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Trophic structure of the bathyal benthos at an area with evidence of methane seep activity off southern Chile ($\sim 45^{\circ}\text{S}$)

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*Through application of carbon (C) and nitrogen (N) stable isotope analyses, we investigated the benthic trophic structure of the upper-slope off southern Chile ($\sim 45^{\circ}\text{S}$) including a recent methane seep area discovered as part of this study. The observed fauna comprised 53 invertebrates and seven fish taxa, including remains of chemosymbiotic fauna (e.g. chemosymbiotic bivalves and siboglinid polychaetes), which are typical of methane seep environments. While in close-proximity to a seep, the heterotrophic fauna had a nutrition derived predominantly from photosynthetic sources ($\delta^{13}\text{C} > -21\text{‰}$). The absence of chemosynthesis-based nutrition in the consumers was likely a result of using an Agassiz trawl to sample the benthos, a method that is likely to collect a mix of fauna including individuals from adjacent non-seep bathyal environments. While four trophic levels were estimated for invertebrates, the fish assemblage was positioned within the third trophic level of the food web. Differences in corrected standard ellipse area (SEA_C), which is a proxy of the isotopic niche width, yielded differences for the demersal fish *Notophycis marginata* ($\text{SEA}_C = 5.1\text{‰}$) and *Coelorinchus fasciatus* ($\text{SEA}_C = 1.1\text{‰}$), suggesting distinct trophic behaviours. No ontogenic changes were detected in *C. fasciatus* regarding food sources and trophic position. The present study contributes the first basic trophic data for the bathyal area off southern Chile, including the identification of a new methane seep area, among the furthest south ever discovered. Such information provides the basis for the proper sustainable management of the benthic environments present along the vast Chilean continental margin.*

Keywords: bathyal megafauna, stable isotopes, trophic ecology, isotopic niche width, methane seeps, Chile Triple Junction

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INTRODUCTION

The deep-sea heterotrophic benthic fauna can be trophically supported by multiple food sources (e.g. plant detritus, animal carcasses, zooplankton remains, bacteria and fungi; Gage, 2003). However, in particular systems like those in which chemically reduced compounds fuel primary production (e.g. hydrothermal vents and methane seeps), chemosynthetic bacteria and archaea may be also an important food source for the heterotrophic fauna (Levin, 2005; Thurber *et al.*, 2012, 2013). At these sites mobile species (e.g. fish, asteroids and echinoids) can export production from reducing environments into adjacent areas (MacAvoy *et al.*, 2002, 2003; Carney, 2010), impacting the local biogeochemical cycles.

The carbon (C) and nitrogen (N) stable isotope ratios of animals have helped to identify the energy flow in deep-sea aquatic food webs, including those in seep ecosystems (Van Dover, 2008). In general, the carbon isotope ratio ($\delta^{13}\text{C}$) is used to discriminate the origin of the carbon sources (e.g. photosynthetic and/or chemosynthetic) in the food web, and the nitrogen stable isotope ratio ($\delta^{15}\text{N}$) provides an estimation of

the trophic positions of heterotrophic organisms (Cabana & Rasmussen, 1996; Carlier *et al.*, 2007). Furthermore, based on these ratios, a series of metrics of trophic structure in the food web have been proposed by Layman *et al.* (2007), and reformulated using Bayesian inference, providing information on isotopic niche width in populations, functional groups (e.g. trophic guilds) and communities (Jackson *et al.*, 2011), allowing more powerful comparisons and inferences in the field of the trophic ecology.

Recent surveys on the continental margin off Chile (shelf, slope and adjacent areas), have described macrobenthic zonation (Gallardo *et al.*, 2004) and diversity patterns of benthic fauna (Sellanes *et al.*, 2010), macro- and megabenthic community structures (Palma *et al.*, 2005; Quiroga *et al.*, 2009) and new bathyal chemosynthetic communities associated with methane seepages off Concepción, central Chile (Sellanes *et al.*, 2004). These seep areas are now known to be among the largest on active continental margins worldwide (Klaucke *et al.*, 2012). New species of chemosymbiotic bivalves (e.g. Holmes *et al.*, 2005; Oliver & Sellanes, 2005; Sellanes & Krylova, 2005), as well as numerous heterotrophic species (e.g. Polyplacophora: Schwabe & Sellanes, 2010; Gastropoda: Houart & Sellanes, 2006; Vilvens & Sellanes, 2006; Fraussen & Sellanes, 2008; Houart & Sellanes, 2010; Vilvens & Sellanes, 2010; Warén *et al.*, 2011; Fraussen *et al.*, 2012; Polychaeta: Quiroga & Sellanes, 2009; Crustacea: Guzman &

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Sellanes, 2011) have been discovered in the last years. New records of species have been also established (e.g. Baez & Sellanes, 2009; Guzman *et al.*, 2009). However, the knowledge about benthic communities living in the deep-sea environment is still quite limited. Only a few studies have reported on the trophic structure of benthic bathyal communities of the south-east Pacific (e.g. Andrade, 1986; Sellanes *et al.*, 2008; Zapata-Hernandez *et al.*, 2013). Hence, the potential role of the benthic fauna in the food web of the Chilean continental margin environments is still poorly understood.

The Chile Triple Junction (CTJ, $\sim 45^\circ\text{S}$), located off the Taitao Peninsula, is an active spreading ridge, which together with adjacent young oceanic crust is subducted beneath the continent of South America (Waseda & Didyck, 1995). Moreover, bottom-simulating reflectors (BSRs), which are indicative of the presence of methane hydrate deposits, have been mapped in the adjacent slope (Brown *et al.*, 1996). Owing to this particular geological context, the presence of chemosynthetically-driven ecosystems (e.g. methane seeps and hydrothermal vents) located in close geographical proximity has been predicted (German *et al.*, 2011). A recent expedition, carried out on the upper slope adjacent to the CTJ, detected evidence of the presence of a methane seep habitat, indicated by the occurrence of carbonate blocks and fresh empty shells of vesicomyid, lucinid and solemyid bivalves, as well as vestimentiferan tubeworms, which are typical chemosymbiotic species inhabiting seep areas. Moreover, a considerable diversity of megafauna, including numerous invertebrate taxa and demersal fishes, were observed in the area.

