

Megafauna community structure and trophic relationships at the recently discovered Concepción Methane Seep Area, Chile, $\sim 36^{\circ}\text{S}$

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The fauna, community composition, and trophic support of the newly discovered Concepción Methane Seep Area (CMSA) are compared with those at a nearby non-seep control. The assemblage of chemosymbiotic bivalves is defined by eight species, including the families Lucinidae, Thyasiridae, Solemyidae, and Vesicomidae. Seep polychaetes are represented by *Lamellibrachia* sp. and two commensal species of the vesicomid *Calyptogena gallardoi*. Although taxonomic analysis is still under way, most of the chemosymbiotic species seem to be endemics. The CMSA is a hotspot for non-seep benthic megafauna too; 101 taxa were present, but most of them are colonists or vagrants (i.e. not endemics of methane seeps). Isotope analysis supported the belief that non-symbiont-bearing species utilize photosynthetically fixed carbon, because they were isotopically distinct from the chemosymbiotic bivalve species present. It is our opinion that, at this site, which underlies one of the most productive coastal upwelling regions of the world, spatial heterogeneity and the availability of hard substratum, generated by the presence of authigenic carbonate crusts, are more important factors in attracting non-seep fauna than the availability of locally produced chemosynthetic food.

Keywords: bathyal benthos, chemosynthesis, Chilean margin, cold seep, stable isotopes.

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Introduction

The discovery of deep-water chemosynthetically fueled systems has been one of the most fascinating scientific findings of the past century (Spiess *et al.*, 1980). Although only discovered two decades ago (Paull *et al.*, 1984; Kennicut *et al.*, 1985), cold-seep communities, those fueled by the emission of methane, have been identified at nearly 100 deep-water sites worldwide, on both active and passive continental margins, in the Atlantic, Pacific, and Indian Oceans, and in the Mediterranean Sea (Sibuet and Olu-Le Roy, 2002; Mazurenko and Soloviev, 2003; Levin, 2005), and most recently, at the shelf below the ice cap near the Antarctic Peninsula (Domack *et al.*, 2005). However, just a small fraction of these sites have been characterized in terms of faunal communities (Levin, 2005). A key area to increase our understanding of seep biogeography is the SE Pacific coast off South America, because to date no studies have described the fauna comprehensively. An area of special interest is the Chilean margin, where, other than the fauna of the oxygen minimum zone (OMZ), little is known about the reducing ecosystems present. The OMZ benthos consists mainly of a community formed by the giant, sulphide-oxidizing bacteria *Thioploca* (Gallardo, 1977), which carpets the shelf sediments that are impinged by the SE Pacific oxygen minimum layer.

However, the realm beyond the shelf break off the Chilean margin has remained mostly unexplored for chemosynthetic systems. Indeed, there have been only a few reports of chemosynthetic taxa off South American coasts: off NE Brazil (e.g. *Calyptogena birmani*; Domaneschi and Lopes, 1990), off Uruguay (e.g. *Lamellibrachia victori*; Mañé-Garzón and Montero, 1986), off Chile (e.g. *Ectenagena australis*; Stuardo and Valdovinos, 1988), and the Yaquina Basin methane seeps off Peru (Olu *et al.*, 1996) are the only ones with detailed biological characterization to date.

Several aspects of the Chilean margin suggest the existence of extensive methane-seep communities: (i) the report of vast gas hydrate fields extending from 35°S to 45°S (Morales, 2003); (ii) the presence of an active subduction front with a well-developed accretionary prism and suggesting fluid expulsion from the sediment (Lagabrielle *et al.*, 2004); and (iii) the early description of the chemosymbiotic clam, *E. australis*, from ~ 1400 m after incidental collection of two specimens by bottom longliners (Stuardo and Valdovinos, 1988).

Based on dredged chemosymbiotic clam fragments and carbonate blocks, the first active seep site off the Chilean coast was recently discovered, the Concepción Methane Seep Area (CMSA; $\sim 36^{\circ}\text{S}$; Sellanes *et al.*, 2004). This study describes the fauna of

this southernmost South American methane-seep area, and compares the roles of chemosynthetic and photosynthetic nutrition in a deep-water seep that underlies one of the most productive photic zones in the world. In this context, we aim to characterize the taxonomic composition of megafaunal communities, including both chemosymbiotic and accompanying non-chemosymbiotic species, and to assess the utilization of chemosynthesis-based food sources by members of the cold-seep community based on C and N stable isotope analysis.

Material and methods

Study area

The CMSA is located 72 km northwest of Concepción Bay ($36^{\circ}22'S$ $73^{\circ}73'W$), on the mid-slope (740–870 m water depth), and on one side of two adjacent mounds separated by a shallow depression (Figure 1). Previous piston-core deployments have found large gas hydrate deposits with carbon isotopic values of $-62.8 \pm 1.0\text{‰}$ for both the hydrates themselves and sedimentary porewater (Coffin *et al.*, 2006); this value is indicative of a biogenic origin of the methane (Ussler *et al.*, 2003). There are abundant blocks formed by carbonate-cemented mud, i.e. mud breccia, containing shell fragments of at least two species of clam known to harbour chemosymbiotic endosymbionts, e.g. a vesicomid and a solemyid (Figure 2; Sellanes *et al.*, 2004). Previous studies have also reported dead vesicomids and other species of chemosymbiotic clam (e.g. *Lucinoma anemiophila*, *Thyasira methanophila*, and *Conchocele* sp.; Sellanes and Krylova, 2005).

A control non-seep site was also sampled to compare the megafaunal composition of the CMSA with the typical bathyal fauna at a similar depth. This site, situated at $36^{\circ}32.54'S$ $73^{\circ}40.05'W$, 798 m deep, and 27 km south of the CMSA, was chosen based on faunal information gathered during a RV “Sonne” cruise (site GeoB 7162; Hebbeln *et al.*, 2001). An underwater video transect, a piston-corer

deployment, and trawled megafauna did not show any evidence of methane-seep activity in the area. Indeed, analysis of the piston corer showed that the upper 345 cm of the sediment at this non-seep site consisted largely of hemipelagic clays (Hebbeln *et al.*, 2001).

