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Variability in fatty acids of two marine copepods upon changing food supply in the coastal upwelling zone off Chile: importance of the picoplankton and nanoplankton fractions

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Fatty acids composition of two marine copepods, Acartia tonsa *and* Centropages brachiatus*, and lipid profiles of natural food assemblages were studied during the austral summer 2006 at three upwelling sites in the coastal upwelling zone off Chile, along with oceanographic conditions. Fatty acids of food supply were assessed for the picoplankton, nanoplankton and microplankton size fractions. There were marked differences in upwelling conditions among locations, as well as in their food supply in terms of quantity and quality. Differences in fatty acid composition were also found, both among food assemblages and between copepod species. Essential polyunsaturated fatty acids (PUFA; linoleic acid) and monounsaturated fatty acids (MUFA; oleic acid) dominated the picoplankton and nanoplankton size fractions of food, and they were highly represented in both species of copepods, indicating these size fractions were the major contributors to their diet. These fatty acids can thus be considered as useful trophic markers for copepods. Variation in lipid profiles between species depended on sampling sites, whereas differences in lipid composition among sampling sites were attributed to distinct upwelling conditions, which drive the changes in food quality, such that trophic response is highly dependent on food offer. Variation in fatty acids compositions of copepods may thus act as an indicator of upwelling variability. Our findings suggest that lipid transfer from primary producers to primary consumers can have a crucial role for carbon cycling in the marine food web, and that picoplankton and nanoplankton fractions are the key items of copepod diet in this upwelling system.*

Keywords: copepods, Chilean coast, fatty acids, food supply, trophic marker, upwelling

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INTRODUCTION

In the highly productive coastal upwelling system of the Humboldt Current, herbivorous zooplankton have traditionally been considered the key link to transfer phytoplankton carbon (C) to higher trophic levels and hence sustain fish production, which in turn supports a strong fishery industry in the region (Cushing, 1990; Mann & Lazier, 1991). This view, however is being challenged by more recent evidence suggesting that at lower trophic levels in coastal upwelling systems, the microbial loop (*sensu* Azam *et al.*, 1983) operates actively in channeling freshly produced C, such that a microbial food web might an important pathway of C towards fish, and even on many occasions should be considered the main pathway (Calbet & Saiz, 2005). Recent studies in the coastal zone off Chile have come to similar conclusions for the Humboldt Current (González *et al.*, 2002; Böttjer & Morales, 2005; Vargas *et al.*, 2007).

In order for the microbial food web to efficiently provide organic matter to fish production, dominant zooplankton, the main prey of fish, should feed on heterotrophic components of the microbial loop, i.e. heterotrophic nanoplankton and microzooplankton, which are incorporating the bacterial carbon (Andersen & Fenchel, 1985; Cuevas & Morales, 2006). In fact, many studies from different regions of the world ocean have shown that in most situations the supposedly herbivorous zooplankton can rather have an omnivorous diet (Gifford, 1993; Vargas *et al.*, 2006), and this capacity would allow them to establish the link between the microbial loop and fish (Calbet & Saiz, 2005). However, the shift from an herbivorous to an omnivorous diet may depend on the food offer in the field, as shown from measurements of food consumption by copepods in the coastal upwelling zone off Chile (Vargas *et al.*, 2006). In this upwelling region food supply, comprising autotrophic and heterotrophic nanoplankton and microplankton, may be highly diverse and sustained year round in the northern zone off Chile (Herrera & Escribano, 2006) where upwelling is a

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permanent process at all seasons (Thomas *et al.*, 2001). By contrast, in the central/southern region, where upwelling is strongly seasonal (Sobarzo *et al.*, 2007), food available for copepods ranges from an extremely high diatom biomass (>20 μg chlorophyll-*a* l^{-1}) during the spring/summer (Montero *et al.*, 2007) to a low-chlorophyll-*a* condition in the winter, but high biomass of heterotrophic nanoplankton and microplankton (Anabalón *et al.*, 2007; Böttjer & Morales, 2007). Dominant copepods in such conditions may then switch their diet from herbivorous to omnivorous behaviour (Vargas *et al.*, 2006) and thus sustain their reproduction and population growth year round (Escribano & McLaren, 1999; Hidalgo & Escribano, 2007). It thus seems that upwelling variation may greatly influence food supply and consequently copepod feeding. This is an important issue, because the upwelling regime is subjected to a strong interannual variability due to the ENSO cycle (Escribano *et al.*, 2004), such that during depleted upwelling the whole autotrophic community may be shifted towards small-sized nanoplanktonic and solitary microplanktonic cells (Iriarte & González, 2004). It is not known how zooplankton may respond to such variability as to sustain their secondary production even during El Niño conditions (Ulloa *et al.*, 2001).

Responses of zooplankton to changing food may be assessed by their fatty acid composition, on the basis of evidence showing that some essential fatty acids are transferred to copepods without major transformations (Fraser *et al.*, 1989). Fatty acids have widely been used to determine trophic relationships in zooplankton (Falk-Petersen *et al.*, 1987, 2000; Kattner *et al.*, 1989; Desvilettes *et al.*, 1994; Cripps & Hill, 1998; Scott *et al.*, 1999; Nelson *et al.*, 2001; Auel *et al.*, 2002; Stevens *et al.*, 2004), such that they are considered to be trophic biomarkers (Ackman & Tocher, 1968; Volkman *et al.*, 1989; Viso & Marty, 1993) as they can be conservative (Lee *et al.*, 1971; Weers *et al.*, 1997; Stevens *et al.*, 2004). In this work, we have used fatty acids as biomarkers to assess whether changing upwelling conditions, which can affect the nanoplankton and microplankton communities, can have an imprinting in copepod food history. We hypothesize that lipid signals in copepods are significantly dependent on a changing food offer. We thus aim at understanding the mechanisms through which upwelling variability may impose constraint on zooplankton feeding and hence secondary production.

MATERIALS AND METHODS

The study sites

Along the Chilean coast, many active upwelling sites can be identified, but depending on latitude the upwelling regimes may vary during the year cycle due to local wind conditions (Strub *et al.*, 1998; Thomas *et al.*, 2001). As to cover some of this variability, three upwelling sites were selected to sample copepods, to assess quantity and quality of the food, and obtain data on oceanographic conditions during sampling. The three sites are shown in Figure 1. These are known to be active upwelling zones. At the central/southern region of Chile (36°S), sampling was carried out at Station