In the present study we provide the first direct evidence of the presence of a seep site off the Taitao Peninsula which, to date, constitutes the southernmost record of this kind of habitat for the Chilean coast. Based on previous observations at other seep sites on the Chilean continental margin (Sellanes *et al.*, 2008; Zapata-Hernández *et al.*, 2013), we hypothesize that the eventual increased availability of food sources should translate into a wider trophic spectrum of benthic communities inhabiting this area. Therefore, using C and N stable isotope ratios we evaluate the trophic structure of the benthic fauna collected at this site in order to understand: (1) the origin of the main trophic sources used by the fauna (i.e. photosynthesis vs chemosynthesis); (2) their trophic position; and (3) the isotopic niche width and ontogenic changes in the use of food sources in selected demersal fish. This information contributes to the understanding of the trophic roles of different species in the community and the energy transfer mechanisms in the benthic food webs along the Chilean continental margin. This study also provides valuable information about the biodiversity present in deep-water environments, which can be used in the implementation of management plans and conservation measures for bathyal ecosystems.

MATERIALS AND METHODS

Samples collection and processing

Samples were collected from the upper slope adjacent to the CTJ, in front of the Taitao Peninsula ($\sim 45^\circ\text{S}$) (Figure 1), during the INSPIRE cruise (February–March 2010) aboard the RV 'Melville' (MV1003, Scripps Institution of Oceanography). At this site, the presence of methane seepage was suggested by anomalously high concentrations

of methane in the water column. At the sampling depths (460–700 m), temperature ranged from 7 to 5.4°C , oxygen between 3.5 to 4.7 ml l^{-1} and salinity was near-constant at ~ 34.3 psu. There was a weak oxygen minimum zone present between 180 to 350 m, with a minimum oxygen value of 1.9 ml l^{-1} .

Multibeam bathymetry data were also considered for the selection of the sampling sites. An Agassiz trawl (AGT) with an opening of $1.5 \times 0.5\text{ m}$ and a mesh of $10 \times 10\text{ mm}$ at the cod-end was deployed twice on the upper slope (Figure 1). Unfortunately, the presence of hard bottoms, potentially associated with authigenic carbonates, precluded sediment sampling using a video-guided multi-corer (TV-MUC) on the continental slope. Samples for sedimentary organic matter (SOM) were obtained from a sediment core (0–3 cm) collected in the CTJ area (3097 m depth) and values of its isotopic composition were pooled with data previously obtained by Hebbeln *et al.* (2000), De Pol-Holz *et al.* (2009) and Sepúlveda *et al.* (2011) at different depths (160–3485 m) on the continental margin between 42° and $\sim 44^\circ\text{S}$.

Immediately after the collection by AGT, invertebrates and fish were sorted. The latter were identified to species, and the standard lengths (L_s) of all individuals were taken. Appropriate amounts of tissue ($\sim 1\text{ mg}$) were dissected from fish and invertebrates, washed with mili-Q water, stored in pre-combusted vials and frozen at -80°C . Voucher specimens of invertebrates and fish were preserved in seawater–formalin solution for further taxonomic study. Once in the laboratory, the samples for stable isotope analysis were dried in an oven (60°C) for 12 h. Lipids were removed from fish tissues using a solution of chloroform:methanol (2:1) (Folch *et al.*, 1957; Bligh & Dyer, 1959) and agitated in a shaker for 30 min and repeated at least three times, until a clear solution (no evidence of lipids) was obtained. Then, the tissues were rinsed with mili-Q water and dried in an oven (40°C) for 12 h. The tissue samples were ground in an agate mortar to a fine powder, and $\sim 0.5\text{ mg}$ was placed in pre-weighed tin capsules and stored in a desiccator.

Stable isotopes analyses

The isotopic composition was analysed at the School of Biological Sciences, Washington State University, using a Eurovector elemental analyser, coupled to a Micromass Isoprime isotope ratio mass spectrometer. 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 $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, 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 $\delta^{13}\text{C}$.

Trophic positions

The calculation of the trophic position was performed for all consumers using the equation detailed by Vander Zanden & Rasmussen (1999):

$$\text{TP}_{\text{consumer}} = 1 + (\delta^{15}\text{N}_{\text{Consumer}} - \delta^{15}\text{N}_{\text{SOM}})3.4^{-1}$$

where $\text{TP}_{\text{consumer}}$ is the estimation of the trophic position of the consumer, $\delta^{15}\text{N}_{\text{consumer}}$ is the measured $\delta^{15}\text{N}$ value in the consumer analysed. Due to the high isotopic variability of potential primary consumers (e.g. suspension feeders and

