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Research Article

Scaling copepod grazing in a coastal upwelling system: the importance of community size structure for phytoplankton C flux

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ABSTRACT. Crustacean zooplankton, often dominated by copepods and euphausiids, are the major phytoplankton grazers in coastal upwelling systems. It has been argued that zooplankton grazing is a size-dependent process, such that models incorporating the size structure of zooplankton are appropriate for describing herbivore C-transfer. Here, based on the size-spectrum theory and on gut-fluorescence experiments, conducted with numerically dominant copepods from two upwelling sites off the Chilean coast, we show that C-specific ingestion rates of copepods are size-dependent. We further show that the size structure of the copepod community, synthesized by the slope of the normalized size spectrum, determines the impact of grazing on phytoplankton. C-specific ingestion rates, depending on species size, were in the range of 0.14-353.97 (ng C µg C^{-1} h^{-1}). A modelled normalized biomass-spectra of a copepod community in the size range of 0.5 to 74.0 µg C showed that C-specific grazing impact can increase by a factor of 4 when small-sized species (0.1-10 µg C ind^{-1}), such as *Paracalanus* cf. *indicus*, *Acartia tonsa*, *Oncaea* spp. and *Corycaeus* spp., dominate the community in terms of biomass. By contrast, when larger-sized copepods dominate (10-100 µg C ind^{-1}), such as *Calanus chilensis*, *Calanoides patagoniensis* and *Rhyncalanus nasutus*, total zooplankton biomass may increase, but with a sharp decrease in the efficiency of C transfer via herbivores. Our findings indicate that processes affecting the size structure of zooplankton communities can substantially impact the phytoplankton C flux through the pelagic food web, thus controlling production of higher trophic levels.

Keywords: copepods, grazing, ingestion rate, body size, upwelling, Chile.

INTRODUCTION

Zooplankton grazing is one of the key processes by which phytoplankton carbon can be transferred to higher trophic levels in the marine ecosystem. Studies on this process have long been carried out to assess the role of herbivorous zooplankton in channeling primary production (Irigoien, 1998; Båmstedt *et al*., 2000). Various works on different systems have suggested that herbivore-grazing exercises limited control over C flux in the marine food chain because most zooplankton are omnivorous & their feeding on heterotrophic diets predominates for a variety of situations, thus favoring microbial C pathways (González *et al*., 2000; Calbet, 2001; Calbet & Landry, 2004; Vargas *et al.*, 2007). Although in strongly seasonal upwelling systems at times of the year (spring-summer) dominant zooplankton can shift their diet, such that herbivorous grazing on diatoms may greatly increase and thus serve as the C channel for higher trophic levels (Vargas *et al*., 2006).

In highly productive coastal upwelling systems, it was initially considered that the zooplankton community was comprised of, and dominated by herbivorous copepods and euphausiids, so that the "classical food chain" is seen as the main C transfer mechanism in the food web. However, several recent works have shown that small-sized omnivorous copepods in fact dominate the zooplankton community in upwelling zones, such that herbivorous grazing accounts for only a minor fraction (<5%) of the primary production incorporated into secondary production (Vargas & Gonzalez, 2004; Vargas *et al*., 2007). This finding challenged the previously established paradigm that grazing by large copepods and euphausiids in upwelling systems represents the major pathway for transferring primary production to fish (Riley, 1947). A further implication is that the community structure (size and species composition) has a critical role in modulating the quantity and rate of C flux from lower to higher trophic levels.

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Nevertheless, the influence of community structure (size and composition) on zooplankton feeding has rarely been considered when assessing C flux in the pelagic food web. Feeding dynamics of zooplankton has been studied in relation to the quantity and quality of food, temperature and body size (Tirelli & Mayzaud, 2005; Vargas *et al*., 2006; Saiz & Calbet, 2007; Vargas *et al*., 2009).