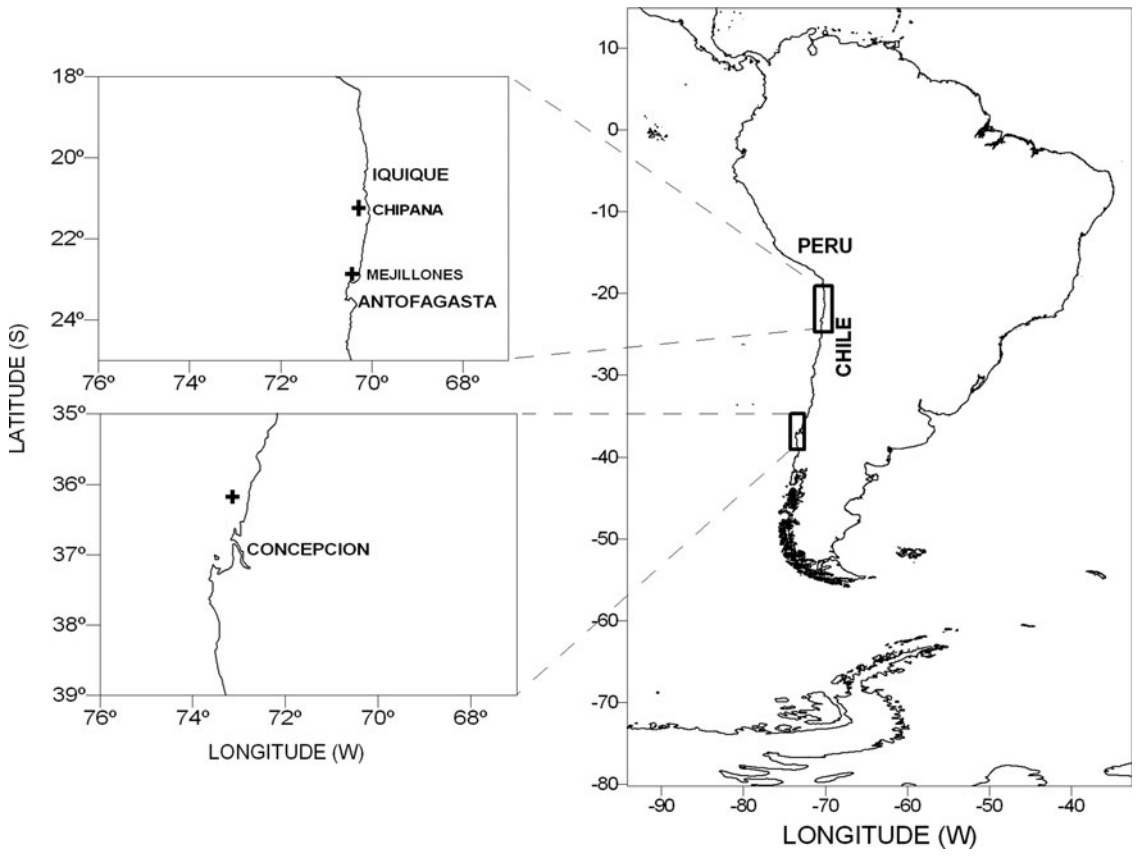

Fig. 1. The coastal upwelling zone off Chile in the eastern South Pacific illustrating the three upwelling sites selected to study fatty acid profiles of two copepod species and the their food supply during the austral summer 2006.

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18 during April 2006, where an ongoing time series study has shown a strongly seasonal upwelling regime (Escribano & Schneider, 2007), and where copepods' food supply may experience drastic seasonal changes as well (Vargas *et al.*, 2006). At the northern upwelling region of Chile, which is characterized by an intermittent, but year-round upwelling (Strub *et al.*, 1998; Thomas *et al.*, 2001), two sites were selected during the second week of January and second week of February 2006. In these, there are also ongoing studies focused on oceanographic variability. The Bay of Mejillones (23°S) is a semi-enclosed highly productive bay (Marín *et al.*, 1993), in which copepods tend to aggregate near-shore during upwelling (Escribano & Hidalgo, 2000), whereas the bay of Chipana (21°) is known as having a highly diverse and continuous supply of autotrophic and heterotrophic nanoplankton and microplankton for zooplankton and fish larvae (Herrera & Escribano, 2006).

Sampling and field studies

Similar procedures were applied at each site. Zooplankton were captured at each site with vertical tows of 200 μm mesh-size plankton net having 0.5 m of opening diameter. The net was towed between 85 m depth and surface at slow speed (<0.5 m s^{-1}), and it was equipped with a non-filtering cod end to obtain undamaged individuals. Once onboard the samples were poured into coolers and diluted with surface seawater for sorting at the laboratory.

Autotrophic and heterotrophic components of the nanoplankton and microplankton were obtained with a 10 l Niskin bottle at 5 depths from surface down to 85 m. Samples were transported to the laboratory in darkened containers.

Oceanographic data at each site were obtained with a SeaBird SBE-19 plus CTDO profiler, equipped with a Wetstar fluorometer, a SeaBird oxygen sensor and a PAR sensor. Additional discrete bottle samples were obtained at 5 depths for measuring size-fractioned chlorophyll-*a* (Chla) and organic nutrients. Size-fractioned Chla concentrations were measured for the fractions 0.7–2.0 μm, 2–20 μm and 20–200 μm, representing picoplankton, nanoplankton and microplankton components of the autotrophic community, respectively. Photosynthetic pigments were measured by fluorometry techniques following procedures of Parsons *et al.* (1984) and using a Turner Designs fluorometer. The macronutrients nitrate, nitrite, phosphate and silicate were analysed by spectrophotometry in accordance to standard methods described by Parsons *et al.* (1984).

Laboratory analyses

Two of the most abundant copepods in the upwelling zone off Chile, *Acartia tonsa* and *Centropages brachiatus* (Hidalgo & Escribano, 2001; Escribano *et al.*, 2007), were selected. Between 18 and 31 individuals of the same stage, usually adult females, were sorted for fatty acids analysis. These individuals were first washed with filtered seawater and then placed onto pre-combusted glass fibre filters. The filters were then placed in sterilized vials and immediately frozen in liquid nitrogen. Fatty acids analysis was carried out by gas chromatography with FID detector. The sample was first mixed with 200 μl of sodium metoxide and 1 ml of hexane following the procedure described by Cantellops *et al.* (1999). Meantime, the fatty acids analysis of the food supply was performed from fractioned seawater samples from Niskin bottles. To do that, between 1.5 and 2.0 l of sample were firstly filtered through a 20 μm sieve and then filtered again with GF/A (1.7 μm) pre-combusted filters, and thereafter filtered again with GF/F (0.7 μm) filters. Additionally 1 l of original sample was directly filtered with GF/F filter for total lipids. Filters were thus frozen in liquid nitrogen and the same procedure as for copepods was applied for analysis of lipid composition.

To study the abundance and composition of the microplankton fraction, about 3 or 7 l of seawater from the Niskin bottles were filtered through a 20 μm sieve and preserved with 4% of buffered (pH = 7) formalin. Thereafter 10 ml of samples were placed in sedimentation chambers for 24 hours. All organisms were identified and counted under an inverted microscope (Nikon, Eclipse TE 2000) following the Utermöhl method (Utermöhl, 1958).

For analysis of the nanoplankton fraction 50 ml of seawater samples were obtained in sterilized centrifuge tubes and fixed with 2% final concentration of glutaraldehyde. Samples were kept at low temperature (5°C) until analysis. Later the samples were processed by taking a 20 ml subsample and placed onto polycarbonate black filters. Before filtering, a fluorochrome solution was added and samples were thereafter analysed by epifluorescence microscopy (Porter & Feig, 1980).