Sample collection and analysis

Sampling was conducted on board the Chilean Navy’s RV “Vidal Gormáz” during October 2004 (VG-04 cruise) and September 2006 (SeepOx cruise), and on the RV “Sonne” (a few samples; Table 1). Samples were collected by an Agassiz trawl (mouth opening 1.5×0.5 m, mesh size 10×10 mm in the codend), in 20-min hauls. Animals were sorted from the non-biological material and preserved on board using appropriate methods for later analysis (e.g. frozen, buffered 10% seawater formalin, glutaraldehyde, and absolute ethanol). Only trawls in which evidence of chemosynthetic activity was observed (e.g. carbonates, shell fragments, or living chemosymbiotic clams) were used for the analysis. No estimates of biomass or abundance were attempted because of the hardness of the substratum and the consequent inadequacy of the collection method for quantitative calculations. Additionally, the collection of large volumes of carbonate blocks (500–1000 kg at each haul) made complete sorting of the samples impractical. However, an indication of the relative frequency of occurrence of each species, based on its relative abundance, was recorded as follows: (i) abundant, present in all hauls and in considerable quantities (e.g. >10 specimens), (ii) common, present in 50% or more of the hauls and sometimes in considerable numbers, (iii) occasional, present in <50% of hauls, in general in small quantities, and (iv) rare, just a few specimens collected in one or two hauls.

Samples for bottom water particulate organic matter (POM) were collected at the CMSA using a Rosette with 12 \times 8 l Niskin

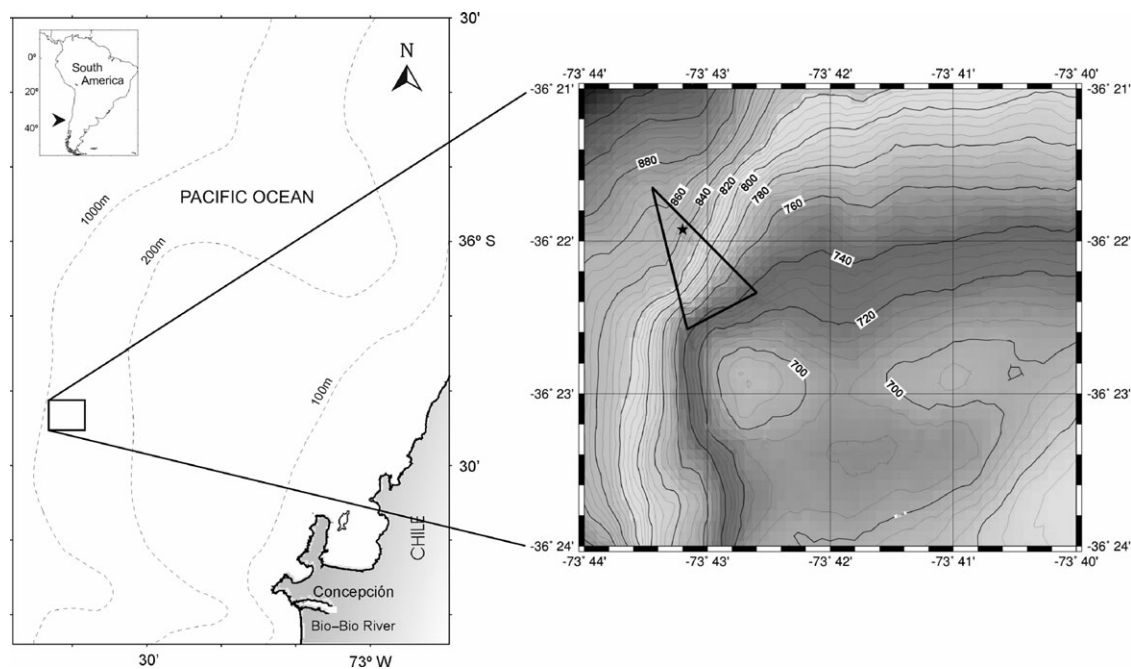


Figure 1. Study area off Concepción Bay, Central Chile. The triangle indicates trawls where evidence of active methane seepage was collected (carbonate blocks, live chemosymbiotic clams, and shell fragments). The star indicates the position in which shallow subsurface gas hydrates have been observed.

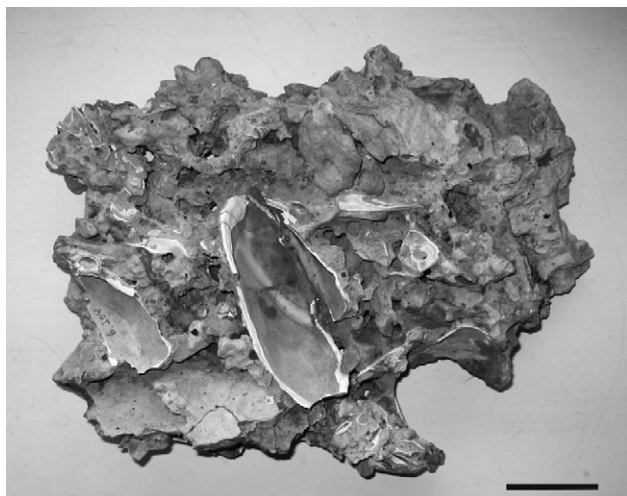


Figure 2. Typical carbonate block collected at the study area. Cemented valves of vesicomysids are also visible. Scale bar = 5 cm.

Table 1. Initial position of trawls (Agassiz trawls, AGT) during VG-04, SeepOx, and SO-156 cruises at the CMSA and the control non-seep site. Observations on the occurrence of chemosymbiotic fauna for AGTs at the seep site are also provided.

