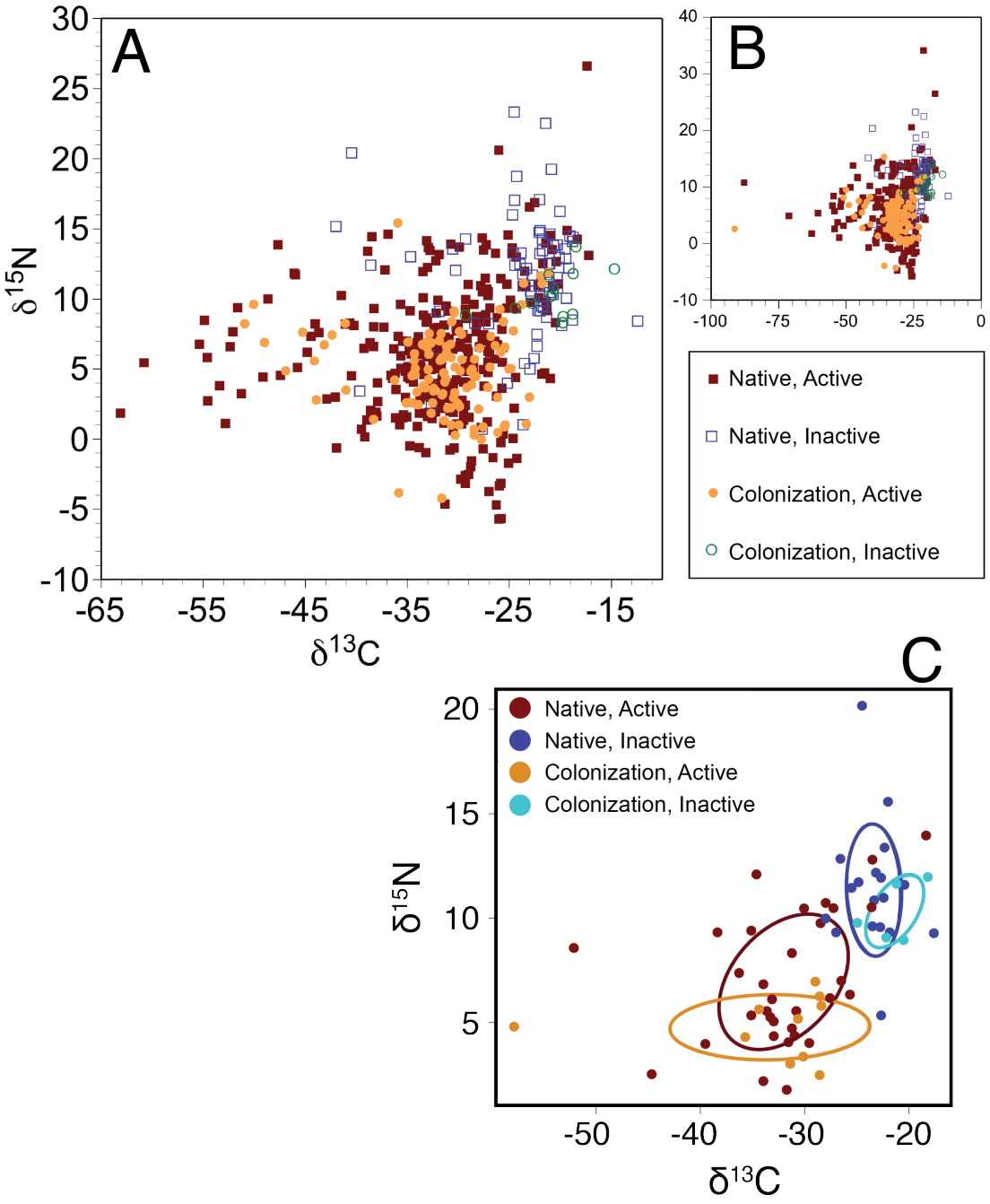

Figure 4.2. Effects of activity and successional stage on macrofaunal stable isotopes. **A)** Each point represents the $\delta^{13}C$ and $\delta^{15}N$ of an individual from native or colonization carbonates at active or inactive sites. Extremely light $\delta^{13}C$ and heavy $\delta^{15}N$ individuals falling outside of plot A) are visible in **B)**. These data were used to calculate community isotope metrics in a Bayesian framework; **C)** Points represent taxon means, and sample-size corrected standard elliptical areas (SEA_C) are drawn to encircle the core isotopic niche utilized by each community. Note, there is less than 50% overlap between successional stages, and no overlap between activity levels.

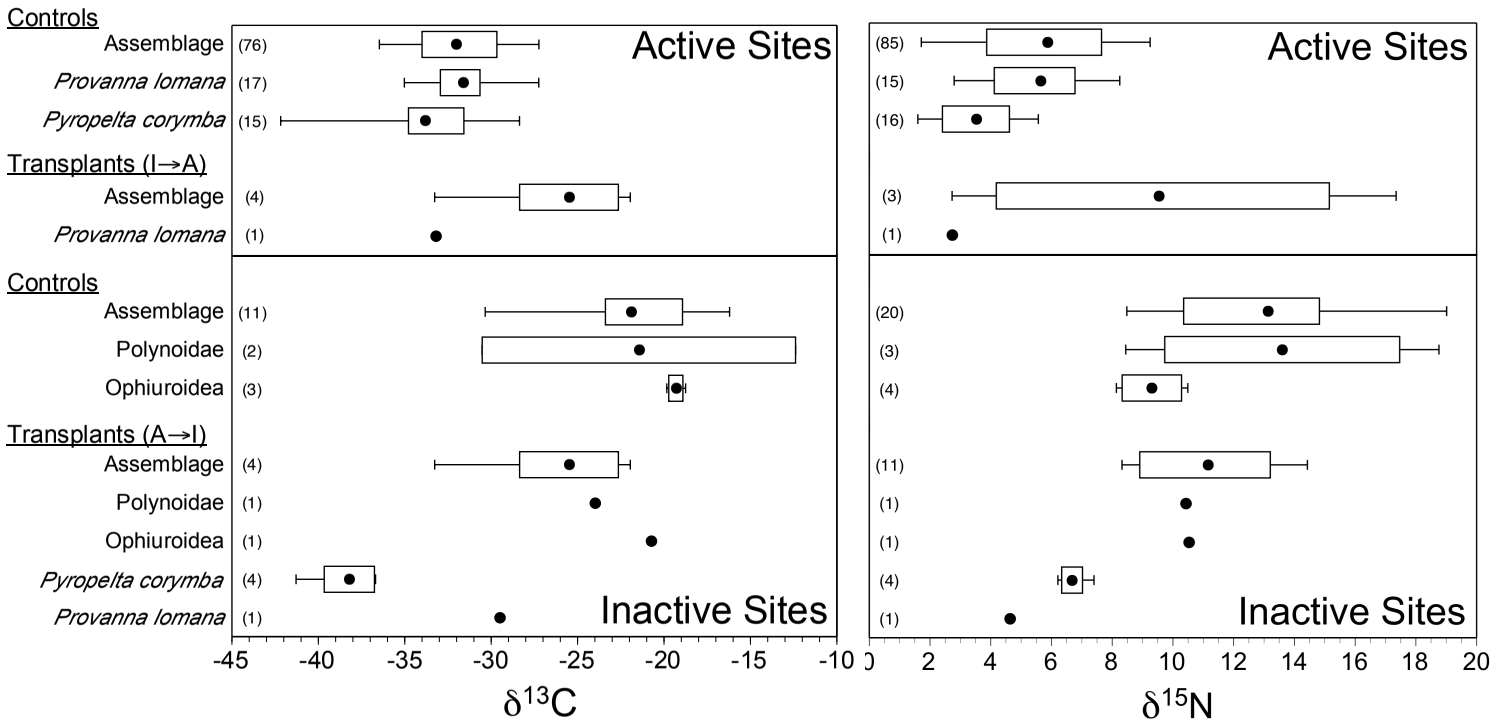

Figure 4.3. $\delta^{13}C$ and $\delta^{15}N$ signatures for entire assemblages and individual taxa on carbonates either transplanted between active and inactive sites, or experimental controls from one site. Points represent the mean $\delta^{13}C$ (left) or $\delta^{15}N$ (right) for each group, and stemmed box plots display 10, 25, 75, and 90 percentiles. Number of individuals analyzed per group is in parentheses at left. All samples are from HR North.

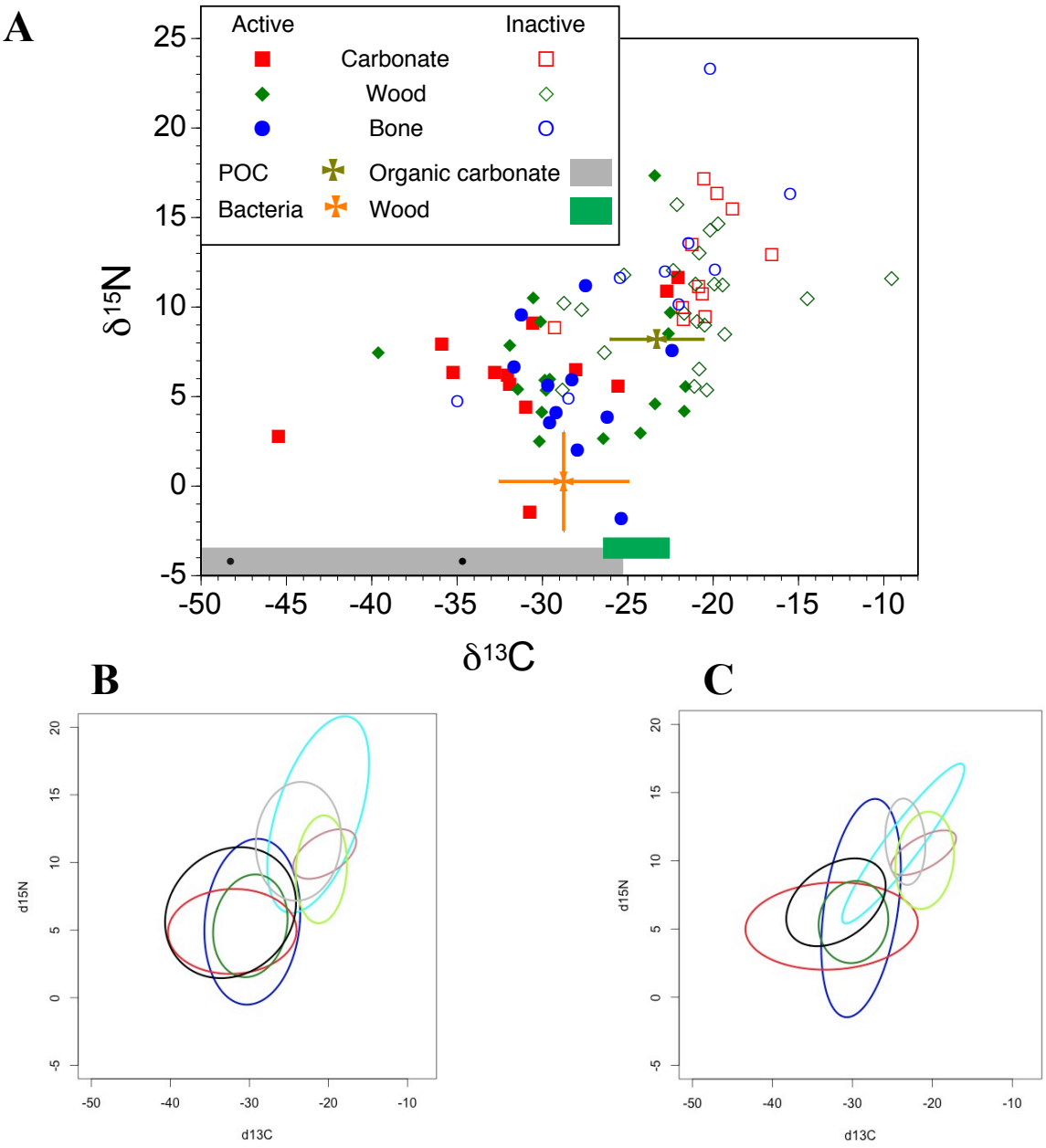

Figure 4.4. **A)** Stable isotopic signatures of macrofauna colonizing different substrates at active and inactive seep environments. Each point is the mean of all individuals of a taxon per treatment (whereas Figure 4.2 plotted signatures of every individual, but only from carbonates). Potential food sources are represented by crosses (± SD) for POC (tan) and filamentous bacteria (orange), and by boxes for organic material in carbonate rocks (grey) and wood (green), for which we measured $\delta^{13}C$ but not $\delta^{15}N$. The width of these boxes represents the $\delta^{13}C$ range (± SD). These data were used to construct SEA_C for each treatment according to Jackson et al. (2011): **B)** using mean isotopic signatures per taxa, and **C)** using data from each individual. Ranges for B) and C) are -50 to -10‰ for $\delta^{13}C$ and -5 to 20‰ for $\delta^{15}N$.