Analysis of data

Daily data of oceanographic variables were averaged for each location to obtain vertical profiles of temperature, salinity, oxygen and light (PAR) representing the condition of each site. From these data mean values of temperature, salinity and oxygen were derived from the whole water column. These derived daily means were also used to test differences among the localities by applying a one-way ANOVA on log-transformed data. Also from the vertical profiles of temperature and salinity, water density was estimated from the standard equation of seawater and then water column stratification for each site was calculated from Bowden (1983) as,

$$\Phi = \frac{g}{H}\int_{-H}^{o}(\rho m - \rho)z dz$$

where Φ is an index of potential energy anomaly (J m^{-3}), H is the water column height (m), ρ is the density at any depth z, and ρm is the mean density of the water column. This index estimates the deficit in potential energy due to a density gradient. A highly mixed water column will present small values of Φ. Integrated values of Φ were obtained for the 0–50 m layer.

Natural food assemblages were considered as functional groups for microplankton fraction including diatoms, dinoflagellates and ciliates, whereas the nanoplankton fraction was separated into autotrophic and heterotrophic nanoplankton. Potential differences in composition among localities and functional groups were tested by means of a two-ways ANOVA applied on log-transformed data.

Fatty acids were distinguished as saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA). Within each of these categories they were also coded as X:Y(n-Z), where X is the number of C atoms, Y is the number of double bonds and (n-Z) is the position of the double bond

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from extreme methyl of the molecule. Lipid composition was then expressed as percentages of these fatty acids for both copepods and their food supply. The correspondent fatty acid profiles were compared among size-fractioned food, copepods according to localities. The non-parametric Friedman test was used to test similarities and differences between profiles. Finally, the association among the different sized-fractions of natural food and both copepod species from the three localities as a function of their correspondent fatty acid composition was assessed by a cluster analysis using the Pearson correlation as a measure of distance.

RESULTS

Environmental setting

At the localities of northern Chile (Mejillones and Chipana) sampling was carried out during austral summer conditions (January/February 2006), whereas in Concepción the sampling took place during autumn 2006. Concepción was colder than Mejillones and Chipana by about 3–5°C as judged by the mean temperature of the water column. These differences are less marked in subsurface water (Table 1). Salinity was greater in the northern localities (Chipana and Mejillones), while oxygen at 10 m depth was markedly lower in the bay of Mejillones compared to Concepción and Chipana. More oxygenated conditions prevailed in Concepción and the OMZ was much deeper than in Mejillones and Chipana, where the 1 ml O_2 l^{-1} isoline was shallower than 20 m depth. Northern localities were also more stratified. Nutrient levels were high at all localities in terms of phosphate and silicate, but nitrate was much lower in Chipana compared to Concepción and Mejillones (Table 1). All these differences in oceanographic conditions among localities were significant after the ANOVA test (Table 2).

Vertical profiles of temperature, salinity, DO and PAR (averaged for each site), are shown in Figure 2. These profiles illustrate differences in the water column conditions among localities and reveal the strong thermal stratification in Chipana and Mejillones (Figure 2A), with increasing salinity with depth (Figure 2B), associated with a much shallower oxycline (Figure 2C). Meantime, illuminated conditions (PAR) appear well restricted to the upper 20 m at all places (Figure 2D).

Table 1. Summary of oceanographic conditions at 3 upwelling sites of the coastal zone off Chile sampled during the summer 2006. Tm, mean temperature of the water column (°C); T50, temperature at 50 m depth (°C); SAL, salinity; DO, dissolved oxygen (ml l^{-1}); OMZ, depth of the oxygen minimum zone (m); Φ, water column stratification (J m^{-3}). NO3, NO2, PO4 and Si (μM).

	Location		
Variable	**Concepción**	**Mejillones**	**Chipana**
Tm (°C)	12.7 ± 0.32	15.3 ± 1.68	17.5 ± 1.61
T50 (°C)	11.5 ± 0.38	12.9 ± 0.03	13.1 ± 0.10
SAL	34.47 ± 0.04	34.59 ± 0.05	34.68 ± 0.06
DO (ml L^{-1})	3.92 ± 0.09	1.71 ± 0.43	3.52 ± 0.43
OMZ (m)	42 ± 32.1	14 ± 3.1	19 ± 3.6
Φ	19.6 ± 4.82	37.7 ± 7.59	57.1 ± 8.54
NO_3 (μM)	14.9 ± 7.15	9.5 ± 4.65	0.4 ± 0.39
NO_2 (μM)	0.5 ± 0.14	0.6 ± 0.25	0.1 ± 0.06
PO_4 (μM)	2.0 ± 0.55	1.6 ± 0.77	2.1 ± 0.65
Si (μM)	12.7 ± 5.74	16.9 ± 10.25	9.3 ± 9.01

Table 2. One-way ANOVA for testing differences in oceanographic conditions among three upwelling sites in the coastal upwelling zone off Chile during the austral summer 2006. Tm, mean temperature of the water column (°C); T50, temperature at 50 m depth (°C); SAL, salinity; DO, dissolved oxygen (ml l^{-1}); OMZ, depth of the oxygen minimum zone (m); Φ, water column stratification (J m^{-3}). NO3, NO2, PO4 and Si (μM).

Variable	**df**	**df error**	**F ratio**	***P***
Tm	2	11	24.6	<0.001
T50	2	11	110.2	<0.001
SAL	2	11	12.6	<0.001
DO	2	11	38.1	<0.001
OMZ	2	11	54.8	<0.001
NO_3	2	9	29.1	<0.001
NO_2	2	9	10.3	<0.010
Φ	2	9	19.9	<0.001
PO_4	2	9	4.4	0.048
Si	2	9	5.3	0.030

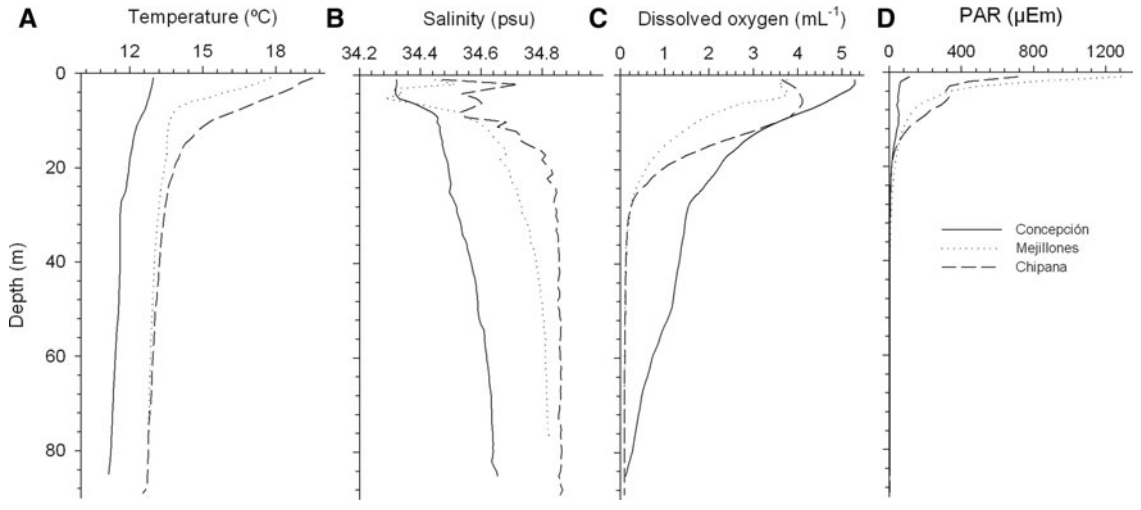

Fig. 2. Vertical profiles of temperature (A), salinity (B), dissolved oxygen (C) and photosynthetic active radiation (PAR) (D) at three upwelling off the Chilean coast during the austral summer 2006. Profiles were averaged for each location from 2 profiles in Concepción and 6 profiles from Mejillones and Chipana.