Cruise	Gear	Latitude S	Longitude W	Depth (m)	Observations*
VG-04	AGT 6	36°21.75'	73°43.55'	726–865	Cg ^{ld} , V1 ^d , Cc ^d
	AGT 7	36°21.64'	73°43.57'	865–926	Cg ^{ld} , V2, Cx ^d , Ax ^l , Tm ^l , Lb ^d
	AGT 8	36°21.80'	73°43.10'	708–854	Cg ^{ld} , V1 ^d , Tm ^{ld}
	AGT 9	36°21.90'	73°43.21'	713–850	Cg ^{ld} , V1 ^{ld} , Lb ^d
	AGT 10	36°22.68'	73°42.46'	708–709	Ax ^d , La ^d
SeepOx	AGT 13	36°21.91'	73°43.21'	728–843	Cg ^{ld} , V1 ^d , Ax ^d , La ^d , Tm ^{ld}
	AGT 6–3	36°21.18'	73°43.89'	919–891	Cg ^{ld} , V2 ^d , Tm ^d
	AGT 6–5	36°21.60'	73°43.88'	728–885	Cg ^d , V1 ^d , Tm ^d
	AGT 6–8	36°21.90'	73°43.21'	710–870	Cg ^{ld} , V1 ^d , Lb ^{ld}
	AGT 7–1	36°32.05'	73°45.02'	879–880	Control, non-seep site
SO-156	AGT 7–2	36°32.54'	73°40.52'	817–820	Control, non-seep site
	AGT 7162	36°32.54'	73°45.72'	798–782	Control, non-seep site

*Cg, *Calyptogena gallardoii*; V1, vesicomysid gen. sp. 1; V2, vesicomysid gen. sp. 2; Cx, *Calyptogena* sp.; Ax, *Acharax* sp.; Tm, *Thyasira methanophila*; Cc, *Conchocele* sp.; La, *Lucinoma anemiophila*; Lb, *Lamellibrachia* sp.; living specimens; ^ddead or shell fragments.

bottles. For each sample, ~2 l of water was pre-sieved through a 63- μ m mesh to remove zooplankton and large detrital particles, then filtered onto pre-combusted (500°C for 4 h) Whatman GF/F filters (nominal 0.7 μ m pore size).

Stable isotope analysis

Stable C and N isotope signatures were analysed for animals, sedimentary organic matter (SOM), bottom-water-suspended POM, and randomly collected potential food sources (e.g. remains of

macroalgae, probably *Macrocystis pyrifera*, at the control site). Additional muscle tissue samples of Patagonian toothfish (*Dissostichus eleginoides*), a large predatory fish known to be present at the seep site but difficult to collect by trawling, were obtained from fish caught by artisanal fishers operating at the CMSA. Samples were frozen (–20°C) and later dried at 60°C overnight. After being ground to a fine powder using an agate mortar, samples were treated with a 1% solution of PtCl₂ to remove inorganic carbon. Because of the elevated lipid content of *D. eleginoides*, small pieces of tissue (<1 g) were rinsed with distilled water, air-dried, soaked in a 1:1 chloroform:methanol solution three times, then rinsed with distilled water to remove lipids before stable isotope analyses (Beaudoin *et al.*, 2001). This lipid removal does not produce significant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ shifts in lipid-free samples (Sotiropoulos *et al.*, 2004).

Isotope composition was analysed in the laboratory of R. Lee (School of Biological Sciences, Washington State University) by a Eurovector elemental analyser (Milan, Italy) coupled to a Micromass Isoprime isotope ratio mass spectrometer (Manchester, UK). Stable isotope ratios are reported in the δ notation as the deviation from standards (Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric N for $\delta^{15}\text{N}$), so $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ = [(R sample/R standard) – 1] $\times 10^3$, where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively. Typical precision of the analyses was $\pm 0.5\text{‰}$ for $\delta^{15}\text{N}$ and $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$.

Results

Chemosymbiotic fauna

The chemosymbiotic assemblage of the CMSA was dominated by eight species of bivalve (Figure 3). Of the Vesicomysidae, *Calyptogena gallardoii* was the most frequently collected. Three other unresolved vesicomysids were present too: a large and slender species (gen. sp. 1), measuring up to 180-mm long, a species of medium size, with an elliptical outline and adherent periostracum (gen. sp. 2), and another species of the genus *Calyptogena*, similar to *C. gallardoii* in appearance, but with a subcircular shell outline (Figure 3). Other chemosymbiotic bivalve families included the Lucinidae (*L. anemiophila*; Holmes *et al.*, 2005), Thyasiridae (*T. methanophila*; Oliver and Sellanes, 2005, and *Conchocele* sp.), and Solemyidae (*Acharax* sp.). Although more living specimens of *Conchocele* sp. and *Acharax* sp. are needed for proper taxonomic studies, upon initial examination they do not correspond to species previously described. Indeed, it is the first time that the genus *Conchocele* has been reported in the SE Pacific (Oliver and Sellanes, 2005).

Three polychaete species were sampled successfully, including one chemosymbiotic and two commensal taxa. Six living *Lamellibrachia* spp. (Siboglinidae), and several unoccupied tubes up to 130-cm long, were collected. A few *C. gallardoii* (~10%) hosted commensal polychaetes belonging to two different families, one to the Nautiliniellidae (*Shinkai* sp.) and the other to the Antonbruunidae (*Antonbruunia* sp.). To our knowledge, this is the first time that two species of commensal polychaete have been reported for a single vesicomysid species. These species are novel and are currently being described.

Heterotrophic fauna

Of the 101 non-chemosymbiotic megafaunal species observed at the CMSA (Table 2), an onuphid polychaete (*Hyalinoecia* sp.),

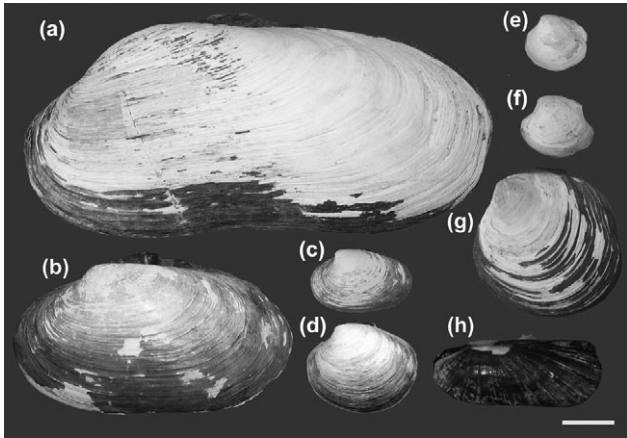


Figure 3. Chemosymbiotic bivalve assemblage of the CMSA: (a) vesicomid gen. sp. 1, (b) vesicomid gen. sp. 2, (c) *Calyptogena gallardoii*, (d) *Calyptogena* sp., (e) *Thyasira methanophila*, (f) *Conchocele* sp., (g) *Lucinoma anemiophila*, and (h) *Acharax* sp. Scale bar = 2 cm.