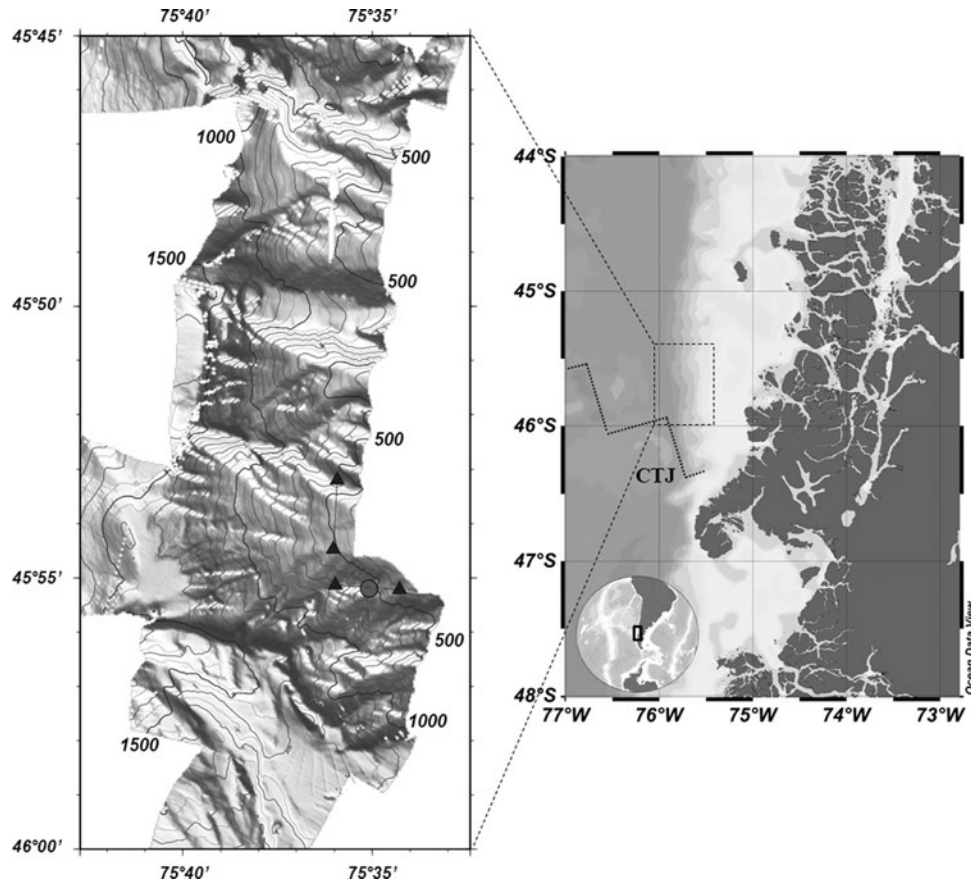


Fig. 1. Map with the location of the two trawls transects at the upper slope off peninsula Taitao. The Chile Triple Junction (CTJ) is located further offshore (Ocean Data View Map; Schlitzer, 2012). The circle represents the site in which an anomalously high concentration of methane has been detected in the bottom water.

deposit feeders), the value of the sedimentary organic matter (SOM) was used as the base signature ($\delta^{15}\text{N}_{\text{SOM}}$) for calculation of trophic position, assuming that this is the main nutritional source for primary consumers at the base of the food web. The constant 1 corresponds to the level of primary sources of the food web (Iken *et al.*, 2010). A value of 3.4‰ is assumed as the average enrichment in $\delta^{15}\text{N}$ per trophic level (Minagawa & Wada, 1984; Post, 2002).

Isotopic niche width and ontogenic trophic changes

Estimations of the corrected standard ellipse area (SEA_C) for the convex hull encompassed in the $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ bi-plot space were performed only for those species with $N > 10$ samples (Jackson *et al.*, 2011). This metric is analogous to the total area of the convex hull (TA) proposed by Layman *et al.* (2007), but unbiased with respect to sample size (Jackson *et al.*, 2011), thus providing quantitative measures of the trophic ecology from animal populations (Jackson *et al.*, 2012). SEA_C measurements were calculated using the routine SIBER (Stable Isotope Bayesian Ellipses in R) incorporated in the statistical package SIAR. Statistical analyses and SIAR calculations were performed using R 2.15.3 software (R Development Core Team, 2013).

In order to detect ontogenic changes in prey selection and the trophic position of the most abundant fish species, the banded whiptail *Coelorinchus fasciatus*, Pearson's correlation

coefficient was used to determine the relation between the standard length (L_S) and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ tissue values.

RESULTS

Faunal composition of bathyal benthos

Evidence of the presence of seep communities recovered in the trawls consisted of authigenic carbonate blocks (Figure 2A), vestimentiferan tubeworms (*Lamellibrachia* sp., Figure 2B), as well as empty shells of vesicomyid (*Calyptogena* aff. *gallardoii*), solemyid (*Acharax* sp., Figure 2C), and lucinid bivalves (*Lucinoma* aff. *metanophila*, Figure 2D). In addition, a live-ingested individual of *Acharax* was collected from the stomach of a macrourid *Coelorinchus fasciatus*. On the other hand, an abundant and diverse assemblage of heterotrophic animals was collected, including 53 invertebrate taxa (i.e. sponges, cold-water corals, nemerteans, crustaceans, pycnogonids, polychaetes, sipunculids and echinoderms; Table 1) and seven species of demersal fish, all of which were analysed for stable isotope analysis (Table 1).

Stable isotope composition of food sources and benthic consumers

Among the potential photosynthetic food sources analysed, the allocthonous macroalgae *Macrocystis* spp. collected on the sea floor and in surface waters had less depleted values