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Food supply conditions

Phytoplankton biomass, estimated as Chla concentration, varied substantially among localities. Chla at 10 m depth was about 22 $\mu g\, l^{-1}$ in Concepción, 6.7 $\mu g\, l^{-1}$ in Mejillones and about 2.2 $\mu g\, l^{-1}$ in Chipana on average. The contribution of the small size-fraction (<20 μm) also varied among localities, on average about 11%, 45% and 52% in Concepción, Mejillones and Chipana, respectively. Differences in food conditions among localities were also reflected in the content of PUFA (Figure 3A). These differences resulted from variable composition of the microplanktonic community, judging by the proportion of diatoms, dinoflagellates and ciliates for each locality (Figure 3B). The nanoplankton fraction did not vary considerably among localities either in quantity or proportion of autotrophic and heterotrophic components (Figure 3C).

The three localities differed significantly in their quantity and composition of microplankton (Table 3), whereas no significant differences in quantity of nanoplankton were found among localities, although the proportion of autotrophic and heterotrophic was significantly different (Table 3). Meantime Chla was different both among localities and between size-fractions (Table 3).

Fatty acid composition in the size fractions of the natural food also exhibited substantial variation among localities (Table 4). Most remarkable differences occurred in the content of PUFA in the large size fraction (20–200 μm), which was extremely high in the fatty acids of Chipana and very low in Concepción. This high content of PUFA in Chipana was determined by large amounts of EPA (20:5 n-3) which was absent in the 20–200 μm fraction of Mejillones and very low in Concepción.

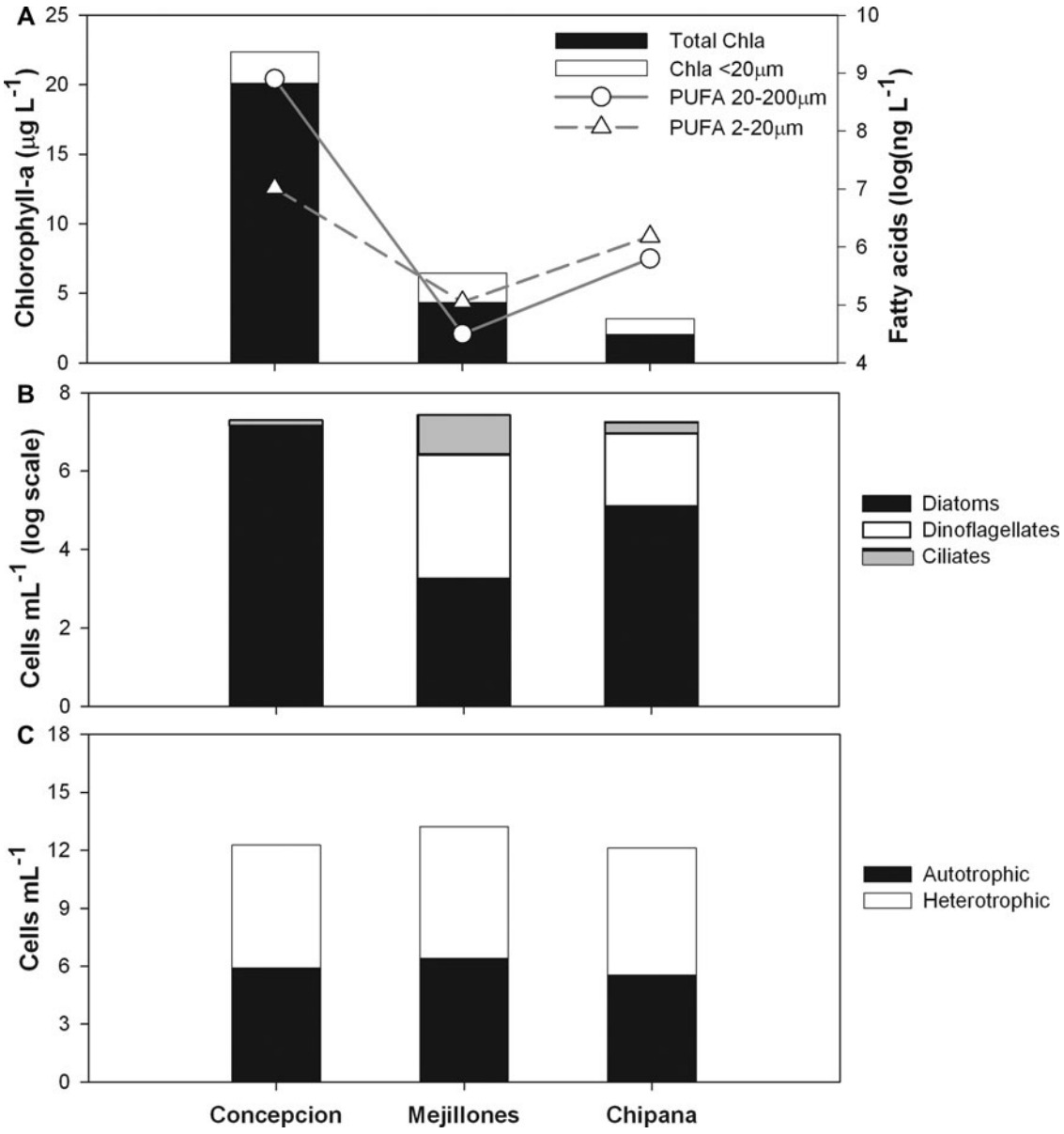

Fig. 3. Quantity and quality of food resources for copepods at three upwelling sites off the Chilean coast during the austral summer 2006. (A) Total phytoplankton biomass and biomass of <20 μm fraction (chlorophyll-*a*) and contents of the fatty acids PUFA for two size fractions; (B) composition of the microplankton in terms of diatoms dinoflagellates and ciliates; and (C) autotrophic and heterotrophic nanoplankton.

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Table 3. Two-way ANOVA to test differences in food composition among three size fractions of natural food assemblages for copepods at three different upwelling sites in the coastal zone off Chile during the austral summer 2006.