a solenocetid shrimp (*Haliporoides diomedea*), and many species of cnidarians, gastropods (*Bathybembix*, *Miomelon*, and *Homalopoma*), annelids, crustaceans, and echinoderms were particularly abundant. Macrourids (grenadiers or rattails) were also common (Table 2). All 24 species collected at the control site were also present at the CMSA, but none was as abundant at the control site as at the CMSA. *Hyalinoecia* sp., *H. diomedea*, and the elasmobranchs *Halaelurus canescens* and *Bathyraja* sp., as well as the rattail *Coelorinchus fasciatus*, were the most conspicuous species at the control site.

Stable isotope signatures

Usually, chemosymbiotic organisms were isotopically distinct from the heterotrophic fauna (Figure 4, Appendix). Vesicomids had the lowest $\delta^{13}C$ and $\delta^{15}N$ signatures, ranging from -36.2 to -35.4 ‰ for $\delta^{13}C$, and from 2.9 to 4.8 ‰ for $\delta^{15}N$, whereas values for $\delta^{15}N$ of *Thyasira methanophila* ($\delta^{15}N = 10.0$ ‰) and for $\delta^{13}C$ of *Lamellibrachia* sp. ($\delta^{13}C = -22.9$ ‰) were close to the range of POM and SOM.

Isotopic signatures did not differentiate the non-symbiont-bearing fauna between the control and CMSA sites (Figure 4). At the CMSA, heterotrophic fauna $\delta^{13}C$ ranged from -19.8 to -11.0 ‰, and $\delta^{15}N$ from 12.6 to 23.5 ‰. Isotopic signatures of the heterotrophic fauna at the control site were within this range (Appendix). This suggests that at both CMSA and control sites, the primary organic food sources are basically the same, most probably POM and SOM. However, some top predators at the CMSA displayed slightly lighter $\delta^{13}C$ values than lower trophic level consumers (Appendix). These include the Patagonian toothfish ($\delta^{13}C = -18.6$ ‰), the octopus *Benthoctopus* sp. ($\delta^{13}C = -17.8$ ‰), and the morid *Antimora rostrata* ($\delta^{13}C = -17.4$ ‰), the latter also with a relatively low $\delta^{15}N$ (15.0 ‰). *Antimora rostrata* collected at the control site displayed heavier isotopic signatures for both isotopes ($\delta^{13}C = -16.0$ ‰ and $\delta^{15}N = 20.1$ ‰).

Table 2. Species collected at the CMSA and control non-seep sites.

Taxa	Seep endemic	CMSA	Non-seep
PORIFERA			
gen. sp.	Y/N	R	–
CNIDARIA			
Anthozoa			
Alcyonaria			
Gorgonacea			
<i>Paragorgia</i> sp.	N	A	–
<i>Callogorgia</i> sp.	N	C	–
<i>Swiftia</i> sp.	N	O	–
gen. sp.	N	R	–
Zoantharia			
Actinaria			
<i>Actinostola</i> sp.	N	R	–
<i>Coralliomorphus</i> sp.	N	O	–
<i>Hormathia</i> sp.	N	O	–
gen. sp.	N	R	–
Scleractinia			
Cariophyllidae			
<i>Bathycyatus chilensis</i>	N	R	–
<i>Caryophyllia huinayensis</i>	N	C	–
Flabellidae			
<i>Flabellum apertum</i>	N	R	–
MOLLUSCA			
Polyplacophora			
Leptochitonidae			
<i>Leptochiton americanus</i>	N	O	–
Ischnochitonidae			
<i>Stenosemus exaratus</i>	N	R	–
Mopaliidae			
<i>Placiphorella atlantica</i>	N	R	–
Gastropoda			
Neolepetopsidae			
<i>Bathylepeta</i> sp.	Y/N	C	–
Fissurellidae			
<i>Puncturella</i> sp. 1	Y/N	C	–
<i>Puncturella</i> sp. 2	N	C	R
Trochidae			
<i>Bathybembix macdonaldi</i>	N	A	R
<i>Margarites huloti</i>	Y/N	R	–
<i>Zetela alphonsi</i>	N	O	–
<i>Calliotropis</i> sp.	N	R	–
Calliostomatidae			
<i>Calliostoma chilena</i>	N	A	–
<i>Calliostoma crustulum</i>	N	R	–
Turbinidae			
<i>Homalopoma panamense</i>	N	C	–
Naticidae			
<i>Natica</i> sp.	N	R	R
Ranellidae			
<i>Fusitriton magellanicus</i>	N	R	–

Continued

Table 2. Continued

Taxa	Seep endemic	CMSA	Non-seep
Muricidae			
<i>Coronium cf. wilhelmense</i>	N	R	–
<i>Pagodula concepcionensis</i>	N	R	–
<i>Trophon ceciliae</i>	N	R	–
<i>Trophon condei</i>	N	R	–
Buccinidae			
<i>Kryptos explorator</i>	N	O	–
Volutidae			
<i>Miomelon philippiana</i>	N	O	R
Turridae			
<i>Aforia cf. goniodes</i>	N	A	R
gen. sp. 1	N	R	–
gen. sp. 2	N	R	–
Bivalvia			
Nuculidae			
<i>Ennucula grayi</i>	N	R	R
Solemyidae			
<i>Acharax</i> sp.	Y	R	–
Limopsidae			
<i>Limopsis ruizana</i>	N	R	–
Lucinidae			
<i>Lucinoma anemiophila</i>	Y	R	–
Thyasiridae			
<i>Thyasira methanophila</i>	Y	O	–
<i>Conchocele</i> sp.	Y	R	–
Vesicomysidae			
<i>Calyptogena gallardoii</i>	Y	C	–
<i>Calyptogena</i> sp.	Y	O	–
gen. sp. 1	Y	O	–
gen. sp. 2	Y	R	–
Scaphopoda			
Dentalidae			
<i>Fissidentalium majorinum</i>	N	O	R
Cephalopoda			
Octopodidae			
<i>Benthoctopus</i> sp.	N	R	–
Sepiolidae			
<i>Semirossia patagonica</i>	N	R	–
ANNELIDA			
Polychaeta			
Onuphidae			
<i>Hyalinoecia</i> sp.	N	A	C
Eunicidae			
<i>Eunice cf. magellanica</i>	N	R	R
<i>Eunice</i> sp.	N	O	–
Aphroditidae			
<i>Aphrodite longirostris</i>	N	R	–
Sabellidae			
gen. sp.	N	R	–
Lumbrineridae			