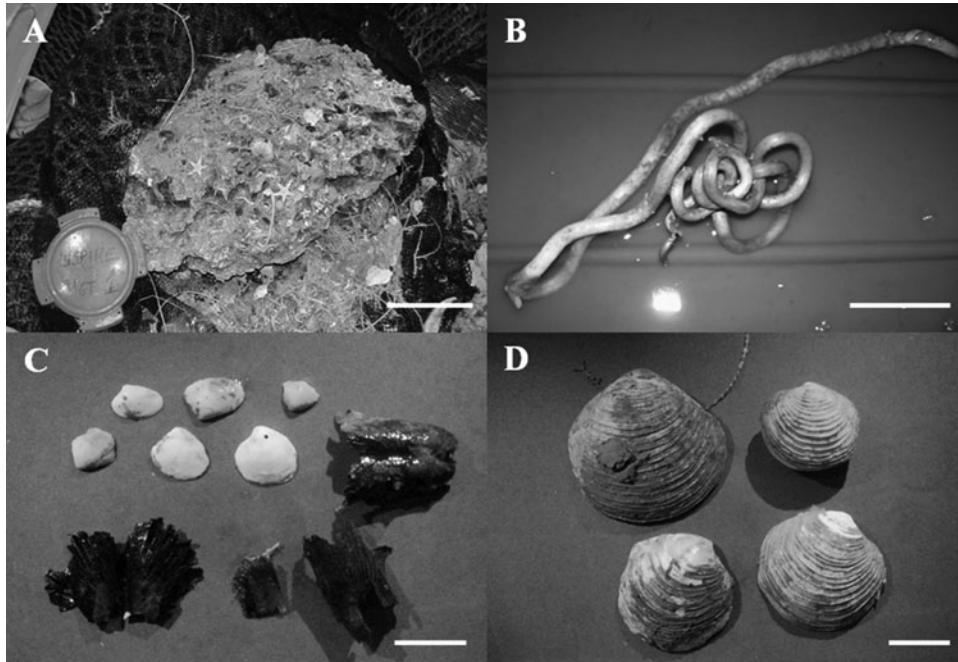


Fig. 2. (A) Authigenic carbonate blocks from the upper slope off Taitao Peninsula (scale bar: 15 cm); (B) empty tube of siboglinid tubeworms *Lamellibrachia* sp. (scale bar: 5 cm); (C) shells of vesicomimid and solemyid bivalves (scale bar: 2 cm); (D) empty shells of lucinid bivalves (scale bar: 2 cm).

for $\delta^{13}\text{C}$ (-12.1 and -15.1‰ , respectively). In contrast, the $\delta^{15}\text{N}$ values was lower in samples collected on the seafloor (8.6‰) compared to samples collected on the sea surface (10.5‰ ; Table 1; Figure 3).

In contrast, their $\delta^{15}\text{N}$ values was lower in samples collected on the seafloor (8.6‰) compared to samples collected on the sea surface (10.5‰ ; Table 1, Figure 3). The values obtained for sedimentary organic matter (SOM) were the most ^{13}C -depleted (mean $\delta^{13}\text{C} \pm \text{SD} = -19.7 \pm 0.8\text{‰}$) and intermediate for $\delta^{15}\text{N}$ values (mean $\pm \text{SD} = 9.3 \pm 0.7\text{‰}$) (Table 1, Figure 3). The solemyid bivalve *Acharax* sp., found in the stomach of an individual of *C. fasciatus*, registered the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (-31.6‰ and 3.4‰ , respectively; Table 1, Figure 3).

The invertebrate megafauna had a narrow $\delta^{13}\text{C}$ range (7.8‰), with the cactus urchin *Dermechinus horridus* having the most ^{13}C -depleted values (-20.9‰) and Ophiuroidea sp. 2 the least ^{13}C -depleted values (-13.1‰) (Table 1; Figure 3). Among the fish fauna, the finless flounder *Neoachirosetta milfordi* had the most negative $\delta^{13}\text{C}$ value ($-17.3 \pm 1.1\text{‰}$), while the narrow necked oceanic eel *Derichthys serpentinus* and the hairy conger *Bassanago albescens* had the least ^{13}C -depleted values ($-14.8 \pm 0.3\text{‰}$; Table 1). The dwarf codling *Notophycys marginata* had the widest overall carbon isotopic niche due to a wide variation of $\delta^{13}\text{C}$ values (range = 8.9‰) (Figure 3).

The range of $\delta^{15}\text{N}$ values was slightly higher (11.2‰) than that reported for $\delta^{13}\text{C}$, with Amphipoda sp.1 having less ^{15}N -enriched values (9.6‰) and Ophiuroidea sp. 2 more ^{15}N -enriched values (20.8‰) (Table 1, Figure 3). Among the $\delta^{15}\text{N}$ values for the fish fauna, the flatfish *N. milfordi* had the most ^{15}N -depleted values ($14.1 \pm 1.4\text{‰}$) and the banded whiptail *C. fasciatus* the most ^{15}N -enriched values ($17.9 \pm 0.1\text{‰}$; Table 1). Despite this, *N. milfordi* showed the wider intraspecific variation of

$\delta^{15}\text{N}$ values (range = 6.5‰), followed by *N. marginata* (5.9‰) and the blackspotted grenadier *L. nigromaculatus* (5.8‰).

Trophic positions of benthic consumers

The primary consumers (trophic position 2), including 37 taxa, were represented mainly by crustaceans (i.e. peracarids and decapods), polychaetes (e.g. polynoid, syllid and eunicid), and echinoderms (i.e. echinoid, ophiuroid and holothuroid), and to a lesser extent by molluscs (e.g. gastropods, bivalves, aplacophora and polyplacophora), cnidarians (only cold-water corals), nemerteans, sponges and fish (Table 1).

The secondary consumers (trophic position 3) included 13 taxa represented mainly by echinoderms (i.e. asteroids, echinoids, holothuroids), fishes (i.e. *L. nigromaculatus*, Ophiidiidae, *D. serpentinus*, *Bassanago albescens* and *Coelorinchus fasciatus*) and some crustaceans, pycnogonid and polychaetes (Table 1).

Only five invertebrate taxa (i.e. sipuncula, the sponge *Pseudosuberites* sp., the echinoid *Austrocidaris* sp., the polychaete Polynoidae sp. and a brittle star Ophiuroidea sp. 2) were categorized into even higher trophic positions (trophic level 4) than those estimated for fish (Table 1).

Isotopic niche width of fish

Sample availability ($N \geq 10$) allowed the estimations of isotopic niche width, through the calculations of the corrected standard ellipses area (SEA_C), for only two fish species (*C. fasciatus* and *N. marginata*). Results indicated that *C. fasciatus* possessed a narrower isotopic niche width ($\text{SEA}_C = 1.1\text{‰}$), than *N. marginata* ($\text{SEA}_C = 5.1\text{‰}$, Figure 4). No overlap was observed between the ellipses of the two fish species, reflecting distinct diets

Table 1. Summary of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the potential food sources and heterotrophic macro- and megafauna. Taxa ordered from lower to higher $\delta^{15}\text{N}$ values, which is a proxy for trophic position (TP). Abbreviations for taxa: Cn, Cnidaria; Cr, Crustacea; Ech, Echinodermata; Mol, Mollusca; Nem, Nemertea; Ost, Osteichthyes; Pol, Polychaeta; Pyc, Pycnogonida; Sip, Sipuncula. Feeding mode (FM): DF, deposit feeder; SF, suspension feeder; G, grazer; O, omnivore; C, carnivore; ?, indeterminate feeding mode. SD, standard deviation; N, number of samples.