Component	Effect	df	F ratio	*P*
Microplankton	Location	2	3.497	0.041
	Group	2	4.946	0.012
	Error	36		
Nanoplankton	Location	2	0.061	0.941
	Group	1	5.092	0.031
	Error	32		
Chla fractioned	Location	2	4.935	0.023
	Group	1	7.726	0.014
	Error	40		

The small-sized fraction 0.7–2.0 μm (picoplankton) was mainly composed of MUFA, especially by oleic acid (18:1 n-9), although palmitoleic acid (16:1) was also abundant in Mejillones and Chipana. PUFA in this small fraction was mainly represented by α-linolenic acid (18:3 n-3), but only at Chipana. The nanoplankton fraction (2–20 μm) on the other hand contained variable proportions of SAFA, MUFA and PUFA depending on locality and SAFA was only considerably present as palmitic acid (16:0) in Concepción, whereas MUFA was well represented by oleic acid in the three locations. PUFA was present in lower proportion in this fraction and represented mainly by linoleic acid (Table 4).

The non-parametric test of Friedman showed significant differences in lipid profiles among localities for the 0.7–2.0 μm fraction (Friedman = 8.65, $P = 0.013$), whereas the nanoplanktonic fraction (2–20 μm) was not different across localities (Friedman = 1.52, $P > 0.05$). The microplanktonic fraction on the other hand did not show significant differences in lipid profiles among localities (Friedman test = 3.351, $P > 0.05$).

Lipid bio-markers of copepods diet

Lipid analysis in individual copepods resulted in variable composition of fatty acids depending on species and locality, although MUFA and PUFA were clearly more abundant in all cases, while proportion of SAFA was always lower than 20% (Table 5). MUFA were in high proportion (>35%) in *Acartia tonsa* and especially represented by oleic acid, whereas PUFA in this species varied from high amounts of linoleic acid in Concepción, stearidonic acid (18:4 n-3) in Mejillones, and EPA in Chipana (Table 5). Oleic acid (MUFA) was also abundant in *Centropages brachiatus*, both

Table 4. Fatty acid composition (%) of three size fractions of food for copepods from 3 upwelling sites at the Chilean coastal zone, during the austral summer 2006. ND, no data available. Size fractions of food were intended to represent natural assemblages of picoplankton (0.7–2 μm), nanoplankton (2–20 μm) and microplankton (20–200 μm).

Fatty acid	Size fractions (μm)								
	Concepción			Mejillones			Chipana		
	07–2	2–20	20–200	07–2	2–20	20–200	07–2	2–20	20–200
Saturated (SAFA)									
12:0	ND	ND	ND	0.0	0.0	7.8	0.0	0.8	0
14:0	2.4	1.7	0.9	1.3	1.1	0.0	0.7	0.7	0.9
15:0	0.2	0.2	0.1	0	0.0	6.5	0.0	0.7	1.7
16:0	22.1	20.3	24.4	7.6	7.3	0.0	4.1	6.4	0.0
17:0	1.0	1.1	0.4	3.0	4.5	0.0	1.6	7.4	0.0
18:0	7.2	5.6	18.6	3.0	2.5	0.0	1.4	2.7	0.0
20:0	0.1	1.0	0.3	0.0	0.3	4.1	0.0	0.1	0.0
22:0	0.3	0.3	0.3	0.0	0.0	22.7	0.0	0.7	0.0
24:0	0.2	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Total	33.5	30.3	45.3	14.9	15.7	41.1	7.8	19.5	2.6
Monounsaturated (MUFA)									
15:1	0.5	0.5	0.2	ND	ND	ND	ND	ND	ND
16:1	3.3	2.8	0.5	23.2	18.1	0.0	17.1	8.8	2.0
17:1	0.6	0.9	0.1	0.0	1.8	3.0	0.0	2.5	0.0
18:1 n-9	39.9	40.8	45.6	42.3	30.2	0.0	25.8	36.5	0.0
20:1	0.8	0.1	0.0	0.0	5.2	7.8	0.0	5.5	0.0
22:1	0.1	0.3	0.2	0.0	0.0	0.0	0.0	0.3	0.0
Total	45.2	45.4	46.6	65.5	55.3	10.8	42.9	53.6	2.0
Polyunsaturated (PUFA)									
18:2 n-6	15.5	18.5	3.4	5.1	5.6	0.0	3.1	13.5	0.0
18:3 n-3	2.1	2.4	1.7	0.8	5.6	18.3	27.0	2.0	0.0
18:4 n-3	1.6	2.2	1.0	3.7	4.9	0.0	12.7	4.8	5.3
20:4 n-3	0.2	0.1	1.3	0.0	0.0	19.4	0.0	0.2	0.4
20:5 n-3	0.5	0.5	0.4	7.8	10.3	0.0	6.4	4.6	81.9
22:3	ND	ND	ND	0.0	0.0	0.0	0.0	1.2	0.0
22:6 n-3	1.4	0.7	0.3	2.2	2.6	10.4	0.0	0.8	7.9
Total	21.3	24.4	8.1	19.6	29.0	48.1	49.2	27.1	95.5

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in Concepción and Mejillones. We did not find this species in Chipana for lipid analysis at the time of sampling, whereas the PUFA components were represented by linoleic acid in Concepción, and variable proportions of acids α-linolenic, stearidonic and EPA in Mejillones (Table 5).

At the locality of Concepción lipid profiles were similar between species with a remarkable presence of the fatty acids oleic (MUFA) and linoleic (PUFA) (Figure 4A). The former was also highly abundant in the three food fractions, whereas linoleic acid was abundant only in the food fractions smaller than 20 μm (Figure 4B). Meantime, the palmitic acid (SAFA) was also present in all the food fractions, but its signal in copepods was rather weak.

At the northern locality of Mejillones the signalling of food in the lipid content of copepods is also remarkable. The MUFA oleic fatty acid was well represented in both species (Figure 5A) as well as in their food (Figure 5B). Another MUFA (palmitoleic acid) was also signalling the food in both species. Both MUFA fatty acids were only present in the small fractions (<20 μm) of food. It was relevant to observe that several fatty acids clearly present in the large-sized fraction of food (>20 μm) were not detected in the copepods, such as the saturated behenic acid and two other PUFA (Figure 5B), suggesting a minor contribution of this size fraction in the copepod diet.

At Chipana site only *Acartia tonsa* was found and its lipid profile is shown in Figure 6A. In this species the most abundant fatty acids were oleic acid and palmitoleic acid (both MUFA), linoleic acid (PUFA) and eicsoatetraenoic acid (PUFA). All of them, except the last one, were present in the smaller food fractions, but not in the microplanktonic fraction (>20 μm).

The high consistency in lipid profiles of copepods and their food, depending on location and species can also be illustrated by a cluster analysis, which is able to combine all the information and highlights the relationships among profiles on the basis of their correlations (Figure 7). This analysis shows that fatty acid contents are closer to each other when belonging to same food fractions, same locations or same species (Figure 7). In this analysis it is also relevant to show that differences in the food offer are more important than differences between species. This reveals the strong dependence of lipid signals of copepods of the available food in the field (Figure 7).