Continued

Table 2. Continued

Taxa	Seep endemic	CMSA	Non-seep
gen. sp.	N	R	–
Antobruunidae			
<i>Antonbruunia</i> sp.	Y	R	–
Nautiliniellidae			
<i>Shinkai</i> sp.	Y	R	–
Siboglinidae			
<i>Lamellibrachia</i> sp.	Y	R	–
CRUSTACEA			
Cirripedia			
Thoracica			
Scalpellidae			
<i>Arcoscalpellum</i> sp.	N	R	–
<i>Scalpellum projectum</i>	N	R	–
Decapoda			
Dendrobranchiata			
Solenoceridae			
<i>Haliporoides diomedea</i>	N	A	C
Pleocyemata			
Oplophoridae			
<i>Acantephyra pelagica</i>	N	O	–
<i>Oplophorus</i>	N	R	–
novaezeelandiae			
Campylonotidae			
<i>Campylonotus semistriatus</i>	N	C	O
Crangonidae			
<i>Paracrangon areolata</i>	N	R	–
<i>Sclerocrangon atrox</i>	N	R	–
Polychelidae			
<i>Stereomastis sculpta</i>	N	R	R
Galatheidae			
<i>Munidopsis quadrata</i>	N	R	–
<i>Munidopsis trifida</i>	N	C	–
<i>Munida curvipes</i>	N	R	–
<i>Munida propinqua</i>	N	R	–
Atelecyclidae			
<i>Trichopeltarion corallinus</i>	N	R	–
<i>Trichopeltarion hystricosus</i>	N	R	–
Lithodidae			
<i>Lithodes turkayi</i>	N	R	–
<i>Paralomis</i> sp.	N	R	–
Isopoda			
Cirolanidae			
<i>Cirolana</i> sp.	N	R	–
<i>Aega</i> sp.	N	R	–
BRACHIOPODA			
<i>Liothyrella cf. scotti</i>	N	O	–
gen. sp.	N	O	–
ECHINODERMATA			
Crinoidea			
<i>Solanometra</i> sp.	N	O	–

Continued

Table 2. Continued

Taxa	Seep endemic	CMSA	Non-seep
Asteroidea			
Ctenodiscididae			
<i>Ctenodiscus australis</i>	N	C	-
Pterasteridae			
<i>Hymenaster</i> sp.	N	R	-
Solasteridae			
<i>Solaster regularis</i>	N	R	-
Zoroasteridae			
<i>Doraster qawashqari</i>	N	O	O
Goniasteridae			
<i>Ceramaster patagonicus</i>	N	O	-
<i>Hippasteria hyadesi</i>	N	C	-
gen. sp. 1	N	R	R
gen. sp. 2	N	R	-
Ophiuroidea			
Gorgonocephalidae			
<i>Gorgonocephalus chilensis</i>	N	C	-
Asteronyxidae			
<i>Asteronyx loveni</i>	N	R	-
<i>Astrodia tenuispina</i>	N	C	-
<i>Astrodia</i> sp.	N	O	-
Ophiuridae			
<i>Ophiura carinata</i>	N	R	R
<i>Stegophiura</i> sp.	N	A	-
Ophiolepididae			
<i>Ophiomusium biporicum</i>	N	C	-
<i>Ophiomusium lymani</i>	N	R	-
Echinoidea			
Schizasteridae			
<i>Tripylaster</i> sp.	N	R	R
Phymosomatidae			
<i>Phormosoma</i> sp.	N	R	R
Holothuroidea			
gen. sp.	N	O	-
SIPUNCULIDA			
gen. sp.	N	R	R
CHORDATA			
Chondrichthyes			
Dalatiidae			
<i>Centroscyllum granulatum</i>	N	C	-
Scyliorhinidae			
<i>Halaelurus canescens</i>	N	O	C
Rajidae			
<i>Bathyraja</i> sp.	N	R	C
Actinopterygii			
Psychrolutidae			
<i>Psychrolutes sio</i>	N	R	-
Macruridae			
<i>Coryphaenoides ariommu</i>	N	C	-
<i>Coelorrhinus fasciatus</i>	N	C	C

Continued

Table 2. Continued

Taxa	Seep endemic	CMSA	Non-seep
<i>Coelorrhinus chilensis</i>	N	A	-
Moridae			
<i>Antimora rostrata</i>	N	O	O
Zoarcidae			
<i>Bothrocara alalongum</i>	N	R	R
Notocanthidae			
<i>Notacanthus sexspinis</i>	N	R	-
Alepocephalidae			
gen. sp.	N	R	-

Organisms endemic to seeps (Y) or not (N), and relative abundance: A, abundant; C, common; O, occasional; R, rare; -, not present.