Table 4.1. Substrates for which macrofaunal stable isotope data were generated. Where experimental substrates did not contribute any individuals to isotope analysis, the number of actual substrates collected is shown in parentheses. Colonization experiments were deployed at Hydrate Ridge for one year. Native carbonates were collected during both 2010 and 2011 cruises. The transplant experiment was conducted only at HR North. HR-**x** refers to local sampling sites (Figure 4.1C & D).

	Active Sites							**Inactive Sites**						
	HR North			HR South			Active	HR North			HR South			Inactive
Colonization Experiment	HR-3	HR-6	HR-7	HR-1	HR-9	HR-11	Total	HR-4	HR-5	HR-8	HR-2	HR-10	HR-12	Total
Carbonate	1	1	1	1	1	1	6	1	1	1	0 (1)	1	1	5
Wood blocks	1	1	1	0 (1)	1	1	5	1	1	1	1	1	1	6
Natural Fir (with bark)	1	1	1		0 (1)		3	1	1	1		1		4
Natural Pine (with bark)	1	1	1				3	1	1	1				3
Bone	1	1		1	1		4	1	1		1	1		3
Native Carbonates	4	1	3	5	3	6	22	2	1	1	3	1	2	10
Transplant Experiment [a]														
Active Control	3		3				6							
Transplanted to Active	1		0 (1)				1							
Inactive Control								2		2				4
Transplanted to Inactive								1 (2)		1 (2)				2

[a] Carbonate transplants occurred between HR-3 and HR-4 (80 m apart) and between HR-7 and HR-8 (89 m apart).

Table 4.2. Results of a three-way ANOVA testing effects of activity, successional stage, and region on stable isotopic signatures of macrofauna on carbonates. Bold *P* values represent significant effects ($\alpha < 0.05$).

Source of Variation	DF	SS	F-ratio	*P*
$\delta^{13}C$				
Activity	1	2562	44.57	**<.0001**
Stage	1	38.65	0.672	0.413
Activity*Stage	1	18.53	0.322	0.570
Region	1	3.725	0.065	0.799
Stage*Region	1	14.67	0.255	0.614
Activity*Region	1	263.9	4.590	**0.033**
Activity*Stage*Region	1	36.05	0.627	0.429
Error	531	30527		
$\delta^{15}N$				
Activity	1	852.2	44.37	**<.0001**
Stage	1	51.38	2.675	0.103
Activity*Stage	1	0.308	0.016	0.899
Region	1	2.519	0.131	0.717
Stage*Region	1	3.948	0.206	0.651
Activity*Region	1	14.32	0.746	0.388
Activity*Stage*Region	1	15.96	0.831	0.362
Error	494	9487		

Table 4.3. Community isotope metrics for macrofauna and foraminifera associated with native and colonization carbonates in active and inactive sites at Hydrate Ridge. Means of 10,000 posterior draws are presented (Layman et al. 2007, Jackson et al. 2011). Abbreviations described in text.

	Native Carbonates				Colonization Carbonates			
Layman Metrics	Active		Inactive		Active		Inactive	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NR (‰)	26.8	17.0	24.2	12.3	14.7	7.1	6.0	4.6
CR (‰)	40.0	16.2	28.7	12.0	31.7	12.7	13.5	5.5
TA (‰^{2})	593.6	389.5	396.8	246.5	262.6	151.3	32.0	37.4
CD (‰)	7.4	0.7	6.0	0.8	6.8	1.4	3.9	1.7
MNND (‰)	1.9	0.5	2.0	0.5	3.0	0.9	3.2	1.2
SDNND (‰)	2.2	2.8	2.5	2.5	3.1	2.9	2.8	2.4

Table 4.4. Results of a two-way ANOVA testing for effects of activity and substrate type on stable isotope signatures of macrofauna on colonization substrates, and on ln (SEA.B) calculated for macrofaunal species on individual substrates. Bold *P* values represent significant effects ($\alpha < 0.05$).

	Source of Variation	DF	SS	F-ratio	*P*
$\delta^{13}C$					
	Activity	1	4212	127.1	**<.0001**
	Substrate	2	15.5	0.234	0.791
	Substrate*Activity	2	34.2	0.515	0.598
	Error	377	12457		
$\delta^{15}N$					
	Activity	1	1651	100.6	**<.0001**
	Substrate	2	149.5	4.554	**0.011**
	Substrate*Activity	2	188.9	5.754	**0.004**
	Error	352	5777		
SEA.B					
	Activity	1	4271	4.97	**0.032**
	Substrate	2	3005	1.75	0.188
	Substrate*Activity	2	1202	0.70	0.504
	Error	352	30928		

Table 4.A1. Mean stable isotope values ($\delta^{13}C$, $\delta^{15}N$), standard deviation (SD), and number of all organisms analyzed from various substrates (colonization substrates, native carbonates, transplant experiments). Averages are calculated according within active and inactive sites.

			Active Substrates						Inactive Substrates					
Major clade	**Minor clade**	**Taxon ID**	**$\delta^{13}C$**	**SD**	***N***	**$\delta^{15}N$**	**SD**	***N***	**$\delta^{13}C$**	**SD**	***N***	**$\delta^{15}N$**	**SD**	***N***
Annelida														
Clitellata														
Hirudinea		Leech							-19.7	–	1	16.4	–	1
Oligochaeta	Tubificidae	*Tubificoides*	-21.4	–	1	9.2	–	1						
Polychaeta														
Canalipalpata	Chaetopteridae	*Spiochaetopterus*							-20.2	–	1	10.8	–	1
	Magelonidae	Magelonidae							-28.4	–	1	8.3	–	1
	Siboglinidae	*Osedax*							-15.6	1.9	5	16.3	8.1	5
	Spionidae	Spionidae	-21.9	1.7	4	9.5	1.1	4	-21.9	0.2	2	15.0	2.9	2
Eunicida	Dorvilleidae	*Dorvillea*	-42.5	23.5	15	0.7	4.8	15						
		Exallopus	-29.0	1.3	2	3.0	0.7	2	-35.5	–	1	6.6	–	1
		Ophryotrocha	-30.5	6.7	5	1.6	2.8	5	-26.7	2.1	2	7.3	6.4	2
		Parougia	-28.6	2.4	3	-1.2	1.2	3						
		Dorvilleidae, unid	-27.9	4.8	10	1.7	4.8	9	-20.0	3.2	7	17.5	8.5	6
Phyllodocida	Glyceridae		-22.7	0.6	2	13.3	4.6	2						
	Hesionidae		-26.1	5.6	10	7.7	4.5	8	-19.4	3.3	2	15.9	–	1
	Lacydoniidae		-38.9	11.9	6	10.7	3.2	5						
	Nephtyidae		-53.5	–	1	3.8	–	1	-23.9	–	1	12.5	–	1
	Nereidae		-33.8	8.0	19	6.9	3.9	19	-21.1	1.2	2	13.6	0.5	2
	Paralacydoniidae		-28.8	–	1	5.3	–	1						
	Phyllodociidae		-27.4	–	1	13.8	–	1						
	Polynoidae		-32.3	13.1	16	8.3	3.4	16	-21.9	3.5	25	11.0	2.8	25
	Syllidae	*Eusyllis* sp.	-35.7	3.9	2	10.7	3.8	2	-21.0	0.6	3	11.3	1.6	3
		Exogone sp.	-29.0	2.0	8	5.7	2.5	8	-32.0	–	1	6.5	–	1
		Sphaerosyllis sp.	-27.9	0.4	3	6.3	2.2	3						
		Typosyllis sp.	-29.0	2.9	4	12.7	2.2	4	-22.2	2.4	4	17.2	3.9	4
		Syllidae, unid	-22.9	4.9	2	11.8	4.4	2	-22.9	–	1	11.9	–	1
Scolecida	Maldaniidae		-26.5	–	1	13.3	–	1	-20.8	–	1	19.3	–	1
	Scalibregmatidae								-21.1	–	1	12.6	–	1
	Capitellidae		-30.6	–	1	10.5	–	1	-20.4	2.2	4	9.3	2.1	4
	Orbiinidae		-32.3	5.2	23	8.2	3.7	23	-21.1	–	1	11.2	–	1
Terebellida	Acrocirridae		-17.4	–	1	13.1	–	1	-21.1	–	1	10.9	–	1
	Ampharetidae	*Ampharete* sp.	-30.1	3.7	3	7.7	0.4	2	-20.7	–	1	10.3	–	1
		Amphisamytha fauchaldi	-31.4	4.7	36	5.3	4.8	32						
		Decemunciger sp.							-20.8	0.3	2	6.6	0.5	2
		Ampharetidae, unid	-32.1	8.2	19	5.4	3.2	18	-25.8	4.2	3	8.5	7.1	3
	Cirratulidae		-31.5	4.1	70	7.4	3.8	66	-21.9	1.8	4	13.6	6.6	4
	Terebellidae		-33.2	–	1	5.9	–	1	-21.5	1.8	7	11.2	1.2	7
	Trichobranchidae		-25.3	–	1	11.0	–	1	-22.9	–	1	10.6	–	1
	Flabelligeridae		-32.5	–	1	7.9	–	1						
Arthropoda														
Chelicerata, Pycnogonida									-22.3	–	1			
Crustacea														
Amphipoda	Gammaridae								-20.6	–	1	13.8	–	1
	Amphipoda, unid		-31.2	3.7	4	8.5	1.5	4	-19.7	1.9	4	14.7	7.0	4
Decapoda	Galatheidae								-20.1	0.5	5	11.2	0.5	5
	Majoidea	Decorator crab							-16.5	2.7	2	12.9	1.1	2
Isopoda	Ischnomesidae		-22.4	–	1	7.6	–	1						
	Munnopsidae		-26.7	6.9	2	7.9	6.6	2						
	Isopoda, unid		-26.3	3.2	5	13.7	9.9	5	-28.2	6.9	5	8.3	4.8	4
Ostracoda									-21.2	–	1	13.5	–	1
Cnidaria														
Actiniaria									-19.9	1.7	2	9.8	6.0	2
Alcyonacea			-21.5	–	1	14.0	–	1						
Hydrozoa									-21.8	1.7	5	11.5	1.5	5
Echinodermata														
Crinoidea									-21.7	–	1	13.5	–	1
Ophiuroidea			-25.6	5.4	6	12.6	11.2	6	-20.1	1.6	21	10.2	1.5	20
Hemichordata														
Enteropneusta									-9.6	–	1	11.6	–	1

Appendix 4.A1. (continued)