DISCUSSION

Fatty acids have long been used as chemical markers of biogeochemical processes and trophic relationships (Ederington *et al.*, 1995; Napolitano *et al.*, 1997). In order to be suitable trophic markers these fatty acids must be synthesized at a lower trophic level and transferred unchanged or in a recognizable form. Since PUFA are essential for marine organisms, they are considered useful as trophic markers (Naoplitano *et al.*, 1997).

Table 5. Fatty acid composition (%) in two copepod species, *Acartia tonsa* and *Centropages brachiatus*, sampled from 3 upwelling sites (Concepción, Mejillones and Chipana) at the Chilean coast during the austral summer 2006.

Fatty acid	Concepción		Mejillones		Chipana
	Acartia	*C. brachiatus*	*Acartia*	*C. brachiatus*	*Acartia*
Saturated					
12:0	ND	ND	0.9	0.7	ND
14:0	0.0	0.0	0.4	0.6	0.8
15:0	0.0	0.0	0.4	0.8	0.1
16:0	2.6	2.5	3.4	3.5	5.1
17:0	0.0	0.0	1.1	1.2	2.3
18:0	1.6	2.1	2.8	1.4	1.9
20:0	2.4	0.0	0.4	0.2	1.0
22:0	3.0	0.0	0.0	0.8	1.0
24:0	0.0	0.0	0.0	9.6	0.4
Total	9.6	4.6	9.4	18.8	12.6
Monounsaturated					
15:1	0.0	0.0	ND	ND	0.3
16:1	0.0	0.0	4.1	7.5	5.6
17:1	0.0	0.0	0.0	0.6	1.2
18:1 n-9	49.2	61.5	45.0	27.3	28.7
20:1	12.4	0.0	0.0	0.7	3.0
22:1	0.0	0.0	0.0	0.5	0.1
Total	61.6	61.5	49.1	36.6	38.9
Polyunsaturated					
18:2 n-6	22.9	33.9	9.3	5.0	8.3
18:3 n-3	5.9	0.0	9.7	10.3	3.7
18:4 n-3	0.0	0.0	17.1	14.2	13.5
20:4 n-3	0.0	0.0	0.8	0.3	20.0
20:5 n-3	0.0	0.0	2.9	10.6	2.2
22:3	ND	ND	1.2	0.0	ND
22:6 n-3	0.0	0.0	0.5	4.3	1.0
Total	28.8	33.9	41.5	44.7	48.7

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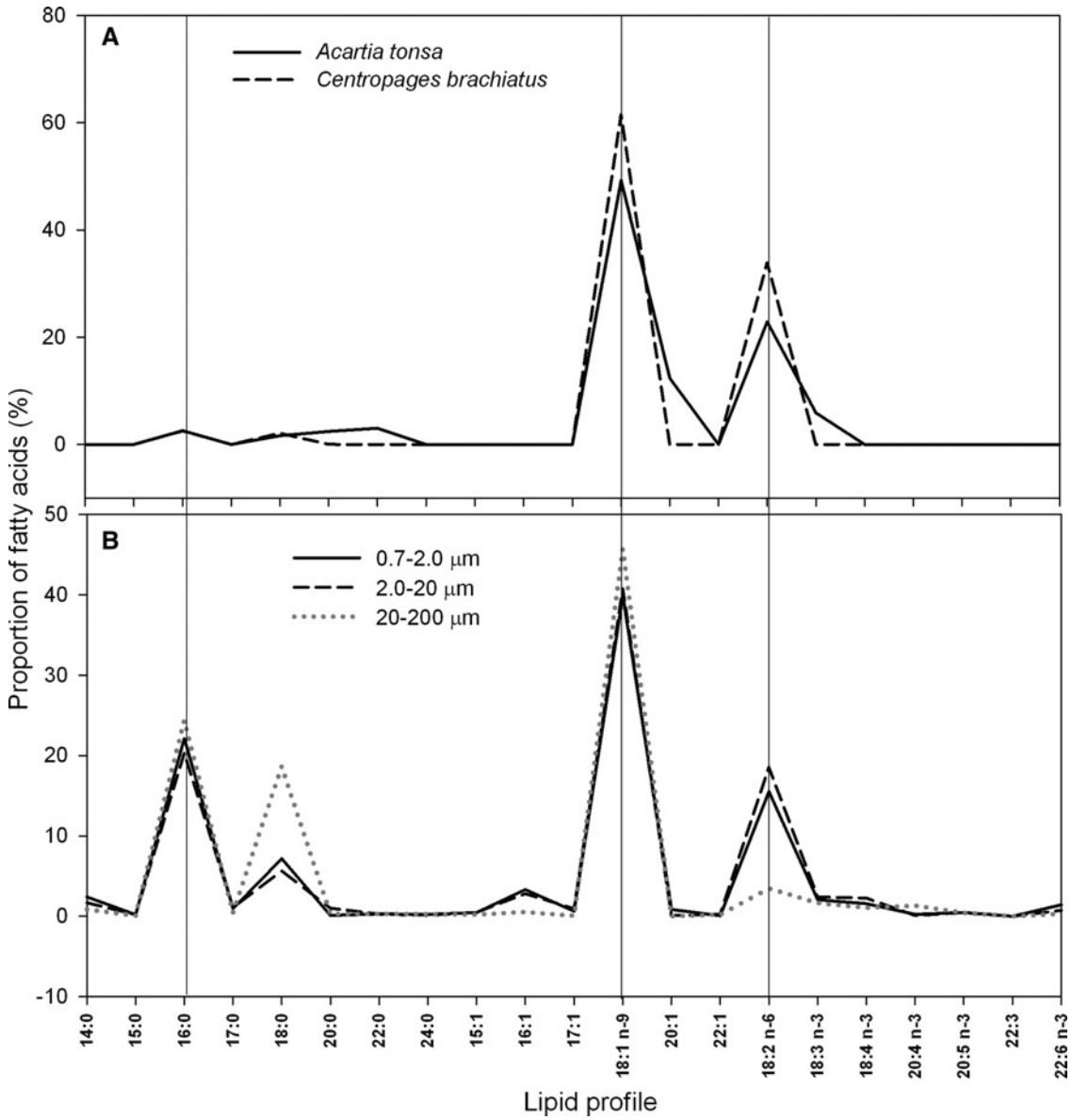

Fig. 4. Fatty acid profiles of the copepods *Acartia tonsa* and *Centropages brachiatus* (A) and three size fractions of natural food assemblages (B) at the coastal upwelling site of Concepción during the austral summer 2006.