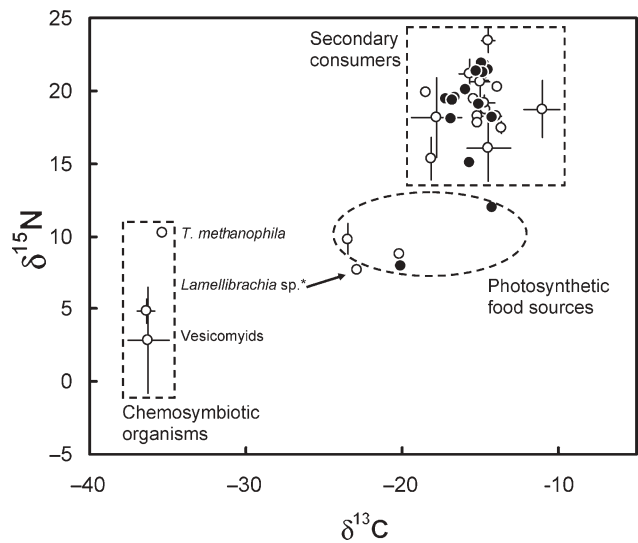


Figure 4. Dual isotope plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean \pm s.d.) of chemosynthetic invertebrates, non-chemosynthetic food sources, and secondary consumers (invertebrates and fish) at the CMSA (white dots) and control non-seep sites (black dots). *See the text for explanation on the comparatively heavy ^{13}C values of *Lamellibrachia* sp.

Discussion

Chemosymbiotic faunal composition

As cold-seep exploration at the SE Pacific margin is just starting, and more seep sites are expected to be discovered there, it is still too early to discuss the degree of endemicity of the CMSA chemosymbiotic fauna, or to consider patterns of latitudinal or depth zonation. However, none of the chemosynthetic species collected here is apparently shared with similar communities off Peru (5–6°S), the closest seep area so far described (~3500 km to the north). Peruvian seeps have been reported from 2630 to 5140 m deep, with their fauna distributed as a function of depth. Their chemosymbiotic fauna are constituted by three vesicomysids, a solemyid bivalve of the genus *Acharax*, and a “pogonophoran” (Olu *et al.*, 1996). The vesicomysid *Calyptogena goffrediae* was recently described, and it resembles *C. gallardoi* in shape and size, but differs in having a shallower escutcheon, a more curved beak, and a more expanded anterodorsal shell region (Krylova

and Sahling, 2006). The other two vesicomids observed still remain undescribed.

Our work has produced the first record of living siboglinids for the Chilean margin. Tubeworms associated with seep sites have been also reported for the Peruvian margin (Olu *et al.*, 1996), but no detail of their taxonomic status was given. The only species of siboglinid reported for the Eastern Pacific is *Lamellibrachia barhami* from the continental slope of southern California and Oregon, the median valley of the Juan de Fuca Ridge, San Clemente Basin, and Monterey Bay, at depths of 600–2400 m (Schulze, 2003), and from the Costa Rica margin, ~8 to 10°N at depths of 1000–2400 m (Mau *et al.*, 2006). However, according to a Cytochrome C Oxidase Subunit 1 (CO1) analysis of the CMSA species, and comparison with other representatives of the genus, the Chilean species is closer to *Lamellibrachia luymeri*. This last species, which inhabits the upper slope of the Gulf of Mexico (<1000 m), has a 3% sequence divergence with the CMSA species, suggesting that it is indeed different (C. Fisher and K. Nelson, pers. comm.).

Our limited knowledge of the Chilean margin seep fauna also suggests that its bivalve-dominated chemosymbiotic community structure is similar to the better-studied counterpart at Monterey Bay on the Northeast Pacific upper slope (~39°N, 500–1000 m). As an example, the most common CMSA vesicomids, *C. gallardoi* and vesicomid gen. sp. 1, morphologically resemble *Calyptogena pacifica* and *Calyptogena kilmeri*, respectively, which are the dominant species at Monterey seep habitats (Goffredi *et al.*, 2004). Three additional vesicomids have been reported for Monterey Bay, but in lesser abundance, and two less abundant vesicomids were also observed at the CMSA. Moreover, as well as the fauna reported in Mediterranean mud volcanoes (Olu-Le Roy *et al.*, 2004), the fauna of the CMSA seems to include symbiotic species that are not restricted to seeps but adapted to organic rich environments (e.g. oxygen-deficient settings), such as thyasirids and lucinids. The proximity of an intense OMZ, where lucinid and thyasirid bivalves are often common (Levin, 2003), may contribute to the adaptation, diversity, and evolution of these groups within seeps. We do not know yet whether all the habitat patch types typical of the Alaska, Oregon, California, Costa Rica, and Peru seeps (mats of filamentous sulphur bacteria, *Calyptogena*, *Acharax* beds, serpulid beds, vestimentiferan aggregations, and siboglinid fields) are present off Chile, or whether there are novel habitat configurations. Finally, it is noteworthy that, of all the known eastern Pacific seeps, only those off Costa Rica support mussel beds (Mau *et al.*, 2006), despite mytilids being common at eastern Pacific vent sites, such as the Galapagos Spreading Centre and the East Pacific Rise (Van Dover, 2000). Vesicomids appear to replace mussels as the dominant biomass at the well-studied eastern Pacific seeps (Levin, 2005).

Megafaunal community structure at the CMSA vs. the control non-seep site

Although we did not attempt to calculate faunal densities, owing to the roughness of the seep-site terrain, it was evident that our site was clearly richer in species and that fauna was more abundant than at the control non-seep site. Only 24 megafauna species were observed at the non-seep site, and all were also present at the CMSA (Table 2). At the control site, the solenocerid shrimp *H. diomedea* was dominant, along with the polychaete *Hyalinoecia* sp. and macrourids, but always in lesser numbers than at the

seep site. The reduced species richness of the control site can in part be explained by the absence of hard substratum, which resulted in a virtual absence of sessile fauna, mainly cnidarians (e.g. gorgonians) and their associated fauna (e.g. brittlestars and galatheid crustaceans). The role of deep-water corals in structuring benthic communities is widely recognized as providing a food source, a perch for suspension-feeders, and protection from predation (Krieger and Wing, 2002).