Regarding the effect of body size, ingestion rates of zooplankton appear to be adequately described by allometric models (Vidal & Whitledge, 1982; Ikeda, 1985; Ikeda *et al*., 2001; Saiz & Calbet, 2007, 2011), with a body mass exponent in the range of 0.7-0.8, although this parameter in some cases can differ significantly from the expected value of 0.75, which is the power scaling of metabolic rates. Saiz & Calbet (2007) found that ingestion rates of calanoid copepods, when related to food availability, temperature and body size, can be explained in about 80% by a combination of these factors, while body size alone explains less than 10% of total variance. Saiz & Calbet (2011) subsequently compiled more data on marine copepod feeding and showed that food availability and body weight were the major factors controlling copepod feeding rate, whereas temperature has a minor effect. In sum, it is suggested that under conditions of high food availability, body size is a suitable predictor of copepod feeding, independent of other species-dependent effects. Nevertheless, the taxonomic effect is an issue hardly been tested in studies focused on ecological rates or processes.

Size-structure and species composition are inter-related ecological attributes in copepod communities. Small-sized copepods (<1.5 mm) like the calanoids *Paracalanus* spp. and *Acartia tonsa*, the cyclopoids *Oithona* spp., *Oncaea* spp. and *Corycaeus* spp. are dominant in the upwelling zone off Chile. By contrast, medium-sized (1.5-2.5 mm) and large-sized (>2.5 mm) copepod communities are mostly comprised of the calanoids *Calanus* spp., *Centropages brachiatus*, *Calanoides patagoniensis*, *Eucalanus* spp. and *Rhyncalanus nasutus* (Hidalgo *et al*., 2010). Therefore, copepod size and species should be assessed together when attempting to characterize the community structure. It is difficult however to quantitatively assign a measurement to variable species composition, or to relate size to ecological processes, although size-structure somehow synthesizes both size-dependent and species-dependent effects.

In this work, we assessed whether size-structure by itself can predict grazing impact of copepods on primary production in two highly productive coastal upwelling sites off Chile. To do so, we estimated species-dependent grazing rates in relation to variable oceanographic conditions, and used the size-spectrum theory (White *et al*., 2007) to determine how changes in the size structure of the copepod community (main grazers in the upwelling zone), independent of the species composition, influences grazing impact on primary production. Thereafter, we used field estimate of copepod abundances from the upwelling zone to examine how an observed copepod size spectrum compares to a theoretical one, and what are the expected consequences for C-fluxes in the pelagic system upon changes in the plankton community structure, potentially driven by climatic/oceanographic forces.

MATERIALS AND METHODS

Experiments were conducted in two upwelling sites during autumn 2010 (May 2010) at Mejillones Bay (22°54´S, 70°24´W) in northern Chile, and in the austral spring of 2010 (October 2010), and winter 2014 (August 2014) off Concepción (36°00’S, 73°07´W) in central/southern Chile (Fig. 1). These two sites are highly productive upwelling centers along the Chilean coast. They are far apart (*ca*. 2000 km), but have similar copepod communities (Hidalgo *et al*., 2010), and their oceanographic conditions have been characterized (Escribano *et al.*, 2012).

Temperature, salinity and dissolved oxygen profiles were obtained in the field with a CTDO Sea Bird SBE-19 for each sampling site (3 profiles). Particulate organic carbon (POC) and chlorophyll-a (Chl-*a*) samples were collected using 10-L Niskin bottles at 5 depths from the surface to the near bottom (*ca*. 90 m). Chl-*a* was measured in duplicate, with the fluorometric technique by filtering 200 mL of seawater through 0.7 µm GF/F filters (Parson *et al.*, 1984), and POC samples from the same depth were analyzed following Bodungen *et al*. (1991) by CHN elemental analysis.