Species	Taxon	$\delta^{13}\text{C}$	SD	N	$\delta^{15}\text{N}$	SD	N	TP	FM	References for trophic categories
<u>Potential food source</u>										
SOM*	-	-19.7	0.8	2	9.3	0.7	19	1.0	-	-
<i>Macrocystis</i> spp. (seafloor)	-	-12.1	-	1	8.6	-	1	0.8	-	-
<i>Macrocystis pyrifera</i> (surface)	-	-15.1	-	1	10.8	-	1	1.4	-	-
<u>Consumers</u>										
Amphipoda sp. 1	Cr	-18.4	-	1	9.6	-	1	1.1	?	-
<i>Ophiomusium</i> sp.	Ech	-17.3	-	1	10.3	-	1	1.3	DF	Denisenko <i>et al.</i> , 2003
Cuspidaridae	Mol	-15.5	0.6	3	11.7	0.3	3	1.7	SF	Quiroga <i>et al.</i> , 2009
Onuphidae	Pol	-15.9	1.4	2	12.4	1.5	2	1.9	O	Fauchald & Jumars, 1979
Psolidae	Ech	-18.4	-	1	12.6	-	1	2.0	SF	McClintock, 1994
Tanaidacea sp.	Cr	-14.7	-	1	12.7	-	1	2.0	?	-
<i>Stegophiura</i> sp.	Ech	-16.7	-	1	12.7	-	1	2.0	O	McClintock, 1994
Maldanidae	Pol	-15.7	1.7	2	12.8	2.8	2	2.0	DF	Fauchald & Jumars, 1979
<i>Dermechinus horridus</i>	Ech	-20.9	-	1	13.1	-	1	2.1	SF	McClintock, 1994
<i>Munida propinqua</i>	Cr	-17.8	-	1	13.2	-	1	2.1	C	Quiroga <i>et al.</i> , 2009
Syllidae sp. 1	Pol	-17.2	0.5	4	13.2	1.2	4	2.1	C	Fauchald & Jumars, 1979
<i>Fusitriton magellanicus</i>	Mol	-20.1	-	1	13.5	-	1	2.2	C	Quiroga <i>et al.</i> , 2009
<i>Aphrodita</i> sp.	Pol	-19.4	-	1	13.5	-	1	2.2	C	Fauchald & Jumars, 1979
<i>Emice pennata</i>	Pol	-16.0	-	1	13.6	0.1	3	2.2	C	Fauchald & Jumars, 1979
Gorgonacea	Cn	-17.1	-	1	13.6	-	1	2.3	SF	Carlier <i>et al.</i> , 2009
<i>Bathybembix macdonaldi</i>	Mol	-17.3	-	1	13.7	-	1	2.3	DF	Quiroga <i>et al.</i> , 2009
<i>Callogorgia</i> sp.	Cn	-14.7	1.3	2	13.8	1.9	2	2.3	SF	Orejas, 2001
Syllidae sp. 2	Pol	-16.8	0.3	3	13.8	0.8	3	2.3	C	Fauchald & Jumars, 1979
<i>Stereomastis sculpta</i>	Cr	-16.7	-	1	13.9	-	1	2.4	C	Cartes & Abello, 1992
<i>Flabellum apertum</i>	Cn	-17.5	-	1	14.0	-	1	2.4	SF	Buhl-Mortensen <i>et al.</i> , 2007
Nemertea sp. 1	Nem	-16.4	1.7	2	14.1	2.7	2	2.4	C	Long & Poiner, 1994
<i>Neoachirosetta milfordi</i>	Ost	-17.3	2.1	4	14.1	2.7	4	2.4	C	Yau <i>et al.</i> , 1996
Paguridae sp.	Cr	-16.2	-	1	14.2	-	1	2.4	?	-
Ophiuroidea sp. 1	Ech	-14.3	-	1	14.3	-	1	2.5	O	McClintock, 1994
Nemertea sp. 2	Nem	-14.6	1.4	2	14.4	0.2	2	2.5	C	Thiel & Kruse, 2001
<i>Stereomastis</i> sp.	Cr	-17.0	-	1	14.6	-	1	2.5	C	Cartes & Abello, 1992
<i>Exogone</i> sp.	Pol	-16.0	0.5	3	14.6	2.8	3	2.5	C	Fauchald & Jumars, 1979
Nemertea sp. 3	Nem	-15.2	0.8	2	14.6	1.0	2	2.5	C	Long & Poiner, 1994
Terebellidae	Pol	-15.6	0.5	2	14.6	0.5	2	2.6	DF	Fauchald & Jumars, 1979
Aplacophora	Mol	-17.6	0.5	2	14.7	1.1	2	2.6	?	-
<i>Thouarella</i> sp.	Cn	-14.5	-	1	14.7	-	1	2.6	SF	Quiroga <i>et al.</i> , 2009
<i>Libidoclaea granaria</i>	Cr	-18.1	-	1	15.0	-	1	2.7	C	Andrade, 1986
<i>Notophycis marginata</i>	Ost	-16.8	2.1	12	15.0	1.7	12	2.7	C	Bailly, 2013
Phyllodocidae	Pol	-19.9	-	1	15.1	-	1	2.7	C	Fauchald & Jumars, 1979
<i>Campylonotus semistriatus</i>	Cr	-14.2	0.4	2	15.2	0.5	2	2.7	C	Quiroga <i>et al.</i> , 2009
Amphipoda sp. 2	Cr	-18.0	0.4	2	15.2	0.2	2	2.7	?	-
<i>Astrotoma agassizii</i>	Ech	-15.3	-	1	15.3	-	1	2.8	C	McClintock, 1994
<i>Munidopsis opalescens</i>	Cr	-15.6	-	1	15.5	-	1	2.8	C	Quiroga <i>et al.</i> , 2009
<i>Sterechinus</i> cf. <i>neumayeri</i>	Ech	-16.0	-	1	15.6	-	1	2.8	G	Norkko <i>et al.</i> , 2007
<i>Leptochiton</i> sp.	Mol	-15.1	-	1	15.7	-	1	2.9	C	Gracia <i>et al.</i> , 2005
Porifera	Por	-20.8	-	1	15.9	-	1	2.9	SF	Ribes <i>et al.</i> , 1999
<i>Lucigadus nigromaculatus</i>	Ost	-16.6	1.5	4	16.0	2.8	4	3.0	C	Stevens & Dunn, 2011
Polychaeta indet.	Pol	-15.8	-	1	16.1	-	1	3.0	?	-
<i>Pallenopsis</i> sp.	Pyc	-17.2	-	1	16.4	-	1	3.1	O	Melzer, 2009
Ophidiidae	Ost	-15.5	-	1	16.6	-	1	3.1	C	Stergiou & Karpouzi, 2002
<i>Ctenodiscus australis</i>	Ech	-15.6	-	1	16.6	-	1	3.1	DF	Shick <i>et al.</i> , 1981
<i>Eurypodius</i> sp.	Cr	-15.7	-	1	16.7	-	1	3.2	O	Mauna <i>et al.</i> , 2011
Echinoidea sp.	Ech	-15.6	0.5	2	16.7	3.0	2	3.2	G	Norkko <i>et al.</i> , 2007
<i>Derichthys serpentinus</i>	Ost	-14.8	0.3	4	16.9	0.4	4	3.2	C	Fitch & Lavenberg, 1968
<i>Psolus</i> sp.	Ech	-14.7	-	1	17.4	-	1	3.4	SF	McClintock, 1994
<i>Hippasteria phrygiana</i>	Ech	-14.7	-	1	17.6	-	1	3.4	C	Castilla & Paine, 1987
<i>Bassanago albescens</i>	Ost	-14.8	0.4	2	17.6	0.7	2	3.4	C	Meyer & Smale, 1991
Astroidea sp.	Ech	-14.2	1.1	3	17.8	1.3	3	3.5	C	Quiroga <i>et al.</i> , 2009