			Active Substrates						Inactive Substrates					
Major clade	**Minor clade**	**Taxon ID**	**$\delta^{13}C$**	**SD**	***N***	**$\delta^{15}N$**	**SD**	***N***	**$\delta^{13}C$**	**SD**	***N***	**$\delta^{15}N$**	**SD**	***N***
Mollusca														
Aplacophora			-21.5	1.6	2	14.0	4.1	2	-21.7	1.7	3	13.8	0.8	3
Bivalvia	Pectinidae								-19.9	0.6	2	13.1	0.7	2
	Propeamussiidae								-14.5	10.1	2	10.5	1.8	2
	Thyasiridae		-26.3	–	1	6.7	–	1						
	Vesicomyidae	*Calyptogena* sp.	-35.2	–	1	1.5	–	1						
	Xylophagidae	*Xylophaga washingtona*	-21.6	1.4	7	5.6	3.3	7	-20.4	1.5	19	5.4	1.5	19
Gastropoda														
Caenogastropoda	Buccinidae	*Neptunia* sp.							-21.7	3.3	13	9.6	1.7	13
	Columbellidae	*Astyris permodesta*	-25.9	0.9	3	7.4	2.9	3						
	Provannidae	*Provanna laevis*	-31.8	6.1	103	4.0	3.1	92						
		Provanna lomana	-30.8	3.4	110	3.8	2.5	96	-32.4	2.8	3	3.9	1.3	3
		Provanna, unid	-32.4	7.5	6	4.3	0.8	6	-28.8	–	1	5.4	–	1
Heterobranchia	Hyalogyrinidae	*Hyalogyrina* sp.	-32.3	5.1	3	4.1	3.7	2						
		Heterobranchia, unid	-25.4	0.3	3	-1.8	2.8	3						
Opisthobranchia	Diaphanidae		-28.0	1.6	3	0.4	–	1						
Patellogastropoda	Lepetopsidae								-27.7	–	1	11.0	–	1
Vetigastropoda	Margaritidae	*Margarites* sp.							-28.5	1.8	5	10.1	1.7	5
	Pyropeltidae	*Pyropelta corymba*	-33.4	5.3	80	5.5	2.8	74	-34.0	4.7	3	6.0	1.0	3
		Pyropelta wakefieldi	-30.8	4.4	65	5.4	2.3	64						
		Pyropelta, unid							-39.7	2.3	2	6.8	0.8	2
		Gastropod egg capsules	-30.3	–	1	7.7	–	1						
Polyplacophora									-21.7	–	1	14.3	–	1
Nematoda			-30.9	1.6	5	1.4	4.0	4						
Nemertea			-26.0	7.6	15	11.9	8.6	14	-20.9	1.1	3	16.4	0.7	3
Porifera		Cladorhyzid sponge							-31.8	–	1	9.2	–	1
		Encrusting sponge	-43.4	11.6	9	7.7	3.7	9	-32.0	8.6	4	15.5	3.6	4
		Solitary, upright sponge	-34.4	13.8	6	6.1	3.3	6	-41.9	–	1	15.2	–	1
		Sponge, unid	-38.9	13.0	4	11.4	4.5	4	-27.0	6.7	3	13.4	1.3	3
Ciliophora		*Folliculina*	-34.9	–	1	-0.5	–	1						
Foraminifera		Agglutinated foraminifera	-27.8	1.3	4	8.8	4.1	4	-22.5	0.8	4	6.2	3.6	4
		Arborescent foraminifera	-29.6	6.3	6	7.7	6.2	3	-24.2	1.9	5	5.7	1.6	5
		Calcareous foraminera	-23.0	4.9	3	13.3	18.8	2						
Bacteria		*Thioploca*	-26.2	1.9	5	1.9	3.0	4						
		Filamentous bacteria	-29.2	4.6	7	0.0	2.2	6						
		Thiomargarita	-31.6	1.1	2	1.5	1.5	2						
Total			-31.3	7.2	746	5.7	4.5	689	-22.5	5.1	211	10.7	4.5	206

CHAPTER FIVE

METACOMMUNITY INSIGHTS FROM COLONIZING MACROFAUNA IN A CHEMOSYNTHETIC COMMUNITY

Abstract

Exploration of metacommunity dynamics in the deep sea is rare, despite the occurrence of multiple systems in which habitat patches are connected by dispersing individuals, and both local and regional processes act to structure patterns of diversity. At Hydrate Ridge, Oregon (600–800 m), we manipulated hard substrates to examine the processes shaping a chemosynthetic metacommunity. Multiple analytical techniques (site-by-species incidence matrices, variance partitioning, abundance rank plots) were employed to examine applicability of idealized metacommunity paradigms.

The observed strong influence of environmental gradients and successional time on community composition suggest contributions of species sorting, mass effects and patch dynamic models to the structure of hard substrate communities around methane seeps. Active fluid seepage was the dominant environmental gradient and it explained patterns of beta diversity better than did substrate type or other factors studied. Of the 36% of community structure explained by distance-based redundancy analysis, environmental parameters (activity, substrate type, successional stage, depth) solely explained 20% while spatial parameters explained only 5% of the variance, suggesting possible prevalence of niche-based over neutral dynamics in this metacommunity at the scales examined. Comparison of the abundance ranks of species on colonization and

native carbonates highlighted differences in colonization dynamics at active and inactive sites, with a possible increase in the influence of stochastic and dispersive structuring as one moves away from active seeps into non-chemosynthetic margin habitats.

Introduction

Development of metacommunity theory over the past decade has improved our understanding of how processes at multiple spatial scales help shape community structure and biodiversity (reviewed in Holyoak and Mata 2008; Logue et al. 2011). We have long recognized that spatial heterogeneity acts to split communities into patches, the dynamics of which vary due to differing environmental and ecological conditions. A metacommunity can be thought of as a network of locally interacting communities ("patches") that are separated spatially, but linked through dispersal of individuals (Wilson 1992; Leibold et al. 2004). This construct leads to consideration of the contributions of both local community dynamics (interspecific interactions, species responses to abiotic conditions) and regional processes (dispersal, habitat fragmentation) to the maintenance of biodiversity, community assembly, and species coexistence.

Four idealized metacommunity perspectives emphasize different processes that may act to promote species coexistence: **species sorting** (coexistence through niche filtering and partitioning); **mass effects** (source-sink dynamics); **patch dynamics** (colonization-competition trade-offs); and **neutral dynamics** (emigration/immigration and speciation/extinction dynamics) (Leibold et al. 2004; Holyoak et al. 2005). However, none of these idealized perspectives will completely explain coexistence and diversity patterns in any metacommunity. Multiple mechanisms are likely to be operating for different species and at different spatial scales, and some have argued that metacommunities are better thought of as gradients along which the relative influences of environment, spatial distance, and ecological redundancy vary (Logue et al. 2011;

Winegardner et al. 2012). The ability of a single metacommunity to display traits consistent with multiple theoretical perspectives is a problem commonly faced by those who have attempted to develop empirical approaches to examine the application of the paradigms in natural communities. While we refer to the commonly recognized metacommunity perspectives in this paper in order to compare the relative importance of different structuring mechanisms, we stress that no one paradigm should be expected to solely explain diversity patterns within any ecosystem or taxonomic group.

In the deep sea, chemosynthetic ecosystems such as hydrothermal vents, methane seeps, whale falls and wood falls, fit well into the conceptual metacommunity framework (Neubert et al. 2006; Cordes et al. 2010). They are patchy and hierarchical in nature, often existing as a mosaic of habitats at scales from decimeters to 100s of kilometers. Their high degree of habitat heterogeneity would seem to result in patches that may have different suitability for each species (Tunnicliffe et al. 2003; Cordes et al. 2010). In addition, the ephemeral natures of vents and seeps, which may last just a few decades or centuries before going extinct, highlights the critical role of dispersal in promoting regional persistence of a species, even if it becomes locally extinct (Mullineaux et al. 2010).

Deep-sea chemosynthetic ecosystems are energetically dependent on reduced compounds that emerge from the seafloor (cold seeps, hydrothermal vents) or originate from the decay of organic carbon on top of the seafloor (wood falls, whale falls, etc.). This results in food webs structured differently from those in photosynthesis-based ecosystems, as both heterotrophic and symbiotic metazoans are fundamentally dependent

on bacteria and archaea that typically use hydrogen sulfide (H_2S), methane (CH_4), or hydrogen to fix carbon. Vents, seeps, and organic food falls are created by drastically different processes, but the presence of H_2S and prolific microbial primary producers is hypothesized to be linked to many taxa that exhibit evolutionary radiations primarily within chemosynthetic habitats (German et al. 2011). After whale falls were discovered to host species evolutionarily similar to those at hydrothermal vents, Smith (1989) proposed a "stepping stone hypothesis", suggesting some species that appeared restricted to particular chemosynthetic environments could actually occupy multiple types of these cognate deep-sea ecosystems. Wood falls are also considered as possible stepping stones for vent and seep biota (Distel et al. 2000). A species' ability to colonize and reproduce at more than one type of habitat increases the likelihood of a larva finding a settlement site, promotes gene flow, allows more rapid colonization following disturbance events, and extends population ranges, each of which may reduce extinction probability and explain the evolutionary histories of taxa that have been associated with chemosynthetic habitats for tens of millions of years (Vrijenhoek 2010; Mullineaux et al. 2010).

Cold seeps, whale falls, and wood falls are typically found along continental margins, including the North American Pacific margin. While each of these systems contain characteristic species that are certainly habitat specialists (e.g. the bone-eating worms *Osedax* spp. and deep-sea wood-boring clams *Xylophaga* spp.), the possibility that metacommunity dynamics could link these ecosystems is an intriguing idea that has not been formally tested (Levin and Dayton 2009), especially given the paucity of research on metacommunities in any marine ecosystem (Logue et al. 2011). In the past few

decades, there are perhaps a dozen species from various taxonomic groups that have been discovered in multiple types of chemosynthetic ecosystems (Craddock et al. 1995; Sasaki et al. 2010; Stiller et al. 2013), but there is still debate as to whether this phenomenon allows for widespread population connectivity among multiple types of chemosynthetic habitats (Cunha et al. 2013).

The research we describe comprises the first study explicitly designed to examine metacommunity dynamics in deep-sea chemosynthetic environments, incorporating both community patterns and a manipulative experiment. While field experiments are often useful to assess the applicability of various metacommunity models, theory development has advanced more rapidly than observations that are able to test that theory, and more empirical data are needed (Logue et al. 2011; Winegardner et al. 2012). This work uses empirical data for hard substrate biota of the Hydrate Ridge methane seeps (Oregon, USA) to examine evidence that metacommunities may connect multiple types of chemosynthetic ecosystems, and assess whether their distinctive physical and chemical setting contributes to distinctive metacommunity dynamics within a single region.

Deep-sea ecologists tend to implicitly use a species sorting mindset when describing assemblages in chemosynthetic ecosystems, which are tightly linked by their reliance on hydrogen sulfide and methane (Tunnicliffe et al. 2003; Neubert et al. 2006; Cordes et al. 2010). These assemblages are typically described in the context of physical and chemical variables, such as species distributions or diversity patterns that correlate with gradients of temperature and reduced compounds at hydrothermal vents or methane and sulfide concentration at cold seeps. However, researchers are well aware that species

have widely differing larval connectivities across the great distances that may separate adjacent habitats (Neubert et al. 2006; Vrijenhoek 2010; Mullineaux et al. 2010; 2012). To this point, no studies have used empirical data to attempt to distinguish among different metacommunity perspectives in these ecosystems, though the dependence on chemoautotrophic energy may cause these metacommunities to operate differently from classical terrestrial and aquatic ecosystems.

We deployed three distinct bare substrates representative of those found at seep, wood and whale falls at active seepage and inactive sites and compared patterns of colonization with those of natural, ambient communities to examine the mechanisms that may shape the metacommunity. We employ three complementary statistical techniques: site-by-species incidence matrices, community variance partitioning, and abundance-rank plots, to investigate the structure of invertebrate metacommunities on hard substrates at deep-sea methane seeps off the Oregon coast (Hydrate Ridge, 580–800 m depth). Simulations have shown different underlying processes can result in similar patterns of community structure and regional diversity (Ruokolainen et al. 2009); the use of multiple analyses should improve our ability to detect possible drivers of metacommunity structure (Meynard et al. 2013). In our investigation of methane seep metacommunities, we hypothesized that:

(1) Substrates consisting of different material (carbonate, wood, and bone) and exposed to different chemical environments (active and inactive sites at seeps) will exhibit distinct metacommunity dynamics, reflecting varying importance of niche-based (species sorting), dispersive (mass effect), or neutral processes.

(2) Rates of community recovery and early successional patterns on bare substrates (representing disturbances) can reveal distinct metacommunity dynamics within an ecosystem in which the relative influence of spatial and environmental processes vary.

Materials & Methods

Field methods

We quantified communities associated both with natural seep carbonates (or "native" as in previous chapters) and experimental, colonization substrates (carbonate, wood, bone) at Hydrate Ridge off the coast of Oregon (44º40'N, 125º6'W, Figure 4.1). The subduction of the Juan de Fuca plate under the North American plate makes this a region of accretion where buried carbon is linked to the thermogenic production of methane (Tréhu et al. 1999). Faults, conduits, and permeable sediments allow hydrocarbon-rich fluids to migrate upwards, eventually reaching the seafloor and supporting dense chemosynthetic communities on several mounds stretched along the 25 km-long ridge (Suess et al. 1999; Tryon and Brown 2001).

We sampled native carbonates and conducted experiments during two cruises to Hydrate Ridge aboard RV *Atlantis* (Figure 4.1). In August 2010 we used DSV *Alvin* to deploy substrates to be colonized and collect *in situ* carbonate rocks (= "native") to quantify the natural macrofaunal community. Just over one year later in September 2011, we used the remotely-operated vehicle *Jason II* to collect our substrate experiments (colonization time of 13 months). Our experimental substrates mimicked *in situ* hard

substrates common to various chemosynthetic systems in the East Pacific Ocean. These included authigenic carbonates originally from seeps at Hydrate Ridge or Costa Rica, different types of wood [natural Douglas fir and pine with bark attached (= "natural wood") and 9x9x17 cm blocks of untreated lumber (Douglas fir)], and bones from cattle and baleen whales. Groups of substrates were deployed at six paired "active" and "inactive" sites, with three pairs each at HR North (peak of mound at 580 m) and HR South (pinnacle at 790 m) (Figure 4.1). Carbonate and wood blocks were deployed at every site, while natural fir and bones were deployed at four pairs of sites and natural pine was deployed only at the three pairs of sites at HR North due to material shortages. The distance between paired active and inactive sites ranged from 30–400 m. Scientists diving in *Alvin* chose sites based on the presence ("active" seep sites) or absence ("inactive" sites) of visual signs of fluid flow (bubbling, shimmering water, bacterial mats, symbiont-hosting fauna such as seep clams). Clusters of substrates were always placed within a 1–2 meter radius. All substrates were collected individually in watertight, insulated bioboxes with partitions to keep substrates and their macrofauna segregated.