Live zooplankton was obtained by vertical net hauls from 0-75 m in Mejillones Bay (2 tows) and 0-50 m in Concepcion (2 tows at each sampling date), with WP-2 nets (200 µm mesh size). Live samples were used for estimating Chl-*a* evacuation and ingestion rates. After capture, the first tow was split immediately into four, approximately, equal fractions to estimate copepod evacuation rates. The fractions were kept in darkness onboard with 5 L of filtered seawater (<20 µm) and incubated for 15, 30, 60 and 120 min for each corresponding fraction, nearly at *in situ* temperature. Once incubation was completed, each fractions were frozen in liquid nitrogen (-196°C) for later analysis in the laboratory. A second zooplankton tow was used to estimate *in situ* copepod ingestion rates. The sample from this tow was preserved in liquid nitrogen for later analysis.

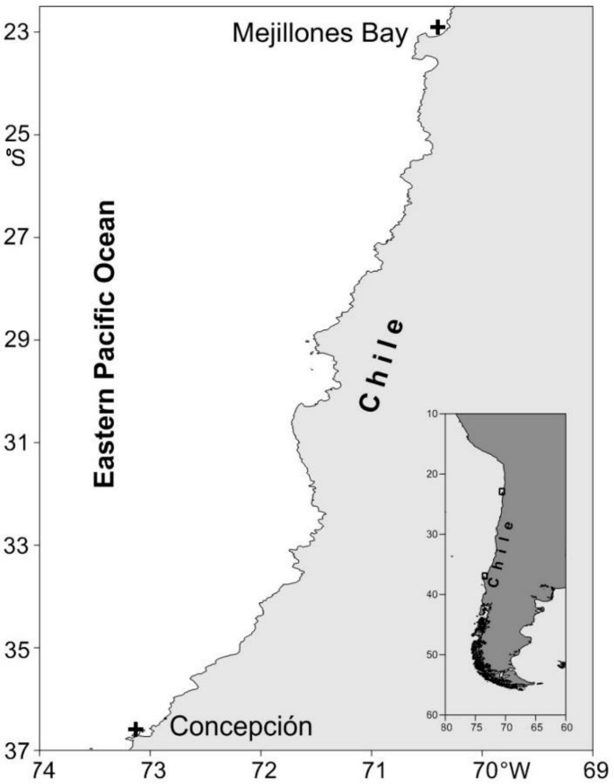

Figure 1. The coastal upwelling zone of Chile indicating the two locations, Mejillones Bay and off Concepcion, where grazing experiments were conducted with the copepod community.

Ingestion and evacuation rates of dominant copepods (listed in Table 2) were determined by gut fluorescence method (Mackas & Bohrer, 1976). In the laboratory, samples in liquid nitrogen were thawed and individuals of dominant species were sorted under a stereoscope microscope. Between 5 and 20 individuals per species were placed into 20 mL vials with acetone (90% v/v) for pigment extraction for 24 h at low temperature (-4°C). Numbers of individuals are indicated in Table 3. Fluorescence was measured with a Turner fluorometer TD 040. Triology and the measurements were done under low-light conditions to avoid pigment degradation (Pasternack, 1994).

Changes in pigment concentration in copepod gut are described by an exponential decay function allowing the following calculation, such that:

$$GC_t = GC_0 \times e^{-kt} \tag{1}$$

where GC_t is the gut content in the time interval t, GC_0 is the gut content in the initial time and k is the evacuation rate. Copepod ingestion rates (I) were calculated by using previously estimated evacuation rates, assuming that a constant proportion of gut content is removed per unit of time.

$$I = GC_0 \times k \tag{2}$$

The daily food (DR) and C rations, ingested per day, expressed as a percentage of body mass, were estimated as described in Båmstedt *et al.* (2000). For this, food ingested was expressed as the percentage of body mass of each copepod species. A mean C/Chl-*a* ratio of 45 was assumed (Båmstedt *et al.*, 2000) to convert Chl-*a* into C units, and to calculate C-specific ingestion and evacuation rates.

The body masses of copepod species were obtained from published data (Table 2). The fitted allometric regression to relate ingestion rates and copepod size was:

$$I_i' = a\,(BM_i)^b \tag{3}$$

where, I_i' is C-specific ingestion rate (ng C x μg C^{-1} h^{-1}), *a* is the y-intercept, *b* is the allometric parameter (Peters, 1983; White *et al.*, 2007) and BM_i is copepod body mass (μg C) for each *i* species.