Continued

Table 1. Continued

Species	Taxon	$\delta^{13}\text{C}$	SD	N	$\delta^{15}\text{N}$	SD	N	TP	FM	References for trophic categories
<i>Coelorinchus fasciatus</i>	Ost	-14.9	0.8	31	17.9	0.5	31	3.5	C	Meyer & Smale, 1991
Sipuncula	Sip	-13.9	-	1	19.4	-	1	4.0	DF	Murina, 1984
<i>Pseudosuberites</i> sp.	Por	-16.2	-	1	19.6	-	1	4.0	SF	Ribes et al., 1999
<i>Austrocidaris</i> sp.	Ech	-15.2	-	1	19.7	-	1	4.0	C	McClintock, 1994
Polynoidae	Pol	-15.5	-	1	20.6	-	1	4.3	C	Fauchald & Jumars, 1979
Ophiuroidea sp.2	Ech	-13.1	-	1	20.8	-	1	4.4	C	McClintock, 1994

*, averaged values of the sedimentary organic matter (SOM) from 1 TV-MUC (this study) and the data from Hebbeln et al. (2000), De Pol-Holz et al. (2009) and Sepulveda et al. (2011) from the continental slope off Chile between 42° and ~45°S and between 160 and 3485 m.

(Figure 4). No correlation between the standard length (L_S) and the $\delta^{13}\text{C}$ ($r^2 = 0.02$, $df = 29$, $P > 0.05$) or $\delta^{15}\text{N}$ values ($r^2 = 0.23$, $df = 29$, $P > 0.05$; Figure 5) was detected in *C. fasciatus* population.

DISCUSSION

The presence of massive methane deposits (i.e. in the form of gas hydrates indicated by bottom simulating reflectors) along the Chilean continental margin (35°–45°S) was documented almost two decades ago (Brown et al., 1996; Morales, 2003). Previous studies in the Chile Triple Junction (CTJ) have confirmed presence of the methane throughout the continental slope, with extremely low $\delta^{13}\text{C}$ values (-86 to -61‰), suggesting a biogenic origin (Waseda & Didyk, 1995). Chemosymbiotic fauna associated with methane seeps often depicts low $\delta^{13}\text{C}$ isotopic values in their tissues. In our study, only empty shells and tubes of seep fauna were directly collected, but a freshly ingested specimen of the solemyid *Acharax* sp. was found in one fish stomach. As expected for this group, which is known to be a facultative heterotrophic

feeder that also has symbiotic sulphide-oxidizing bacteria (Barry et al., 2000), this specimen exhibited a low $\delta^{13}\text{C}$ value (-31.6‰), similar to values reported for other congeners from other chemosynthetic environments (e.g. Gulf of Alaska–Sea of Okhotsk, Sahling et al., 2003, Unimak Margin, Levin & Mendoza, 2007; Gulf of Cadiz, Rodrigues et al., 2012).

Several studies, conducted to date at methane seeps from different regions reveal benthic food webs supported largely by chemosynthetic production, where the tissues of heterotrophic organism typically have highly negative $\delta^{13}\text{C}$ values (<-50 to -21‰; summarized in Levin & Michener, 2002), in contrast to species dependent on phytoplankton-derived organic matter ($\delta^{13}\text{C} = -25$ to -15‰; Fry & Sherr, 1984). Some examples of these chemosynthetic-based methane seeps have been documented for the Oregon margin, Eel River margin and Gulf of Alaska (Levin & Michener, 2002), Blake Ridge (Van Dover et al., 2003), Aleutian Islands (Levin & Mendoza, 2007), Gulf of Mexico (Levin & Mendoza, 2007, Demopoulos et al., 2010), Hikurangi margin, New Zealand (Thurber et al., 2010), eastern Mediterranean Sea (Carlier et al., 2010), Norwegian margin (Decker & Olu, 2012) and Chilean margin off Concepción (Zapata-Hernández et al., 2013). However, for this study the C isotopic values of most of the heterotrophic species were within the typical range of organisms that depend on C fixed by photosynthesis (>-25‰) and similar to that reported by Sellanes et al. (2008) and Zapata-Hernández