Aboard the ship, substrates were stored in a cold room (4ºC) until processing, during which all macrofauna associated with the substrate or its box were collected or sieved from sediments on a 300-µm screen. The substrate was allowed to soak in room temperature seawater for 24 hours, after which it was again washed and the water sieved to collect endolithic fauna that crawled out of the substrate. Samples were sorted at sea under dissecting microscopes, or preserved in 3% buffered formalin until they could be sorted in the laboratory. All specimens were identified to the lowest possible taxonomic

level and counted. Since some samples were processed live during separate cruises with non-identical personnel, records for individual taxa were grouped at a level we could ensure would be consistent for both years. For example, we could confidently identify many gastropods to at least genus and often species, but most polychaetes could not be identified to species quickly except by experts, and thus are often (but not always) binned at the family level. Taxa from other phyla are often binned at much broader levels (e.g., Nemertea, Ophiuroidea, etc.).

Surface area was calculated for regular wood blocks (L x W x H) but determined with aluminum foil weight for irregular substrates (carbonates, bone, natural wood). These were completely covered with a single layer of foil, which was then weighed and converted to surface area based on the weight of 25 cm^2 of foil. Densities here are expressed as individuals per 100 cm^2. Additionally, we analyzed the $\delta^{13}C$ signature of the organic fraction carbonate and wood to use as an explainer variable for community structure (methods in Chapter 4).

Statistical approach

We used three complementary statistical analyses to investigate the structure of metacommunities on hard substrates at methane seeps: site-by-species incidence matrices (SSIMs), variance partitioning, and ranked abundance of colonizers (Table 5.1).

1. Site-by-species incidence matrices

The use of site-by-species incidence matrices (SSIM) to examine metacommunity patterns was developed by Leibold and Mikkelson (2002), and extended by Presley et al.

(2010). For our purposes, each deployed substrate unit is considered a site, and a taxon presence-absence matrix was constructed for all our substrates. We use the term "taxon" instead of "species" because most of our macrofauna were not identified to the species level. We used the metacom package in R v3.0.2 (Dallas 2014; R Core Team), which constructs a SSIM with sites (substrates, in our case) arranged along a primary axis of ordination. (In a metacommunity perfectly described by species sorting, this primary axis in the SSIM may correspond to measurable environmental gradients.) The types of patterns in metacommunity structure were then measured: (1) coherence, or the level to which taxa all correspond to the same primary gradient in the matrix; (2) species range turnover, corresponding to how frequently taxa replace each other between sites; and (3) boundary clumping, which exists when many range boundaries are aligned at the same place in the SSIM. Coherence and turnover are evaluated through comparisons with a null model, in which the ordinated SSIM is compared to the mean of 1000 simulated matrices, and boundary clumping is based on Morisita's index (Table 5.1).

2. Variance partitioning and multivariate community analysis

Multivariate data analyses were performed in PRIMER 6.1 (McArdle and Anderson 2001; Clarke and Gorley 2006; Anderson et al. 2008). A Bray-Curtis dissimilarity resemblance matrix was constructed from taxonomic densities (fourth-root transformed), and analysis of similarity (ANOSIM) was used to test for an effect of activity, substrate, successional stage or region on the distribution of beta diversity. For the factor substrate, native carbonate and colonization carbonate were separated as different variables so the effects of substrate and succession could be tested together.

SIMPER analysis was used to determine which taxa contributed most to dissimilarity between treatment groups, and non-metric multidimensional scaling (MDS) plots were constructed so that we could visualize community differences among the treatment groups.

We considered Bray-Curtis dissimilarity as a measure of between-substrate beta diversity (Borcard et al. 1992; Anderson et al. 2011). We carried out distance-based linear modeling (DISTLM) in PERMANOVA+ for PRIMER 6.1 using distance-based redundancy analysis (dbRDA) to assess the relative contributions of spatial and environmental variables in structuring substrate metacommunities (Legendre and Anderson 1999; McArdle and Anderson 2001). DISTLM was used to conduct significance testing on individual variables (marginal tests) before performing variation partitioning on the Bray-Curtis dissimilarity matrix. The AIC Best selection criterion was used to select subsets of variables for the spatial and environmental explanatory sets based on 9999 permutations in PERMANOVA+.

Our spatial parameters were linked to each substrate's unique sets of coordinates (X and Y based on a UTM grid and recorded from *Alvin*'s and *Jason II*'s navigational systems). These data were used to create a set of polynomials (XY, X^2, Y^2, XY^2, X^2Y, X^3, and Y^3) to test for spatial effects at varying scales (Borcard et al. 1992). Categorical environmental parameters input as binary explanatories included seepage activity (active or inactive), region (Hydrate Ridge North or South), substrate (carbonate, wood, natural fir, natural pine, or bone), successional stage (colonization or native), and seafloor type (hard ground, soft sediment, intermediate). Continuous environmental variables included

ln-transformed depth, ln-transformed substrate surface area, and log-transformed $\delta^{13}C$ of the organic fraction of the substrate. The variables selected for the spatial parameterized model was based on minimizing the Akaike information criterion (AIC), to select the most parsimonious model without selecting too many parameters that might artificially increase the adjusted-R^2 (R^2_{adj}) (Kissling and Carl 2008). Environmental model selection was based on the forward selection of variables to minimize AIC. Additional predictor variables were added one at a time until the contributed variable became non-significant ($p > 0.05$).

The amount of variation in the Bray-Curtis resemblance matrix explained by the sets of spatial and environmental variables were tested by fitting the variables sequentially with DISTLM in PERMANOVA+, and significance was based on 9999 permutations. Using both sets of variables at once produced an R^2_{adj} that represented total explained variation (E + S). Since some variance can be explained by multiple sets of predictors, we determined the variance attributable to environmental variables by sequentially modeling the spatial variables, and then measuring the increase in R^2_{adj} resulting from adding in the environmental variables as a second set of predictors. The reverse was done to calculate variance attributable to spatial but not environmental variables.

3. Abundance–rank scatter plots

We created scatter plots comparing the abundance rank of species at two successional stages: when they occurred on 13-mo-old colonization carbonates and when they occurred on native carbonates. The structure of these plots provides information

about metacommunity dynamics since it incorporates temporal processes (dispersal and recruitment). A positive correlation between experimental and native rocks indicates similar structure (relative species abundances) in communities of different successional ages and suggests a species sorting or mass effect perspective. Conversely, a negative correlation indicates successional age strongly influences the community structure and implicates patch dynamics, while a random scatter of points may reflect a neutral metacommunity.

Results

Colonization patterns

Generally, experimental substrates were colonized by species from the native community and in similar densities (Table 5.A1). Comparison of shared and unique taxa across treatments (Figure 5.1) reveals that differences between successional stages generally influence the species pool more so than differences among substrate types. Of the 72 taxa we documented on native carbonates, 32 (44%) did not colonize any of our experimental substrates (Figure 5.1A). In contrast, 22 of the 62 taxa that colonized our substrates were absent from all native carbonates (Figure 5.1B). The most abundant taxa usually occurred on all types of substrates (the center of the Venn diagrams). Exceptions included several substrate specialists that recruited to wood and bone substrates (Figure 5.1). Bone at inactive sites was colonized by three species of *Osedax*, a siboglinid polychaete with endosymbionts that can digest collagen or lipids from bone (CITATION). Wood was dominated by *Xylophaga washingtona*, a wood-boring clam, especially at inactive sites where densities surpassed 8000 individuals 100 cm^{-2}. Recently

settled juveniles were abundant on the outside of wood (and sometimes carbonate and bone), but adults always had burrowed into wood.

Site-by-species incidence matrices (SSIMs)

Ordination of species composition for all substrates in a SSIM showed structural distinctions based primarily on activity and successional stage, and to a lesser degree substrate type (Figures 5.2, 5.3). Generally, inactive native carbonates cluster at one side of the ordination, while active colonization substrates cluster at the opposite end. In the middle, there is a fair degree of mixing between inactive colonization substrates and active native carbonates. The matrix with all substrates exhibits a significant degree of coherence ($p = 0.01$) and clumped range boundaries ($p < 0.0001$; Figure 5.2). When SSIMs are analyzed for groups of substrates (e.g. active and inactive sites, colonization and native carbonates), all remain coherent with a high degree of boundary clumping (Figure 5.3). Rates of species turnover are usually not significantly different from the randomized (null) matrices, except for substrate at inactive sites and for colonization substrates. In those cases, species turnover is significantly positive, which means species tend to replace each other from site to site (Leibold & Mikkelson 2002).

Variance partitioning

Beta diversity as defined by Bray-Curtis dissimilarity was better explained with measured environmental variables than by spatial arrangement, although nearly two-thirds of the variation was unexplained by either set. Across all communities, the environmental and spatial variables explain a combined 36.5% of the variation in the

fitted data from the resemblance matrix. Environmental parameters explain 21.5% of the variation independent of spatial parameters, whereas spatial parameters only explained 5.2% of the variation independent of environmental parameters (Table 5.3).

Since DISTLM selected activity, successional stage, and substrate type as parameters that each explained a significant amount of variation in the community on their own (Table 5.3, Figure 5.A1), ANOSIM was used to further examine the effect of these variables on community structure. Within colonization communities, activity and substrate type both had a significant effect on beta diversity, with activity explaining about three times more variation (Global $R_{activity} = 0.785$, Global $R_{substrate} = 0.239$, $p = 0.001$). However, because *Xylophaga* densities were often 3–4 orders of magnitude above the next most abundant taxon on wood substrates, our multivariate data set was severely skewed even after transformation. Therefore, after analyzing patterns for the whole macrofaunal community, we excluded *Xylophaga* and re-analyzed the data, reported in Figure 5.4. This removal from the analysis reduced the explanatory power of substrate more than it did for activity (Global $R_{activity} = 0.746$, Global $R_{substrate} = 0.137$, $p = 0.001$). When only carbonates were included in the analysis, activity and successional stage significantly affected beta diversity (Global $R_{activity} = 0.615$, $p = 0.001$; Global $R_{stage} = 0.304$, $p = 0.001$). When considering all treatment combinations, community composition was more variable for inactive substrates than for active substrates (2-D MDS plots, Figure 5.4, Table 5.A2).

Abundance ranks for colonizers versus native carbonates.

At active sites, we observed a positive relationship between the rank abundances of early colonizers and native species ($R^2 = 0.48$). The top 15 most abundant colonizing taxa were present on native carbonates except for *Astyris permodesta*, which was the 11th ranked species on colonization carbonates, but was only the 46th ranked species on native carbonates. At inactive sites, there was no relationship between rank abundance of colonizer and native assemblages, indicating a lack of influence of successional stage. Some abundant species on native carbonates colonized our experiments in high abundances (e.g., amphipods, ophiuroids, isopods, polynoid polychaetes), while others did not colonize any of the inactive substrates (e.g. hydroids and the polychaete groups *Sphaerosyllis* (Syllidae) and Cirratulidae] (Figure 5.5). Gastropods common to active seep settings (*Provanna* spp, *Pyropelta* spp.) had much higher abundance ranks on colonization carbonates (6th – 13th) than on native carbonates at inactive sites (33rd – absent, Figure 5.5).

Discussion

We can make several generalizations regarding metacommunity dynamics at methane seeps based on a manipulative experiment that measured colonization patterns and the native assemblage of macrofaunal communities with statistical methods that attributed community variation to specific causes. Our sites were separated by up to 400 meters within a region, and by 13 km between our two seep regions (HR North and HR South), and yet all of our data suggest that substrates throughout these regions are connected via their component species. SSIMs did not emphasize any splits between HR

North and HR South sites (Figure 5.2), many taxa co-occurred in active and inactive sites (Table 5.A2), and despite clear differences in the dynamics structuring their communities, there were several occasions in which taxa common to one chemical environment colonized substrates in the other (Figure 5.5). Thus, these hard substrate communities at Hydrate Ridge appear to function as a single metacommunity influenced by varying factors depending on spatial scale.