When compared with other seep sites worldwide, the CMSA epibenthos species number ranks among the highest reported. Just 25 species have been reported for Mediterranean mud volcanoes (Olu-Le Roy *et al.*, 2004), 83 taxa for the clam-bed and microbial-mat habitats on the northern California slope (Levin *et al.*, 2003), 86 taxa for the San Clemente cold seep (Baco and Smith, 2003), and 66 species associated with vestimentiferan aggregations in the Gulf of Mexico (Bergquist *et al.*, 2003). However, due to each of these studies only examining a subset of the total fauna (i.e. some were more focused on the macrofauna and others on the megafauna), and methodological approaches differing, comparisons should be made with caution. Nevertheless, ecosystem-wide species counts are likely to be much higher, because it is expected that the CMSA also hosts a rich meiofaunal and macrofaunal assemblage. Once assessed, these groups will probably increase the species number of animals associated with our site considerably.

According to Carney (1994), colonists are heterotrophic species attracted to vent or seep sites by the aggregation of chemo-autotrophically derived organic matter, endemics are species never found outside reducing environments, and vagrants occur uniformly within and outside vents and seeps. The very limited knowledge of Chilean background bathyal fauna prevents us evaluating whether the non-chemosymbiotic fauna we found are seep endemics, although most of them seem to be colonists or vagrants. Many of the species we collected, like *Pagodula conceptionensis*, *Otukaia crustulum*, and *Margarites huloti*, are new to science (Houart and Sellanes, 2006; Vilvens and Sellanes, 2006). Representatives of some of these genera have been reported for seep sites off Japan (e.g. *Margarites shinkai*). However, excluding the antonbruunid and nautiliniellid polychaetes, commensal of *C. gallardoi*, no other species of non-chemosymbiotic fauna endemic of seeps (e.g. provannid gastropods, alvinocarid shrimps), were found. Hence, the CMSA assemblage shares some characteristics of the shallow-water methane seeps off California (Levin *et al.*, 2000) and the North Sea (Dando *et al.*, 1991), which have dense faunal populations but few seep endemics. This contrasts with observations in the Gulf of Mexico, where relatively high levels of faunal endemism are tightly associated with vestimentiferan aggregations (MacAvoy *et al.*, 2005).

Primary sources driving the heterotrophic foodweb

Except *Lamellibrachia*, chemosymbiotic animal signatures did not overlap with photosynthetic or sediment organic carbon isotopic signatures. Sediment and suspended matter organic carbon isotopic values were nearly in the range for photosynthetically fixed material ($\delta^{13}\text{C}$ from -20.2 to -23.47‰ ; Appendix). There were also relatively high organic carbon (2.6%) and chlorophyll *a* ($4.07 \mu\text{g g}^{-1}$) contents in the control-site sediments (Quiroga *et al.*, in press). This information, along with sediment isotopic signatures, suggests that a large fraction of the partially undegraded phytodetritus reaches the mid-slope seabed off Concepción. Moreover, trawled fragments of *Macrocytis* at the

control site also indicate that inputs of other photosynthetic sources are present (Appendix). Chemosymbiotic animals showed $\delta^{13}\text{C}$ values lighter than -35‰ , except *Lamellibrachia* sp., with a $\delta^{13}\text{C}$ of -22.8‰ . Similar ^{13}C -enriched values have been reported for other siboglinids (e.g. -20.1‰ for *L. luymesii* in the Gulf of Mexico; MacAvoy *et al.*, 2005). This enrichment relative to other cohabiting chemosymbiotic fauna (e.g. vesicomids) is likely a consequence of metabolic and morphological differences. Symbionts in the two groups use different forms of ribulosebiphosphate carboxylase–oxygenase, which fractionate carbon to different extents (Fisher *et al.*, 1990). It has also been suggested that the different groups take up dissolved inorganic carbon (DIC) that is quite dissimilar in carbon isotopic signature. Although bivalves take up DIC at the sediment surface, which is low in ^{13}C , tubeworms grow with their plumes well above the sediment and hence are able to take up DIC with signatures more typical of seawater (MacAvoy *et al.*, 2005). The similarity between ^{13}C signatures of *Lamellibrachia* sp. and other photosynthetic food sources (Figure 4) make it difficult to discriminate whether heterotrophs also consume *Lamellibrachia*. However, siboglinids (e.g. *L. luymesii*) seem to be unpalatable for predators, probably because they contain chemical compounds that deter consumption (Kicklighter *et al.*, 2004).

The general distribution of stable isotope signatures of the fauna at both the CMSA and control site indicates that primary organic food sources are the same, and mainly of photosynthetic origin (e.g. phytodetritus and SOM). Because the CMSA is located beneath highly productive waters, photosynthetically originated C is expected not to be a limiting factor even at such depths, overriding the potential significance of other locally fixed carbon sources for heterotrophic consumers. However, it is interesting that $\delta^{13}\text{C}$ values of top predators such as *A. rostrata* ($-17.4 \pm 0.1\text{‰}$), *Benthoctopus* sp. ($-18.9 \pm 1.5\text{‰}$), and even *D. eleginoides* ($-18.6 \pm 2.2\text{‰}$) are more depleted than their expected prey (i.e. background fauna $\delta^{13}\text{C} = -14.3 \pm 1.3\text{‰}$ on average), suggesting partial or occasional inputs from lighter sources (Figure 4). However, although high ^{15}N values for *D. eleginoides* and *Benthoctopus* sp. do not really support chemosynthetic food sources, lighter values of *A. rostrata* at the seep site (Appendix) suggest that this species at least could ingest some chemosynthetic production.