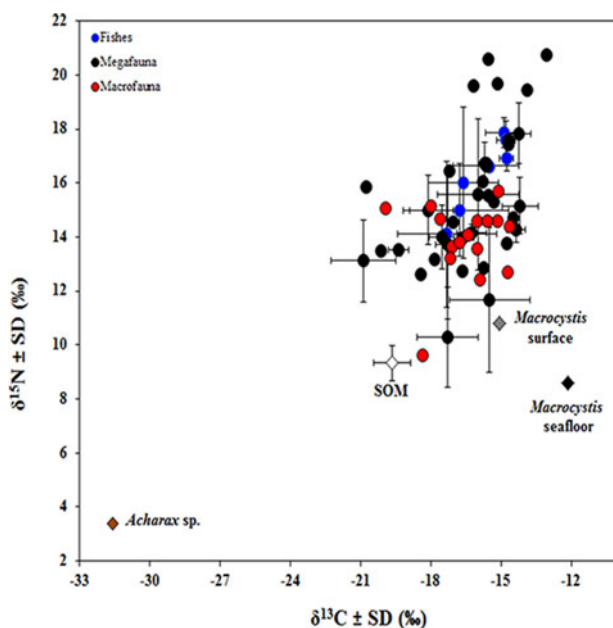


Fig. 3. Plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of potential food sources and invertebrate macro- and megafauna and the fish species collected in the upper slope off peninsula Taitao. SOM, sedimentary organic matter.

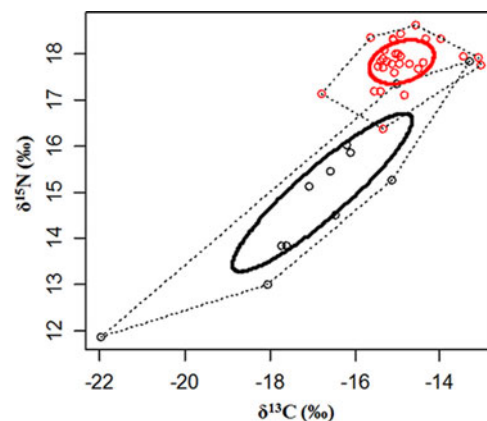


Fig. 4. Convex hulls (polygons) and corrected standard ellipse areas (SEAc) in the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ bi-plot space for two fish species analysed at upper slope area: *Coelorinchus fasciatus* (grey ellipse) and *Notophycis marginata* (black ellipse).

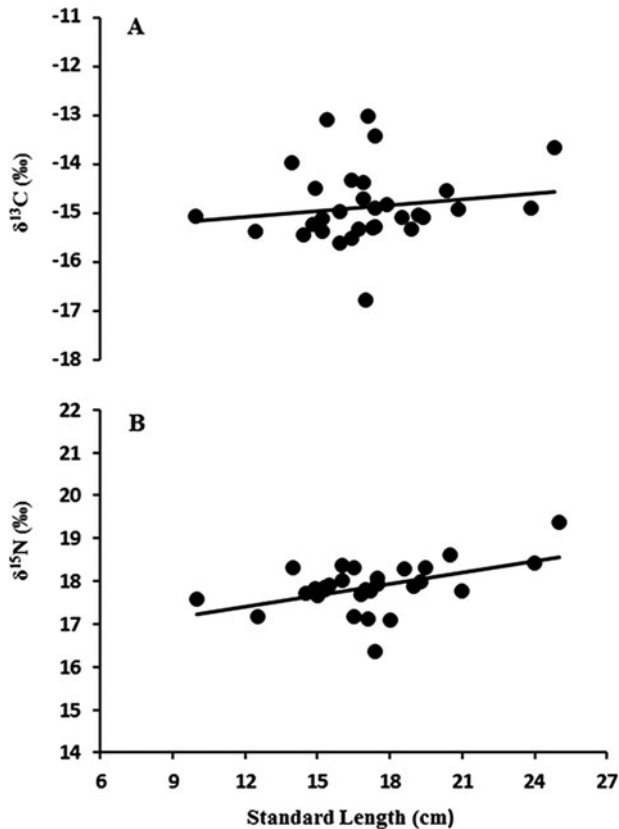


Fig. 5. Regressions between standard length (L_S) and $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) for *Coelrorinchus fasciatus*. The curves were fitted to: (A) $y = 0.039x - 15.54$ ($r^2 = 0.022$) and (B) $y = 0.088x + 16.35$ ($r^2 = 0.233$).

et al. (2013) in some megafaunal taxa from the Concepción Methane Seep Area (CMSA).

On the other hand, it is recognized that the different biogeochemistry of multiple local microhabitats (e.g. clams bed, siboglinids fields and microbial mats) leads to heterogenic isotopic signatures in the fauna (Bernardino *et al.*, 2012). In this sense, the absence of a signature derived from chemosynthetic carbon ($\delta^{13}\text{C} = < -25\text{‰}$), could also be an effect of the lack of selectivity of the benthic trawl, which samples mobile fauna that feed and dwell in areas comprising both seep and non-seep slope habitats. Therefore, other devices (e.g. ROVs, TV-MUCs and TV-Grabs) that allow selective sampling of carbonate rocks and distinctive biogenic features at seeps could provide samples from specific microhabitats, in which the incorporation of chemosynthetic production could be substantial among the heterotrophic benthic fauna.

In contrast with typical bathyal communities further north, whose structure is mainly controlled by the south-east Pacific permanent oxygen minimum zone (Sellanes *et al.*, 2010), the normoxic conditions at the study area, associated with the presence of the well-oxygenated Antarctic Intermediate Waters (AAIW) covering the continental slope (~400–1200 m depth; Quiroga & Levin, 2010), could largely explain the diverse assemblage of heterotrophic fauna observed. Furthermore, the presence of carbonate blocks and habitat-forming taxa (e.g. chemosymbiotic clams, tubeworms, sponges and cold-water corals) could also be facilitating colonization by several organisms (Cordes *et al.*, 2010) from different trophic levels, which could be using these sites for

settlement, food, refuge and nursery (Sellanes *et al.*, 2010; Treude *et al.*, 2011), increasing the biodiversity in the area and generating more complex trophic interactions over time (Zapata-Hernandez *et al.*, 2013).