Each of the statistical approaches employed implicated species sorting (and possibly mass effects, since they are difficult to distinguish) as playing an important structuring role in the seep metacommunity, especially at sites with active fluid flow. Structure in diversity among sites was more explainable by environmental than spatial variables, a result for which one explanation is strong species sorting or mass effects (Cottenie 2005). SSIM ordinations showed positive coherence suggesting a metacommunity response to some sort of one-dimensional non-random gradient in the environment (Leibold and Mikkelson 2002). Newly assembled (13-mo-old) communities at active sites largely resembled the native carbonate communities, and the presence or absence of seep activity was clearly the measured factor than best differentiated colonization communities (Figures 5.4, 5.A1). These results are not unexpected. The heterogeneity of cold seeps has been well-described (Menot et al. 2010; Cordes et al. 2010), and chemosynthetic ecosystems frequently exhibit tight associations between physicochemical conditions and community structure (Desbruyéres et al. 2000; Decker et al. 2012).

Perhaps more surprising was absence of substrate type influence on colonization communities. Substrate did not contribute to beta diversity at active sites (Figure 5.4) and was relatively non-influential across the entire seep metacommunity (Table 5.3). Most taxa acted as substrate generalists. Exceptions were seen in the bone specialist *Osedax priapus* and two other congeners, and in the wood specialists *Xylophaga washingtona*, and a new species of *Neptunea* (A. Waren, personal communication.

Additionally, the fact that several seep endemics colonized wood and bone not only next to active seeps, but also at inactive sites hundreds of meters from the nearest fluid flow lends support to the stepping stone hypothesis. If provannid snails and pyropeltid limpets, the dominant taxa on Hydrate Ridge carbonates (unpublished data, SIMPER results in Table 5.A2) recruit to small, isolated habitat patches far from areas of seepage, it increases the likelihood their larvae might also recruit to whale falls or wood falls distributed along much of the continental margin. This observation is noteworthy but not unique. Several species of provannids, including *Provanna laevis*, have been identified from more than one chemosynthetic habitat. In similar colonization experiments in the Mediterranean Sea, Gaudron *et al.* (2010) reported colonization of organic substrates in inactive areas by the chemosynthetic mussel *Idas modiolaeformis*, and vestimentiferans have even been collected from shipwrecks where degradation of food stores had led to high sediment concentrations of hydrogen sulfide (Dando et al. 1992). Other researchers point out that even if a few individuals can "invade" a stepping stone habitat, they are likely to be overwhelmed by endemic fauna, thus limiting their

reproductive potential and reducing the potential for such stepping stones to connect distant populations (Cunha et al. 2013).

Although inactive substrates were identified as being part of the larger Hydrate Ridge metacommunity, they are structured by a different set of dynamics than substrates at actively seeping sites. The SSIM of colonization substrates (Figure 5.3C) created an ordination that neatly split active and inactive substrates. While active substrates were consistently colonized by the same taxa resulting in few embedded absences, inactive substrates were more heterogeneous, creating a shotgun effect in the matrix, as species occurrences are scattered with little regard to the primary ordination (Figure 5.3), and among-group dissimilarity is higher than for active communities (MDS, Figure 5.4). At inactive sites the abundance ranks of colonizers are not related to those of macrofauna on native carbonates, unlike the patterns on active carbonates (Figure 5.5). Thus, in inactive areas around the seeps, local native communities on carbonates seemingly provide no predictive power of which species are likely to successfully recruit to a bare substrate, reflecting neutral or patch dynamics.

This last example highlights the value in our use of multiple statistical approaches to address questions regarding metacommunity dynamics. Multivariate methods, incidence matrices, and abundance ranks of colonizers point toward a similar conclusion. The Hydrate Ridge carbonate metacommunity becomes more dominated by stochastic processes or colonization-competition tradeoffs (patch dynamics) away from active seeps. Since spatial variables among sites explained little of the structure in beta diversity (Table 5.3), we argue that these data do not support neutral dynamics so much as they

highlight colonization-competition tradeoffs (patch dynamics) combined with slow temporal successional processes. Many of the species on native inactive carbonates that were not part of the colonizing assemblage were sessile suspension feeders such as corals, anemones, and hydrozoans, which might trade-off dispersal ability in favor of stronger competition. Cirratulid polychates provide another example; they were very common inhabitants on the inside of native carbonates, and their absence from colonization carbonates could be related to limited dispersal rates.

The stochasticity in our patterns for inactive substrates could also be interpreted as neutral dynamics, but the neutral model should involve spatial coherence through autocorrelation processes(Diniz-Filho et al. 2012). The lack of explanatory power attributed to space in the variance partitioning routine seems to not support neutrality. It may be that the spatial scale of our study was too limited to show distance decay patterns in the communities, though this dynamic may likely appear as spatial scales expand beyond larval dispersal distances (Vrijenhoek 2010). One caution in concluding that neutral dynamics are not important in this metacommunity is related to our need to bin taxa at higher groups (genera, families, and even phyla in some cases). Previous work on damselfly assemblages has shown that even if niche partitioning allows coexistence among higher taxa (genera, families), species within a genus may still be structured by neutral dynamics (Siepielski et al. 2010). The inability to resolve all of the most abundant groups at the species level may limit our ability to tease apart metacommunity dynamics, if they are scale dependent within taxa.

To what can we attribute the distinction in metacommunity pattern and dynamics between active and inactive sites? The clear ecological distinction is that active sites are associated with a source of reduced chemicals, and thus a high, chemosynthesis-based food supply in a normally food-poor community. Nearly all the macrofauna on active substrates were heterotrophs dependent on microbes in some way for their food, and microbial communities in reducing systems also are highly adapted and tend to appear to exhibit species sorting dynamics (Fagervold et al. 2012; Meyer et al. 2013). Niche-based dynamics become more evident when organisms respond to heterogeneity in habitat patches. If patches in active seeps exhibit gradients in primary production, it makes sense that metazoans could be sorted along the same gradients. Inactive sites, in contrast, do not typically contain dense populations of chemoautotroph grazers,, and a number of their fauna consume particulate organic matter from the ocean's surface. Faunal diets at inactive sites are not spatially linked to a source of reduced fluids, so the success of species in these habitat patches might be more dependent on the presence or absence of interacting species on a carbonate, leading to competition-colonization dynamics.

While chemosynthetic ecosystems in the deep sea might seem far from the terrestrial and aquatic realms where metacommunity theory has been developed, the features of methane seeps we address might have terrestrial analogues. At cold seeps, metacommunities are overlaid across a varied productivity landscape with multiple sources of organic carbon. Concentrations of hydrogen sulfide are linked to sulfide-oxidizing bacteria, which represent food for heterotrophic seep endemics, but sulfide also reduces oxygen-binding capabilities and is a chemical to be avoided in species not

adapted to reducing environments. Seeps increase regional diversity because endemic species have evolved to live in these chemically stressful habitats (Cordes et al. 2010; Bernardino and Smith 2010). Serpentine soils can be described similarly, as endemic herbs have adapted to the extremely high Mg:Ca ratio and act to increase regional diversity, while generalists in continuous non-serpentine soils avoided these soil conditions and exhibit different dynamics and diversity patterns in the region surrounded serpentine outcrops (Harrison 1998). While hydrothermal vents and cold seeps are often dominated by symbiont-hosting fauna (which might have their own metacommunity dynamics), carbonates at Hydrate Ridge seeps are dominated by heterotrophic gastropods, polychaetes, peracarid crustaceans, and other invertebrates that are dependent on chemosynthetic autochthonous production from the seep. In contrast, surrounding inactive habitats see gradual declines in autochthonous seep production and allochthonous, sinking photosynthetic production becomes increasingly important. Parallel dynamical systems might occur in other metacommunities in which some species move between patches that contrast in productivity, such as lakes with differing ratios of pelagic-to-benthic production, or canopy ponds influenced by varying levels of forest litter (Vander Zanden and Vadeboncoeur 2002; Binckley and Resetarits 2007).

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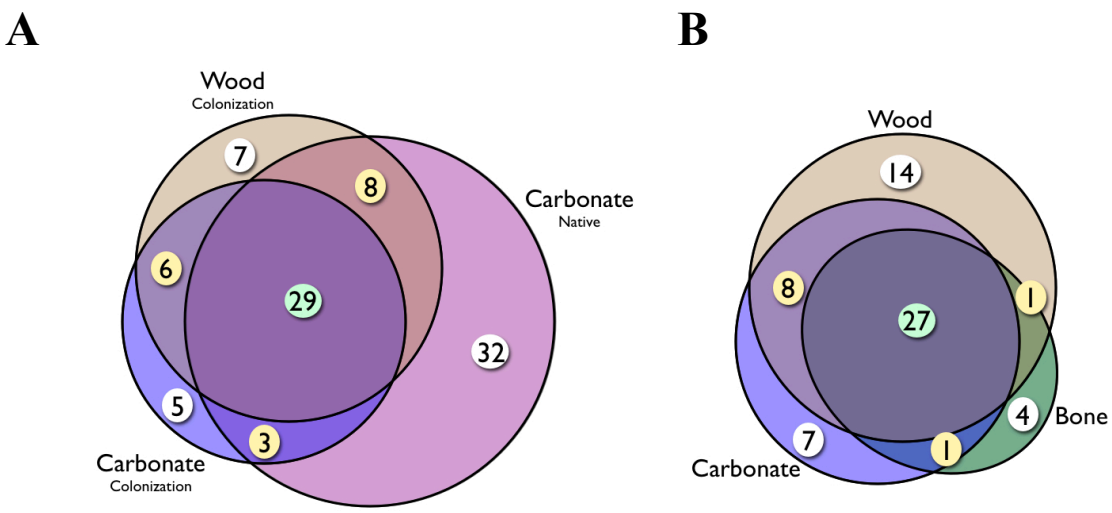

Figure 5.1. Venn diagrams showing taxa shared among all substrates and distinct to particular substrates. **A)** Comparison of native carbonates, colonization carbonates, and colonization wood. **B)** Comparison among all colonization substrates. Bone is not included in A), as nearly all taxa from bones occurred on all other substrates (27 of 33) or were unique to bones (3 species of *Osedax* and *Neptunea amianta*, though the latter was frequently observed *in situ* on native carbonates, and occurred on several carbonate samples whose communities we did not quantify).

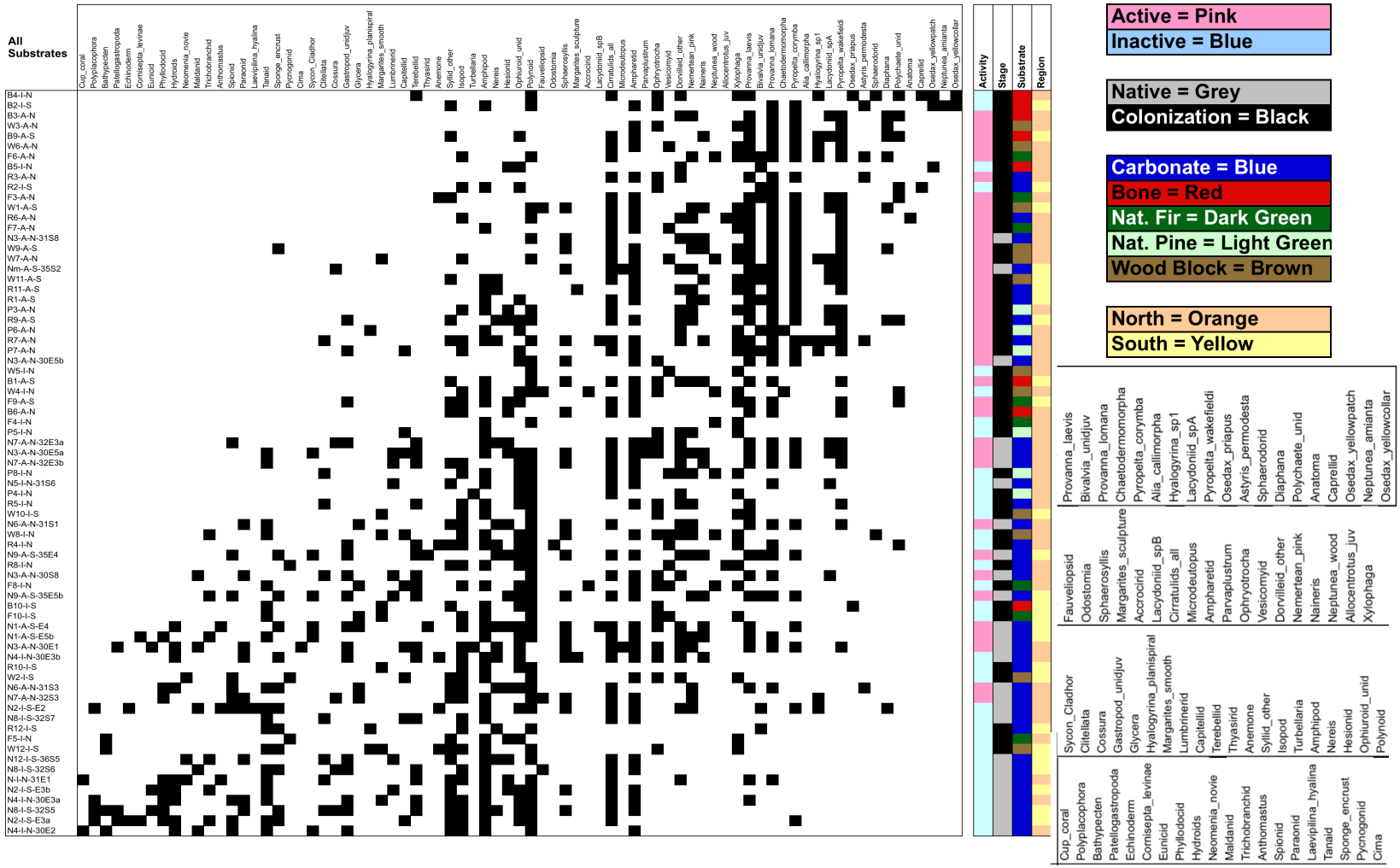

Figure 5.2. Ordinated incidence matrix showing presence of taxa (columns) across substrates (rows). Colored columns convey environmental information for each substrate including activity, successional stage, substrate type, and Hydrate Ridge region. Species list is shown larger for clarity, and should be read from top to bottom, left to right to match the matrix.