Mechanisms promoting faunal aggregation at the CMSA

Levin and Michener (2002) hypothesized that as food becomes limiting, seep-related resources should comprise a larger part of the diet of non-seep vagrants. For example, at the Oregon margin seeps, also located beneath a highly productive eastern boundary system, the isotopic signatures of mobile sea urchins and crabs closely resemble non-seep production, whereas there is significant incorporation of chemosynthetic material into the benthic foodweb from methane-based communities underlying the oligotrophic waters of the Gulf of Mexico (Levin and Michener, 2002; MacAvoy *et al.*, 2003). At the CMSA, large predatory fish are frequent, and this site seems to be a preferred fishing ground. This is evidenced by abundant lost fishing gear (hooks and weights) in many of the trawls which collected living chemosymbiotic fauna (Sellanes and Krylova, 2005). However, the overall increase in abundance, biomass, and diversity of the megafaunal communities, including those top predators, is not a function of increased local primary production, because there is no reliance on *in situ* production. Instead, we suggest that the presence of

methane-derived authigenic carbonates provides a suitable habitat for sessile organisms and associated fauna. This hard substratum may provide a rich feeding ground for species such as *D. eleginoides*, because much of their prey (e.g. rattails, cephalopods, and crustaceans; Oyarzún *et al.*, 2001) are present in large quantities there. This has been suggested for the Gorda Escarpment off northern California, where multispecies aggregations of octopus (*Benthoctopus* sp. and *Graneledone* sp.) and blob sculpins (*Psychrolutes phrictus*) brood at seep sites. This preference has been ascribed to the interaction of local topography, physical, and geological settings (Drazen *et al.*, 2003). Females of a local species of blob sculpin (*Psychrolutes sio*), in an advanced reproductive stage, have been also caught at the CMSA (Table 2), suggesting similar behaviour to their NE Pacific counterparts.

The Chilean margin seep environments seem to act as nuclei for increased diversity and abundance for invertebrates and fish, including commercial species. Damage to these environments by anthropogenic activities may affect the populations of associated species, some of which have been found exclusively at this site, at least up to now. Future studies should describe the extent of seeps across and along the Chilean margin to clarify the species associations and interactions within them and the surrounding non-seep environment, including nearby OMZ. In addition, further studies to elucidate patterns of energy transfer within the system need to include unresolved constituents of the foodweb such as mat-forming bacteria, infauna, and fish. The whole information should facilitate better comparison with other seep settings along the Pacific margin and contribute to improve our understanding of endemism, biogeographic, bathymetric, and latitudinal zonation patterns of these particular systems.

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Appendix

Carbon and nitrogen stable isotope composition of selected CMSA and control site invertebrates, sedimentary organic matter (SOM), and bottom-water suspended particulate organic material (POM). Samples ordered from higher to lower $\delta^{15}\text{N}$, as a relative indicator of trophic position.

Seep site taxa and parameters	$\delta^{15}\text{N}$ (‰)		$\delta^{13}\text{C}$ (‰)		n
	Mean	s.d.	Mean	s.d.	
<i>Psychrolutes sio</i>	23.5	0.8	−14.5	0.4	2
<i>Dissostichus eleginoides</i> (>85 cm)	22.1	1.1	−18.6	2.2	5
<i>Centroscyllium granulatum</i>	21.8		−14.7		1
<i>Coelrorinchus fasciatus</i>	21.8		−14.8		1
<i>Coryphaenoides ariommus</i>	21.2	1.0	−15.7	0.7	2
<i>Stegophiura</i> sp.	20.7		−15.0		1
<i>Calliostoma chilena</i>	20.6	1.1	−15.0	0.6	4
<i>Miomelon philippiana</i>	20.3		−13.9		1

Continued

Continued

Seep site taxa and parameters	$\delta^{15}\text{N}$ (‰)		$\delta^{13}\text{C}$ (‰)		n
	Mean	s.d.	Mean	s.d.	
<i>Coralliomorphus</i> sp.	19.9		−18.5		1
<i>Munidopsis trifida</i>	19.4	0.3	−15.4	0.2	3
<i>Homalopoma panamense</i>	19.3	0.2	−14.3	0.7	4
<i>Hyalinoecia</i> sp.	19.1	0.5	−14.8	0.7	3
<i>Astrodia tenuispina</i>	18.9		−15.5		1
<i>Asteronyx loveni</i>	18.8		−13.5		1
<i>Ctenodiscus australis</i>	18.7	2.0	−11.0	1.1	3
<i>Bathybembix macdonaldi</i>	18.7	0.4	−14.6	0.2	4
<i>Campylonotus semistriatus</i>	18.3	0.2	−14.0	0.3	2
<i>Haliporoides diomedea</i>	18.2	0.4	−15.1	0.3	3
<i>Benthoctopus</i> sp.	18.1	2.7	−17.8	1.6	4
<i>Limopsis</i> sp.	17.8	0.2	−15.2	0.3	2
<i>Aforia</i> cf. <i>goniodes</i>	17.4	0.4	−13.6	0.1	3
<i>Callogorgia</i> sp.	16.5		−19.4		1
<i>Paragorgia</i> sp.	16.5		−19.7		1
<i>Gorgonocephalus chilensis</i>	16.1	2.3	−14.5	1.4	3
Turridae	16.0		−14.3		1
<i>Ophiura carinata</i>	15.3	1.4	−18.1		2
<i>Antimora rostrata</i>	15.0	0.8	−17.4	0.1	3
<i>Ophiomusium biporicum</i>	12.6		−17.0		1
<i>Thyasira methanophila</i>	10.2		−35.4		1
POM	9.8	1.1	−23.5	0.0	2
SOM	8.8		−20.18		1
<i>Lamellibrachia</i> sp.	7.6		−22.8		1
<i>Calyptogena gallardoi</i>	4.8	0.8	−36.4	0.6	5
Vesicomidae gen. sp. 1	2.9	3.7	−36.2	1.3	2
Control site taxa and parameters					
<i>Bathyraja</i> sp.	21.9		−14.9		1
<i>Bathybembix macdonaldi</i>	21.4		−14.5		1
<i>Halaelurus canescens</i>	21.4		−15.2		1
<i>Coelrorinchus fasciatus</i>	21.3	0.6	−14.8	0.2	2
<i>Antimora rostrata</i>	20.1		−16.0		1
<i>Fissidentalium majorinum</i>	19.4		−17.2		1
<i>Stereomastis sculpta</i>	19.3		−16.8		1
<i>Haliporoides diomedea</i>	19.1		−15.1		1
<i>Puncturella</i> sp.	18.1		−14.3		1
+ <i>Benthoctopus</i> sp.	18.0		−16.9		1
<i>Ennucula grayi</i>	15.1		−15.7		1
<i>Macrocystis</i> sp. <i>debris</i>	12.0		−14.2		1
SOM	8.0		−20.1		1

s.d., standard deviation; n, number.

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