Cold-water corals form important habitats for many species in the deep sea (Buhl-Mortensen *et al.*, 2010). In the south-eastern Pacific numerous cold-water coral reefs have been detected in southern Chile fjords (Jantzen *et al.*, 2013), on the continental slope (Sellanes *et al.*, 2008, 2010) and on seamounts (Cañete & Haüssermann, 2012 and references therein). Some coral species associated with methane seeps have previously been reported for the CMSA at central Chile (Sellanes *et al.*, 2008, Zapata-Hernandez *et al.*, 2013). These studies have found no trophic relationship between the corals and the chemosynthetic production, but corals seem to benefit from the presence of a hard substrate (pavements of carbonate) generated through the anaerobic oxidation of methane (AOM) (Sibuet & Vangriesheim, 2009). As at the CMSA, the cold-water corals at the seep site off Taitao Peninsula (e.g. *Gorgonacea*, *Callogorgia* sp. and *Flabellum apertum*) were classified as primary consumers, consistent with suspension feeder feeding mode, possibly consuming the sinking particulate organic matter (Carlier *et al.*, 2009 and references therein).

Among the crustacean taxa, the galatheids *Munida propinqua* and *Munidopsis opalescens* were positioned as primary consumers. It has been reported that galatheid species show a wide spectrum of feeding modes; they can be detritivores, suspension feeders, carnivores, scavengers and cannibals (Romero *et al.*, 2004; Vinuesa & Varisco, 2007). In some deep-water environments off central Chile (i.e. methane seeps and seamounts), the galatheid crabs are typically found associated with the branches of cold-water corals (e.g. *Callogorgia* sp.), using them as substrate (Cañete & Haüssermann, 2012). Therefore, it is feasible that both species could be feeding on particulate organic matter (POM), using corals as a perch to capture their food at higher positions in the water column, or they may feed directly on the tissues of these organisms (Macpherson & Segonzac, 2005) and gain protection from the strong bottom currents and predators (Etnoyer & Morgan, 2005).

To date, the majority of deep-water polyplacophorans reported for Chile have been found associated with hard substrates (authigenic carbonates) near methane seeps (Schwabe & Sellanes, 2010), but their trophic ecology has not been properly studied. In general littoral chitons are considered as grazers, but they nevertheless display a high trophic versatility, including detritivorous, carnivorous, herbivorous and omnivorous lifestyles (Latyshev *et al.*, 2004), as well as feeding on decaying wood (Sirenko, 2001). In the present study, the polyplacophoran *Leptochiton* sp. has an intermediate trophic position (TP = 2.9), suggesting a detritivorous or omnivorous feeding mode, in which they obtain their food from the hard substrate where are confined (i.e. authigenic carbonates) (Lyons & Moretzsohn, 2009).

The echinoderms, including several taxonomic classes, were positioned in all the trophic levels estimated in this study, suggesting an important role in the transfer of energy within the benthic food web. The wide variability in the $\delta^{15}\text{N}$ values and trophic position of ophiuroids probably corresponds to the broad range of feeding strategies of this group, which include suspension-feeding, deposit-feeding, scavenging and predation (Stöhr *et al.*, 2012); similar

variability was also detected in the echinoid taxa. However, the asteroid species were all positioned at higher trophic levels, highlighting their important role as predators in benthic ecosystem.

In general, fish were positioned in the third level of the food web. There are records of some species of shark, hake, and conger off the Taitao Peninsula (>500 m) (Sielfeld & Vargas, 1999 and references therein), also including the Patagonian toothfish *Dissostichus eleginoides*, which has been observed further north at seep sites off Concepción (Sellanes *et al.*, 2012). These species were not captured during the present study, probably because of the insufficient sampling effort and the type of gear used, or because of their relative scarcity in the area. Therefore, a greater sampling effort in the area would help to better understand the role of the fish assemblage in the food web of this bathyal environment.

The large isotopic niche width estimated for *N. marginata* indicates that it is probably a generalist consumer, despite the limited number of samples obtained. Additional samples could increase the isotopic niche width, reaffirming our hypothesis. However, the high variability within a population is a typical feature of some predators, due to a high degree of intraspecific competition (Quevedo *et al.*, 2009). Moreover, ontogenetic differences (e.g. Taylor & Mazumder, 2010), sex (Bolnick *et al.*, 2003), nutritional conditions (Bearhop *et al.*, 2004), and/or the variability of prey availability in the environment (Frédérich *et al.*, 2009) affect the trophic niche of animal populations. In contrast, our results suggest that *C. fasciatus* has an extremely narrow isotopic niche width which, together with an absence of ontogenetic change in carbon source and trophic position, indicates that this species is likely a trophic specialist throughout most of its life. This type of trophic behaviour could be the result of a trophic niche separation driven by a strong evolutionary pressure associated with competition for food (Iken *et al.*, 2001), or be associated with the abundance of a particular prey in their environment (Gartner *et al.*, 1997).

Finally, it is also important to note that our knowledge concerning the biological and ecological history of bathyal species is quite limited and restricted to taxonomic lists or records of by-catch of specific fisheries. Therefore, future studies in the trophic ecology field should consider the incorporation of fatty acids analysis, molecular sequencing of the stomach contents and the analysis of sulphur stable isotopes. Given increasing pressures on continental margins from human activities (Levin & Sibuet, 2012), such studies, together with the characterization of these ecosystems in terms of microhabitats, biodiversity levels, community structure, trophic guilds and ecological interactions, will provide a basis for the proper sustainable management of the suite of benthic environments present along and across the vast Chilean continental margin.

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