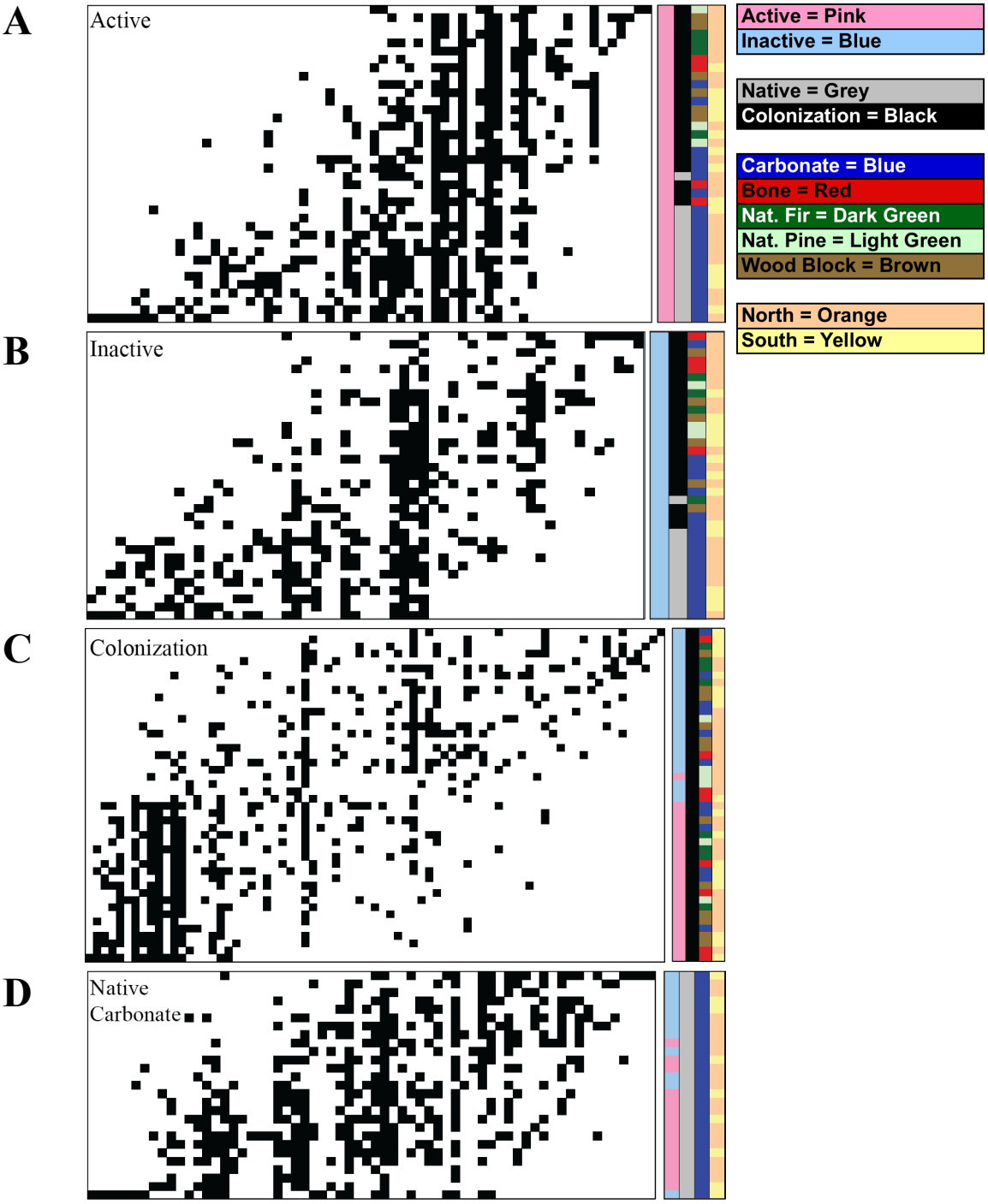

Figure 5.3. Ordinated incidence matrices for substrates: **A)** at active sites; **B)** at inactive sites; **C)** colonization experiments; and **D)** native carbonates showing presence of taxa (columns) across substrates (rows). The color-coded columns at right convey the environmental information for each substrate including activity, successional stage, substrate type, and Hydrate Ridge region.

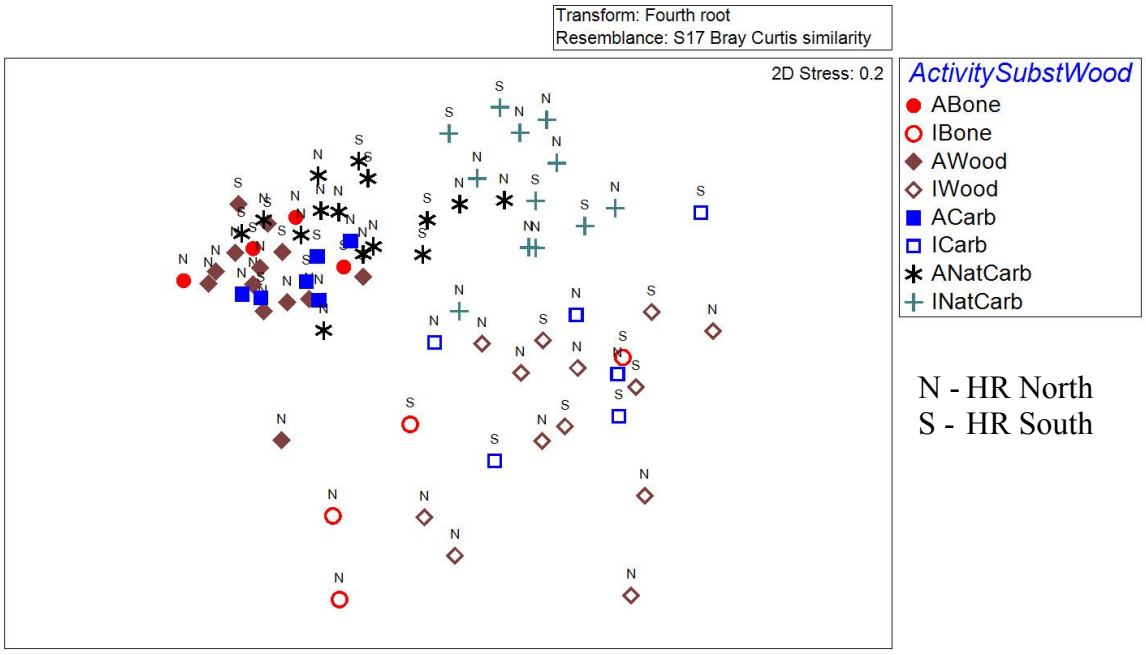

Figure 5.4. MDS plot showing dissimilarity among communities identified by activity, substrate type, and regions. Substrates at active locations have filled symbols or darkened stars (in the case of native carbonates). Substrates at inactive locations have open symbols or crosses (for native carbonates). N = HR North, S = HR South. Because stress was poor (0.20) the three-dimensional MDS was inspected visually to make sure general spatial relationships between groups were similar.

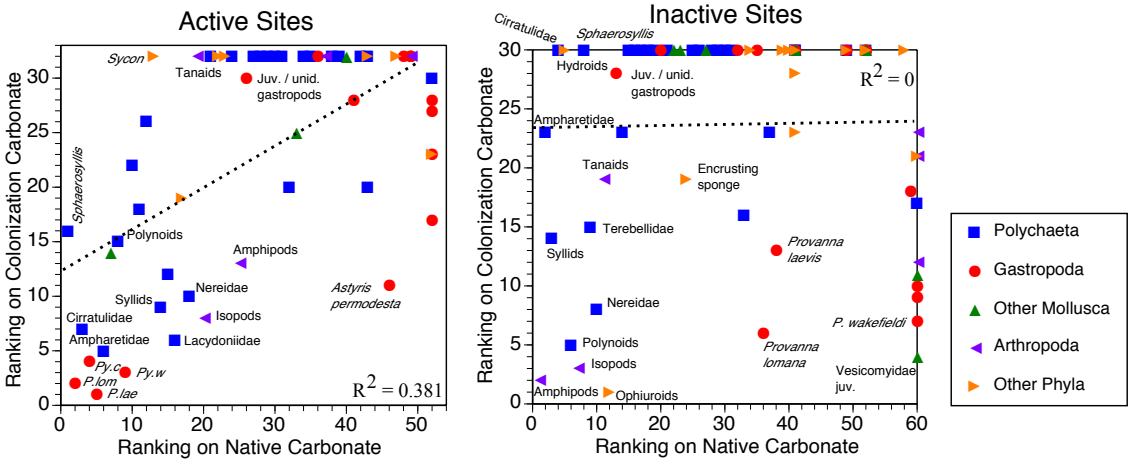

Figure 5.5. Ranked abundance of macrofauna occuring on experimental colonization carbonates and native carbonates at active (left) and inactive (right) sites. Major taxonomic groupings are denoted by symbol, and selected taxa are labeled. Dashed lines represent linear regressions for all points. The points in the lower left portion of the plots represent taxa that were highly abundant on both native and colonization carbonates. Points falling along the right or top edge of the plots are taxa that were absent on either native or colonization carbonates, respectively. Abbreviated species names in the active plot include *Provanna lomana, Provanna laevis, Pyropelta wakefieldi,* and *Pyropelta corymba.*

Table 5.1. Methodological approaches employed for the analysis of metacommunity patterns in natural communities

Method and References	Statistical Approach	Application for Our Data	Metacommunity patterns or dynamics that may be distinguished
Site-by-species incidence matrices (SSIM); Leibold & Mikkelson (2002), Presley et al. (2010)	Species presence-absence data from many sites is ordinated to find the dominant axis of variation, and three aspects of the incidence matrix are measured: coherence, species turnover, and boundary clumping.	SSIMs were applied to all substrates sampled at Hydrate Ridge. Additionally, we analyzed SSIMs for distinct treatment groups to look for evidence of altered metacommunity structure as a function of environment or successional time.	Presley *et al.* (2010) speculate on metacommunity dynamics that may lead to specific SSIM patterns. Some interactions (e.g. competition) may lead to negative coherence and checkerboard patterns, while neutral dynamics should result in lack of coherence in the matrix. Environmental filtering or successional dynamics are expected to contribute to positive coherence and positive species turnover, in which case boundary clumping might reveal the presence of species-specific (Gleasonian) or assemblage-wide (Clementsian) patterns in terms of how range boundaries in the metacommunity respond to gradients.
Variance partitioning of community composition among environmental and spatial variables; Legendre & Legendre (1998), Legendre & Anderson (1999), Cottenie (2005) Anderson *et al.* (2008)	Distance-based redundancy analysis (DistLM routine in the PERMANOVA+ package run in PRIMER 6) is used to analyze the relationships between a similarity matrix (Bray-Curtis resemblance of species composition from each sample) and two sets of explanatory predictors: one constructed from environmental parameters (E), and one from spatial parameters (S) associated with each sample.	Environmental parameters include categorical (activity, substrate type, region) and continuous variables (substrate surface area, depth, organic $\delta^{13}C$ of carbonate). The spatial parameters include the complete trend surface regression constructed from X and Y coordinates from a meter-scale grid along with second- and third-order polynomials (XY, X^2, X^2Y, X^3, etc.)	Variance parititioning helps tease apart the roles of spatial structure and environmental filtering in their influence on community assembly. Spatial structure should be stronger in metacommunities exhibiting neutral or patch dynamics, compared to those in which many species respond to environmental heterogeneity in a way that influences assemblage composition (species sorting, mass effects).
Ranked-abundance scatterplots comparing successional stages	Regressions are run on scatterplots constructed from the ranked-abundance of species, paired between colonization and native substrates. A positive relationship indicates similar patterns of species dominance on substrates of different successional stages, while a negative relationship implies community turnover with time due to successional processes.	Our data set includes carbonates colonized over one year and native carbonates from Hydrate Ridge, so we compared the ranked-abundance of species occuring on these substrates.	In contrast to the SSIM and variance partitioning methods, this test incorporates a temporal component and should reveal whether recruitment dyanamics have a strong influence on community assembly. If environmental filtering is strong, we would expect early successional assemblages to quickly resemble more mature assemblages on native carbonates. Deviations from a positive 1:1 line might be explainable by larval dispersal and implicate mass effects. A negative 1:1 line or many species occuring in one suceessional stage and not the other would suggest patch dynamics may contribute to different communities maintained through colonization-competition tradeoffs.