In zooplankton studies the conservative transfer of fatty acids from phytoplankton to copepods was early demonstrated by Lee *et al.* (1971) from laboratory feeding experiments of *Calanus helgolandicus*. Later studies have evidenced the signalling capacity of phytoplankton lipids in herbivorous zooplankton (Fraser *et al.*, 1989; Sargent *et al.*, 1995; Desvilettes *et al.*, 1997). Some of these works have shown changes in fatty acid profiles of zooplankton upon changing diet, as shown in Arctic calanoids (Graeve *et al.*, 1994). It now becomes clear that such essential lipids not only act as just chemical markers, but their composition may play a crucial role for copepod nutrition and physiological rates (Klein Breteler *et al.*, 2005), as well as for copepod population dynamics (Vargas *et al.*, 2006). Therefore, our knowledge about factors causing variability in copepod lipids is highly relevant to understand variation in their growth and physiology, which in turn govern their population dynamics. In this context, it became clear that food supply is the major determinant of fatty acid profiles of copepods, and as shown from our work not only PUFA serve as trophic markers, but MUFA and even SAFA can leave their signals in copepods. As noted above, some of these fatty acids are essential, are they are in most cases the main contributors to the animal profiles, indicating that they are efficiently incorporated by copepods from their available food resources. It is important to note that such food resources comprise a complex of food particles containing both autotrophic and heterotrophic components, as shown in our analyses, and copepods seem able to combine these in their diet (Vargas *et al.*, 2006). Therefore, it is impossible to establish a species-to-species trophic relationship between copepods and food (Desvilettes *et al.*, 1997). However, coexisting predators might select distinct food items from this mixture and such selection could be reflected in the fatty acid profiles of the species. In our study, both copepod species are rather small copepods and known as typically omnivorous

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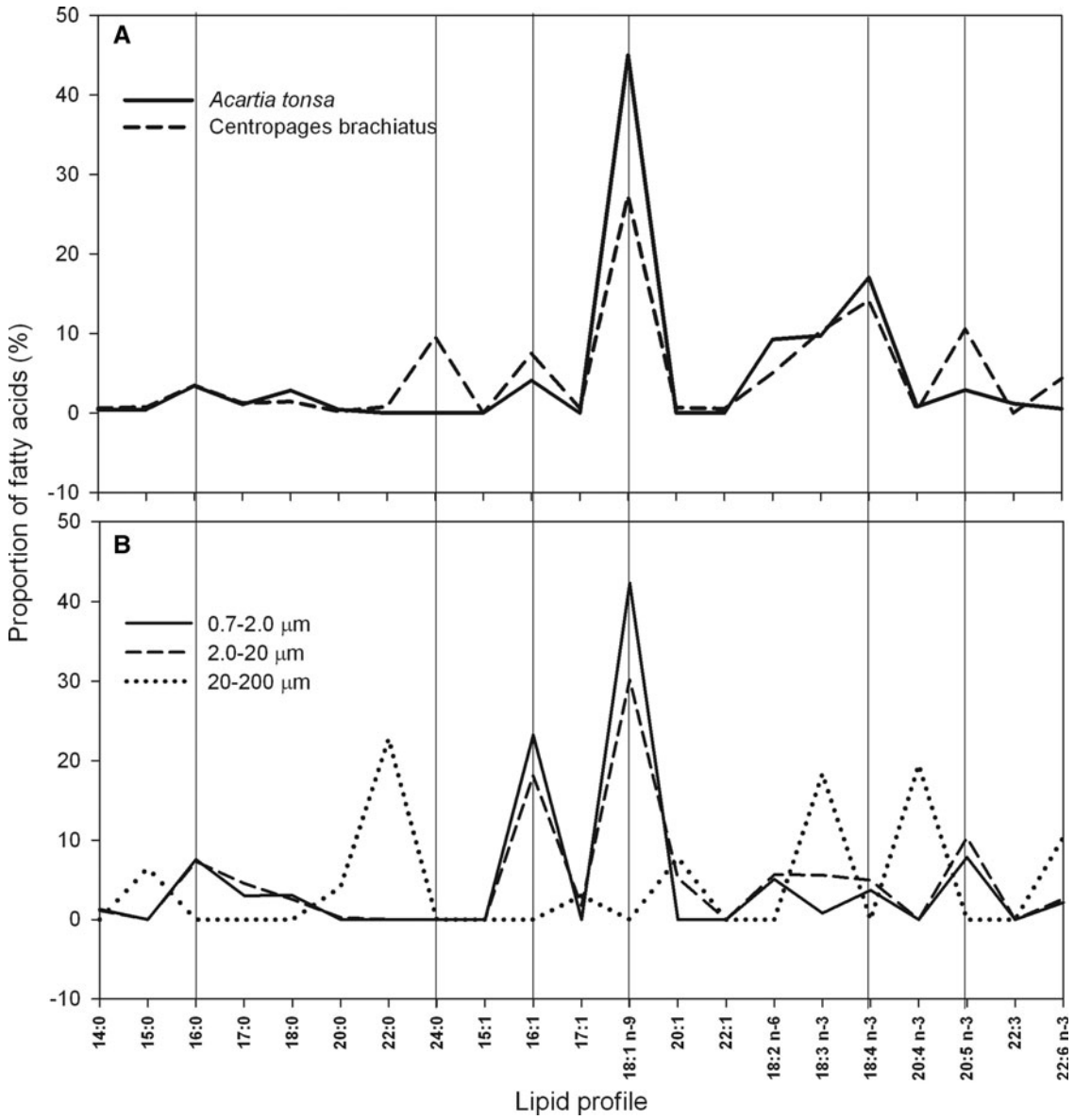

Fig. 5. Fatty acid profiles of the copepods *Acartia tonsa* and *Centropages brachiatus* (A) and three size fractions of natural food assemblages (B) at the coastal upwelling site of Mejillones during the austral summer 2006.

(Roman, 1984; Berggreen *et al.*, 1988; Vargas *et al.*, 2006), such that they might exhibit a similar feeding preference upon the same offer. Indeed, at the locality of Concepción both species showed virtually the same fatty acid profile (Figure 4) dominated by the MUFA, oleic acid and the PUFA linoleic acid, which were also the dominant fatty acids in the food supply. By contrast, at Mejillones where both species were present their fatty acid profiles differed considerably (Figure 5A) suggesting the utilization of distinct food items. Consistently the food supply in this location was more diverse in fatty acid composition (Figure 5B) as compared to Concepción. It is, thus, likely that resource partition in relation to food operates when the food offer is sufficiently diverse, but under a condition of a more uniform food spectrum coexisting species tend to use it without selectivity. Similar feeding responses (little selectivity) for the area of Concepción were found by Vargas *et al.* (2006) and shifts in the diet were just a function of variation in the food offer. Our cluster analysis also showed that differences in food offer can be more important in determining the lipid profiles than differences between species (see Figure 7).