Table 5.2. Coherence, species turnover, and boundary clumping in the Hydrate Ridge metacommunity. Embedded absences and replacements were calculated in ordinated site-by-species matrices and compared to the mean from 1000 simulations of randomly constructed matrices. For boundary clumping, values of Morisita's Index >1 indicate clumping of range boundaries, and values <1 indicate over-dispersion of range boundaries. Note: several matrices contained many taxa that were present at a single site, preventing the R routine from running a complete analysis. For these cases, taxa with single presences were combined at higher taxonomic levels, slightly reducing overall richness.

	All Substrates	**Active**	**Inactive**	**Colonization**	**Native**	**All Carbonate**
Number of communities (substrates) in analysis	73	38	35	46	27	39
Taxa richness	77	64	57	75	64	80
Coherence	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**
Actual Embedded Absences	2902	812	831	1725	717	1387
Simulated Mean (± SD)	3304 ± 12	1121 ± 9	1040 ± 7	1942 ± 10	855 ± 7	1734 ± 10
z	2.47	3.75	3.90	2.34	2.75	3.37
P	**0.013**	**<0.001**	**<0.001**	**0.019**	**0.006**	**<0.001**
Species Turnover	N.S.	N.S.	**Positive**	**Positive**	N.S.	N.S.
Actual Replacements	166282	55277	60688	135104	24221	48496
Simulated Mean (± SD)	139172 ± 214	42048 ± 105	31703 ± 96	78457 ± 155	16725 ± 73	58544 ± 142
z	-0.59	-1.19	-3.13	-2.37	-1.43	0.50
P	0.552	0.232	**0.002**	**0.018**	0.153	0.617
Boundary Clumping	**Strong clumping**	**Strong clumping**	**Clumping**	**Clumping**	**Clumping**	**Strong clumping**
Morisita's Index	2.73	2.05	1.33	1.76	1.56	2.59
P	**<0.001**	**<0.001**	**0.027**	**<0.001**	**0.012**	**<0.001**
df	75	35	54	73	61	80

Table 5.3. Distance-based linear modeling (DistLM in PRIMER 6.1) results with beta diversity partitioned among environmental (E) and spatial (S) sets of parameters. Beta diversity is interpreted from a Bray-Curtis resemblance matrix of 4^{th}-root transformed macrofaunal density. Marginal tests represent the influence of each variable individually, whereas results of sequential tests give the cumulative effect of environmental parameter sets. These combined models were constructed with AIC stepwise selection for environmental parameters and the AIC best solution for spatial parameters, and the adjusted-R^2 (R^2_{adj}) criterion is reported. * $p < 0.05$, ** $p < 0.01$

Parameter	Prop.	Prop. (cum.)	R^2_{adj} (cum.)
Marginal tests			
log ($\delta^{13}C_{fauna}$) **	0.1334	-	-
Successional Stage **	0.0688	-	-
Activity **	0.2306	-	-
Substrate Type **	0.0949	-	-
log ($\delta^{13}C_{carbonate}$)	0.0180	-	-
ln (Depth)	0.0170	-	-
Seafloor cover	0.0301	-	-
ln (Substrate SA)	0.0139	-	-
X	0.016	-	-
XY	0.015	-	-
X2	0.015	-	-
Y	0.015	-	-
X3	0.015	-	-
Y3	0.015	-	-
Y2X	0.015	-	-
X2Y	0.015	-	-
Y2	0.015	-	-
AIC parameter selection			
Environment: AIC stepwise forward selection			
Activity **	0.231	0.231	0.220
Successional stage **	0.070	0.300	0.280
Substrate type **	0.063	0.363	0.306
ln (Depth) *	0.016	0.379	0.313
Spatial: AIC best solution			
8 groups (all except Y^3)			0.153
Variance Partitioning (DISTLM)			
Parameter Set	R^2_{adj}		
E + S	0.365		
E	0.329		
S	0.153		
E, independent of S	0.215		
S, independent of E	0.052		
Unexplained	0.635		

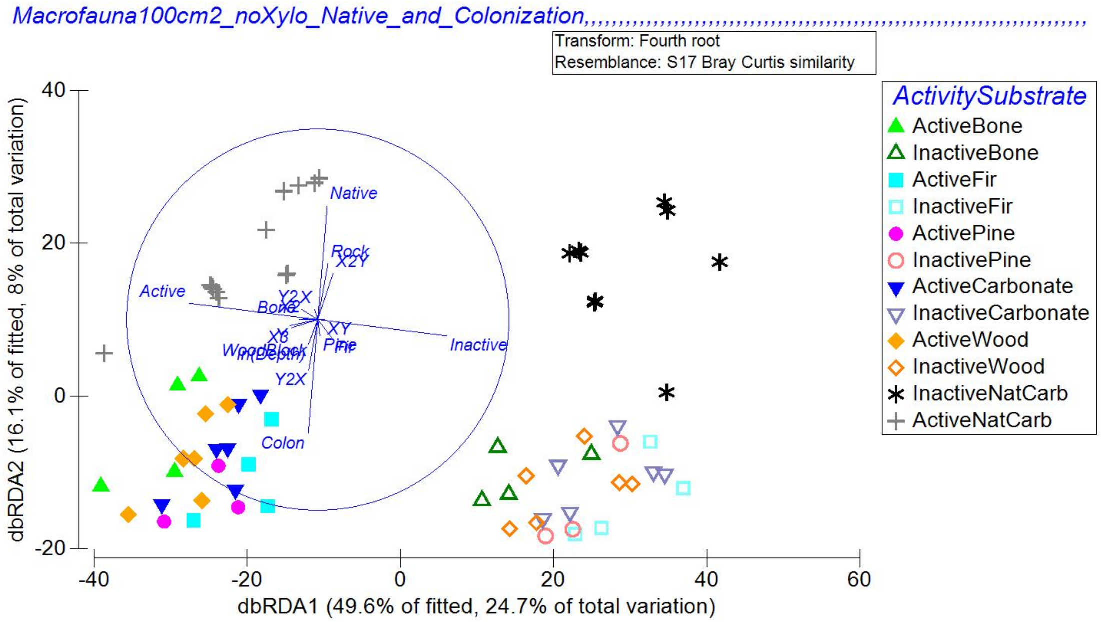

Figure 5.A1. Results of distance-based redundancy analysis (dbRDA), displaying relationships among communities on substrates and environmental parameters evaluated for distance-based linear modelling. Note the communities are divided into four clusters that are well defined by their level of activity and successional stage (colored shapes are all colonization substrates, while black stars and grey crosses are active and inactive native carbonates, respectively).

Table 5.A1. Densities of macrofauna (individuals 100 cm^{-2}) on colonization and native substrates at Hydrate Ridge. Colonization substrate densities are totaled across sites and regions.

		Active	**Inactive**
Colonization Substrates			
	Carbonate	18.2	4.0
	Bone	49.5	13.4
	Native Fir	47.9	4.2
	Native Pine	54.9	7.1
	Wood Blocks	17.7	8.7
Native Carbonate		31.2	11.3
	HR North	37.9	11.0
	HR South	17.8	11.9

Table 5.A2. Taxa contributing most to dissimilarity in colonization substrates between activity levels, and to dissimilarity in colonization of different substrates. Displayed are the average taxa densities (individuals 100 cm^{-2}), dissimilarity (Diss), taxon percent contribution to dissimilarity (Contr), and cumulative contribution to dissimilarity (Cum), based on SIMPER analysis (PRIMER 6.1).

Groups: Active and Inactive					
Average dissimilarity = 77.99					
Species	Act. Dens.	Inact. Dens.	Diss.	Contr	Cum
Provanna_lomana	1.48	0.1	7.13	9.14	9.14
Provanna_laevis	1.35	0.1	6.24	8	17.14
Pyropelta_corymba	0.91	0.06	4.54	5.82	22.96
Pyropelta_wakefieldi	0.8	0.03	4.09	5.24	28.2
Ampharetid	0.81	0.47	3.19	4.09	32.29
Amphipod	0.27	0.78	2.93	3.76	36.05
Cirratulids_all	0.83	0.34	2.62	3.37	39.42
Sphaerosyllis	0.66	0.14	2.43	3.12	42.53
Polynoid	0.44	0.66	2.41	3.09	45.62
Ophryotrocha	0.21	0.38	2.25	2.88	48.51
Isopod	0.28	0.49	2.18	2.79	51.3
Dorvilleid_other	0.42	0.26	2.16	2.77	54.07
Ophiuroid_unid	0.28	0.49	2.15	2.75	56.82
Syllid_other	0.33	0.37	2.11	2.71	59.53
Nemertean_pink	0.46	0.1	2.06	2.64	62.17
Lacydoniid_spA	0.3	0.03	1.36	1.75	63.92
Tanaid	0.11	0.29	1.32	1.69	65.61
Naineris	0.32	0.02	1.31	1.67	67.28
Hesionid	0.24	0.11	1.18	1.51	68.79
Vesicomyid	0.06	0.2	1.17	1.49	70.29
Bivalvia_unidjuv	0.15	0.06	1.03	1.32	71.6
Gastropod_unidjuv	0.18	0.14	1.02	1.31	72.91
Nereis	0.21	0.13	0.99	1.27	74.18
Capitellid	0.06	0.14	0.98	1.26	75.45
Neptunea_wood	0.04	0.15	0.98	1.25	76.7
Terebellid	0.14	0.22	0.97	1.24	77.94

Groups: Bone & Carbonate (Colonization only)					
Average dissimilarity = 62.04					
Species	Bone Dens.	Carb. Dens.	Diss.	Contr	Cum
Isopod	0.1	0.55	3.47	5.6	5.6
Ophryotrocha	0.6	0.16	3.21	5.18	10.78
Dorvilleid_other	0.51	0.34	3.13	5.05	15.83
Syllid_other	0.63	0.27	3	4.83	20.66
Osedax_yellowpatch	0.39	0	2.53	4.08	24.74
Ophiuroid_unid	0.29	0.54	2.52	4.06	28.8
Amphipod	0.32	0.53	2.51	4.05	32.85
Polynoid	0.49	0.48	2.38	3.84	36.69
Pyropelta_corymba	0.45	0.44	2.06	3.32	40.01
Pyropelta_wakefieldi	0.65	0.39	1.96	3.16	43.16
Polychaete_unid	0.32	0.15	1.89	3.05	46.21
Sphaerosyllis	0.48	0.08	1.89	3.04	49.25
Osedax_yellowcollar	0.31	0	1.65	2.65	51.91
Nereis	0	0.34	1.64	2.65	54.55
Provanna_lomana	0.96	0.85	1.64	2.64	57.19
Osedax_priapus	0.27	0	1.63	2.63	59.82
Cirratulids_all	0.5	0.36	1.58	2.55	62.36

Groups: Bone & Wood					
Average dissimilarity = 61.59					
Species	Bone Dens.	Wood Dens.	Diss.	Contr	Cum
Dorvilleid_other	0.51	0.23	3.29	5.34	5.34
Syllid_other	0.63	0.21	3.19	5.18	10.52
Ophryotrocha	0.6	0.3	3.06	4.97	15.49
Osedax_yellowpatch	0.39	0	2.65	4.3	19.78
Pyropelta_corymba	0.45	0.54	2.45	3.97	23.76
Ampharetid	0.45	0.6	2.41	3.92	27.67
Amphipod	0.32	0.47	2.39	3.88	31.55
Polynoid	0.49	0.42	2.3	3.73	35.28
Ophiuroid_unid	0.29	0.27	2.18	3.54	38.82
Isopod	0.1	0.36	2.11	3.43	42.25
Sphaerosyllis	0.48	0.1	2.09	3.4	45.65
Cirratulids_all	0.5	0.35	1.82	2.96	48.61
Osedax_yellowcollar	0.31	0	1.69	2.74	51.35
Osedax_priapus	0.27	0	1.69	2.74	54.08
Polychaete_unid	0.32	0.09	1.66	2.69	56.78
Provanna_laevis	0.88	0.79	1.61	2.61	59.39
Hesionid	0.2	0.09	1.59	2.58	61.97

Groups: Wood & Carbonate (Colonization only)					
Average dissimilarity = 57.39					
Species	Wood Dens.	Carb. Dens.	Diss.	Contr	Cum
Ophiuroid_unid	0.27	0.54	3.29	5.74	5.74
Isopod	0.36	0.55	2.76	4.81	10.54
Dorvilleid_other	0.23	0.34	2.5	4.36	14.9
Amphipod	0.47	0.53	2.42	4.21	19.11
Ampharetid	0.6	0.47	2.3	4.01	23.12
Ophryotrocha	0.3	0.16	2.25	3.92	27.05
Vesicomyid	0.28	0.14	2.17	3.78	30.82
Provanna_lomana	0.9	0.85	2.11	3.67	34.5
Syllid_other	0.21	0.27	1.98	3.45	37.95
Polynoid	0.42	0.48	1.94	3.38	41.33
Pyropelta_wakefieldi	0.56	0.39	1.88	3.28	44.61
Nereis	0.03	0.34	1.87	3.27	47.88
Nemertean_pink	0.18	0.28	1.74	3.03	50.91
Provanna_laevis	0.79	0.83	1.7	2.96	53.87
Bivalvia_unidjuv	0.13	0.17	1.64	2.86	56.73
Neptunea_wood	0.25	0	1.62	2.82	59.55

Groups: Native Carbonate & Colonization Carbonate					
Average dissimilarity = 63.00					
Species	NatCarb. Der	ColCarb. Der	Diss.	Contr	Cum
Cirratulids_all	0.36	0.96	3.09	4.91	4.91
Sphaerosyllis	0.08	0.83	3.02	4.8	9.7
Ampharetid	0.47	0.83	2.62	4.15	13.86
Ophiuroid_unid	0.54	0.44	2.41	3.82	17.68
Syllid_other	0.27	0.44	2.2	3.49	21.17
Polynoid	0.48	0.71	2.12	3.37	24.54
Amphipod	0.53	0.61	1.96	3.12	27.65
Provanna_lomana	0.85	0.69	1.93	3.06	30.71
Isopod	0.55	0.4	1.9	3.01	33.72
Nereis	0.34	0.29	1.78	2.83	36.55
Tanaid	0.06	0.37	1.71	2.71	39.26
Hydroids	0	0.34	1.69	2.68	41.94
Terebellid	0.11	0.38	1.69	2.68	44.63
Provanna_laevis	0.83	0.63	1.68	2.67	47.3
Pyropelta_wakefieldi	0.39	0.26	1.56	2.48	49.78
Ophryotrocha	0.16	0.25	1.45	2.31	52.08
Gastropod_unidjuv	0.1	0.32	1.44	2.29	54.37
Pyropelta_corymba	0.44	0.51	1.38	2.2	56.57
Nemertean_pink	0.28	0.39	1.38	2.19	58.76
Hesionid	0.06	0.32	1.25	1.99	60.75
Dorvilleid_other	0.34	0.39	1.24	1.96	62.71

CHAPTER SIX

CONCLUSIONS

Cold seeps are characterized by many types of heterogeneity – geological, geochemical, spatial, biological – a point that has been echoed by many other investigators (Orphan et al. 2004; Levin 2005; Cordes et al. 2010; Bernardino et al. 2012). This dissertation set out to elucidate the consequences of several types of that heterogeneity for macrofaunal communities at methane seeps by investigating the community composition, dynamics, and trophic structure of hard substrate assemblages. Despite being separated by 6000 km and each occurring in distinct geotectonic settings, seeps at Costa Rica's Mound 12, Del Mar, and Hydrate Ridge were found to have consistencies that are likely be extrapolated to other cold seep ecosystems, especially where carbonate substrates are abundant. In general, habitat heterogeneity correlates with species richness, and this was observed at each of the three seep locations. Even the Del Mar Seep, located on the edge of the oxygen minimum zone (OMZ) and with a limited central area of activity (10–15 m across), had a rarefied species richness that rivaled any other seep in a meta-analysis by Bernardino et al. (Orphan et al. 2004; Levin 2005; Cordes et al. 2010; Bernardino et al. 2012).

Colonization experiments introduced novel forms of heterogeneity to seeps as substrates intended to invoke various chemosynthetic ecosystems: authigenic carbonates (seeps), wood (untreated lumber and natural pieces of wood intended to simulate sunken wood falls), bones (to simulate whale falls or other sunken megafauna), and shells and tubes (biological habitat providers). The chemically heterogeneous landscape allowed

comparison of successional dynamics in active and inactive environments. The finding that chemical heterogeneity was dominant in structuring the resulting communities at Mound 12 and Hydrate Ridge was expected because of the relationship between fluid flow and microbial producers, upon which most faunal species at active seeps depend (Levin and Michener 2002; Tunnicliffe et al. 2003). Macrofauna colonized wood, bone, and shell substrates at rates usually as high as on carbonate, a result mirrored in other chemosynthetic environments (Gaudron et al. 2010; Cunha et al. 2013; Cuvelier et al. 2014). The occurrence of many species on multiple substrates indicates flexibility in habitat requirements, but also reflects the ability of similar microbial populations to be productive on different types of surfaces with different sources of H_2S.

Away from the influence of seepage, substrate sometimes took on a more important role in shaping macrofaunal colonization patterns. At Hydrate Ridge, at least five species of substrate specialists recruited only to wood or bone. In contrast several seep gastropods recruited to these organic substrates and were able to obtain chemosynthetic resources, based on stable isotope signatures. Thus, at short distances from methane seeps, the regional fauna does respond to substrate heterogeneity, and some species may even utilize certain substrates as stepping stones to other reducing ecosystems (Smith et al. 1989). These findings are germane since sunken wood is common at Mound 12 and other Costa Rica seeps (B. Grupe & L. Levin, personal observations) and winter storms commonly wash wood from the abundant forests in Oregon and Washington into the Pacific. Whale falls may also be common in these regions due to migratory routes of several species of baleen whales that migrate or feed

along the west coast of North and Central America (Calambokidis and Barlow 2004; May-Collado et al. 2005).

Carbonates and other substrates we deployed tended to be dominated by seep-endemic gastropods, which are rapid colonizers onto new seep habitats. Some of these species ranged across all of our sites (*Provanna* spp., *Pyropelta* spp.), while others only appeared in Costa Rica seeps, which support particularly diverse site for gastropods and other taxa. In fact, though the rarefied richness (ES_{100}) for the active colonization communities at Mound 12 was only half that of inactive colonization communities, it was still greater than both active and inactive carbonates (native or colonization treatments) at Hydrate Ridge. Even so, diversity metrics of colonization communities lagged those on native substrates, both in active and inactive settings.

Stable isotope data from macrofauna at Mound 12 suggest that dietary flexibility might be related to the ecological success of colonizers. Multiple production pathways lead to a wide range of $\delta^{13}C$ in heterotrophic fauna, seen both in early and late successional assemblages. Thus, a dietary generalist like *Provanna laevis* not only is likely to be able to select from multiple microhabitats and food types within a seep, but as a new recruit is more likely to be able to quickly find nutrition no matter where it is. This is not to say trophic specialists are excluded from early successional communities. At Mound 12, a chiton species, *Paralepetopsis* sp., and *Parougia* sp. all colonized active carbonates and had respective $\delta^{13}C$ values of -48‰, -60‰, and -93‰. These were feeding on very different food sources than most macrofauna (generally -22 to -40‰), and were almost certainly incorporating methane-derived carbon. At both Mound 12 and

Hydrate Ridge, the general trophic structure recovered quickly on colonization substrates, with the possible exception of Mound 12 carbonates, in which trophic diversity and range of $\delta^{13}C$ and $\delta^{15}N$ fell far short of native communities on inactive carbonates.

Results from this dissertation could influence future management strategies in deep-sea chemosynthetic environments, which may possibly be a target of trawling, mining, oil, gas or gas hydrate extraction, or bioprospecting in the 21st century (Thornburg et al. 2010; Van Dover 2010; Levin and Sibuet 2012). The metacommunity perspective that is applicable to seeps and vents emphasizes dispersal among patches. Small-scale disturbances within an active seep probably see rapid recovery to a community that is similar to what existed before. The macrofauna observed to colonize active substrates were usually the same species already present in the local community, and might have originated locally, representing self-recruitment. Even so, after one year, some species from the surrounding communities at Mound 12 and Hydrate Ridge had not recruited, so the rapid colonization of dominant species and recovery of the food web should not be interpreted as perfect ecological resilience. Inactive sites were colonized at slower rates, and resulting communities did not necessarily reflect those on native substrates. In addition, trophic structure on inactive carbonates seems to recover more slowly than at active sites. The margin environments surrounding seeps often contain sessile, suspension-feeding species such as corals and crinoids. They are probably longer lived and slower growing than most endemic seep species, since they occupy less productive sites, so these areas could be more sensitive to disturbance than seeps themselves.

Ramifications of larger disturbances that would negatively affect an entire mound or network of seeps, however, would involve a longer recovery time, be more difficult to predict, and would likely be heavily influenced by the distance to other seeps. Seeps – especially those along active margins – are probably susceptible to large-scale natural disturbance from earthquakes and turbidity flows, and methane hydrate destabilization, but human actions are increasing the frequency of such large-scale disturbances. Exploration of cold seeps on the New Zealand margin revealed evidence of deep-water trawling at many sites, the intensity of which may correlate with reductions in chemotrophic fauna such as long-lived tubeworms (Baco et al. 2010; Bowden et al. 2013). If seeps, whale falls, and wood falls interact in metacommunities, as this investigation suggests is plausible, human may have already impacted their structure in a regional sense where whaling has led to population declines and a reduced number of sinking whale carcasses, or along coasts where deforestation has reduced the amount of wood subsidies to the deep sea (Butman et al. 1995).

A metacommunity can only maintain its biodiversity and overall ecosystem function if a sufficient number of patches remain intact to allow populations to remain connected. This underscores a need to improve our understanding of connectivity patterns and to explore and discover new sites that may act as important sources or sinks of larvae (Mullineaux et al. 2010). Since seep larvae are able to disperse on surface currents (Arellano et al. 2014), it may be that negative impacts at any seep could indirectly affect other communities negatively hundreds of kilometers away. A cautious but prudent principle would be to refrain from performing activities that would ruin the integrity of

large swaths of active seeps or permanently damage structures (e.g. complex carbonate structures, habitat forming tubeworm bushes) that might not recover as fast as the populations in this study, especially in regions where seeps might be widely spaced with a greater degree of beta diversity, or where rates of larval dispersal and connectivity might be lower.

A major outstanding question for the ecology of chemosynthetic ecosystems is to what extent they interact with surrounding non-chemosynthetic ecosystems. At each seep in this study, scientists using submersibles or ROVs have observed invertebrate and vertebrate megafauna near active seeps, and sometimes even species targeted by fisheries. Longspine thornyhead and Pacific dover sole were abundant at the Del Mar seep, and sablefish were observed near the active pinnacle at the Hydrate Ridge North mound. That densities of thornyhead increase closer to the Del Mar seep is an association and not necessarily habitat dependence, but chemosynthetic productivity is prolific enough to be exported to sediments of the OMZ surrounding the seep, and it could well form as aspect of the diet of these fish as well. Other investigators have also observed higher densities of predatory fish near seeps, and whether attracted by the productivity or structural aspects of the environment, it is important we be aware of the potential for ecosystem services that specific cold seeps in the deep sea provide to humans before extractive activities began.

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