We do not know if observed changes in copepod lipid profiles may actually affect nutrition or physiology of individuals. In all cases, essential PUFA and MUFA were found in the examined copepods indicating that copepods are able to obtain them from the picoplanktonic and nanoplanktonic fraction, with no need to use larger sized chain-forming diatoms, which are known to be rich in essential PUFA (Ackman & Tocher, 1968; Sargent *et al.*, 1988). In this respect, it has been found that heterotrophic diet (protozoan) may also substantially contribute with essential fatty acids (Klein Breteler *et al.*, 1999). This possibility may provide support to assume that our examined copepods at all locations were well fed and in good nutritional condition, also supporting earlier suggestions that copepods in the upwelling zone off Chile may grow under non-limiting of food for most

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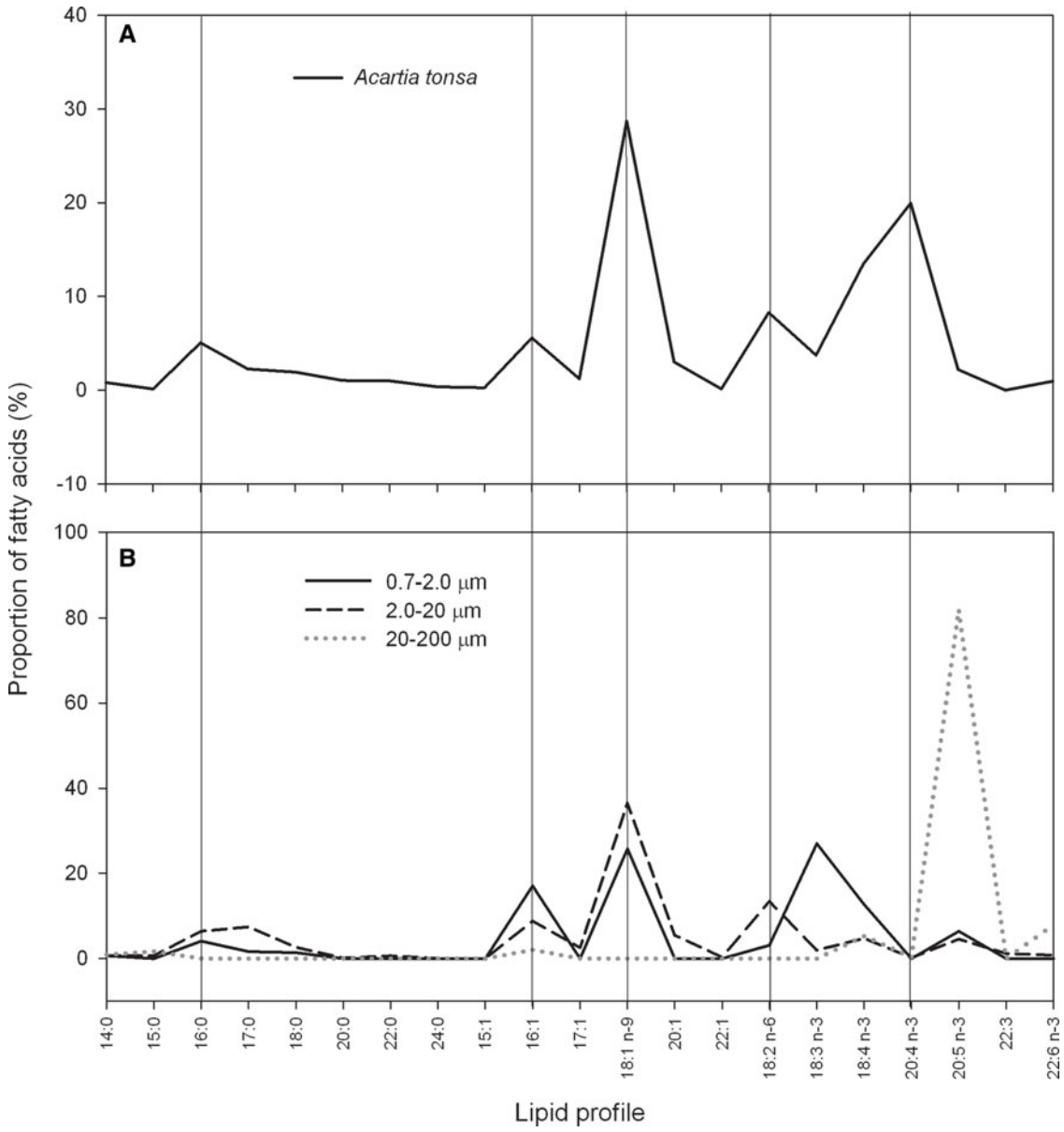

Fig. 6. Fatty acid profiles of the copepod *Acartia tonsa* (A) and three size fractions of natural food assemblages (B) at the coastal upwelling site of Chipana during the austral summer 2006.

situations (Escribano & McLaren, 1999; Hidalgo & Escribano, 2007).

Changes in lipid profiles of copepods depended on food variability in the field (different location). This variation in food supply seemed related to distinct upwelling conditions, as reflected in significant difference in temperature, water column stratification, depth of the oxycline (OMZ depth), and nutrients. Different oceanographic conditions among locations could have influenced phytoplankton biomass and composition, as well as abundance and diversity of heterotrophic components in the food fractions. In the upwelling zone off Chile, several studies have shown the strong link between upwelling variation and microplankton and nanoplankton biomass and composition (Herrera & Escribano, 2006; Anabalón *et al.*, 2007; Böttjer & Morales, 2007). It is, thus, reasonable to expect that any factor causing changes in the upwelling regimes can also greatly affect copepod feeding after alteration of quality of food supply. These effects, as suggested by our work, may be reflected in changes in lipid profiles of primary consumers. The biogeochemical consequences of such changes may certainly deserve further attention, because the understanding of mechanisms controlling the flux of C in the marine food web constitutes a pending and highly relevant task in the context of global C cycling. Our work also provides strong evidence that small-sized fractions of plankton, such as nanoplankton and microplankton seem to be the dominant diet of these numerically dominant copepods of the upwelling zone. Copepods have traditionally been viewed as feeding on diatom diets, and this might be true for most systems. However, copepods dominating the coastal zone of the Humboldt Current System may rather feed on heterotrophic and autotrophic flagellates, including dinoflagellates, as shown in this work and previously in Vargas *et al.* (2006).

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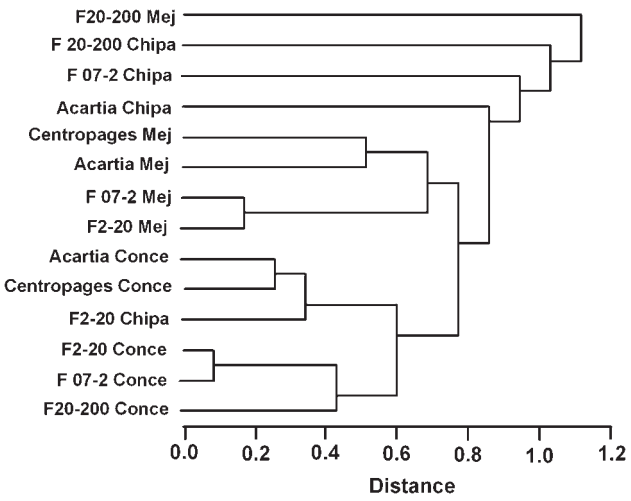

Fig. 7. Cluster analysis to relate the fatty acid profiles of the copepods *Acartia tonsa* and *Centropages brachiatus* and three size fractions of natural food assembles from 3 upwelling sites off the Chilean coast during the summer 2006. The measuring distance for clustering was the Pearson correlation. Fo7-2, food fraction 0.7–2.0 μm; F2-20, food fraction 2.0–20 μm; F20-200, food fraction 20–200 μm; Acartia, *Acartia tonsa*; Centropages, *Centropages brachiatus*. Conce, Mej and Chipa represent the locations of Concepción, Mejillones and Chipana, respectively.

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