To assess the effect of changes in the size structure of the copepod community on grazing impact, we simulated a copepod size spectrum in the range of copepod body mass of all the species considered in this study. This normalized size spectrum was represented by size classes with their corresponding abundances (n_i) and associated body masses (BM_i), such that,

$$\mathrm{Log}_2\,(N) = \mathrm{Log}_2\,(a) - b\log_2\,(BM) \tag{4}$$

where *a* is the y-intercept, *b* is the slope of the regression and BM is the mean body mass of each copepod size class. To simulate changes in the slope of this size spectrum, we used the range of variation of the spectrum slope, as reported in other works (*e.g.*, Manríquez *et al.*, 2009), and the assumed range was -0.25 and -1.76. The lowest BM was assumed to be 0.5 μg C and the highest 74 μg C. A total number of 28 size classes (SC) were assumed at 2.5 μg C intervals of BM. We started the size spectrum with an arbitrary number of 100 individuals per size class. This initial condition assumes a slope = 0, which is unreal, but we used it as the initial value to estimate the abundance of each size class (Nc) by increasing the slope of the size spectra in 0.1 units, such that,

$$\mathrm{Log}_2\,(Nc) = \log_2\,(100) - s\,\log_2\,(BMc) \tag{5}$$

where Nc is the abundance (number m^{-2}) of each size class, s is the slope of the size spectrum and BMc is the mean biomass of the corresponding size class. Grazing exerted by each of these size classes was estimated after fitting equation (3) (shown in Fig. 4), such that,

$$Ic = a \times (BMc)^b \tag{6}$$

where Ic is the C-specific ingestion rate of a given size class (ng C × μg C^{-1} d^{-1}), *a* and *b* are the allometric parameters, and BMc as defined above. Finally, grazing impact by the copepod community was estimated as,

$$GI = \sum_{c=1}^{28} (I_c\, N_c)/TB \tag{7}$$

where GI = standardized grazing impact by the copepod community (µg C × mg C^{-1} d^{-1}), Ic and Nc as defined above, and TB is total added biomass of the copepod community (mg C m^{-2}) after changing the slope of its size spectrum.

In order to assess a natural size spectrum of copepods, we used data on copepod abundances from a zooplankton time series at Station 18, located over the continental shelf off Concepción (36°30'S). We used monthly data from August 2002 to December 2005. Details on zooplankton sampling and analyses were provided in Escribano *et al*. (2007), while a detailed description of the copepod community from the same data can be found in Pino-Pinuer *et al*. (2014). About 50 copepod species, including those for which grazing rates had been estimated, were assigned to 10 size classes, in terms of mean species biomasses (µg C). Same size interval (2.5 µg) was used to assign these size classes to coincide with the theoretical size spectra. Mean copepod biomass for each species was obtained from weight-length regressions provided in Chisholm & Roff (1990) and using mean adult length. Monthly normalized size spectra were thus constructed with their corresponding slopes. C-specific ingestion rates (Ic) were then estimated for each size class applying Eq. (6) and thereafter the total grazing impact (GI) for the whole community was calculated by using Eq. (7). Chlorophyll-*a* data from Station 18, as reported in Anabalón *et al*. (2007) were converted to phytoplankton C, as indicated above, and then used to assess grazing impact by daily C removal per square meter from the phytoplankton community. Seasonal and interannual changes in GI as related to a changing copepod size spectrum could thus be studied.

RESULTS

Oceanographic conditions of both sampling areas (Fig. 2) have been characterized in previous studies.

For instance, Marín *et al*. (2001) described Mejillones Bay as a highly productive upwelling site with year-round upwelling conditions, and a shallow (<50 m) low-oxygen layer constraining a highly aggregated copepod community in the upper layer (Donoso & Escribano, 2013). Off Concepción in central southern Chile, upwelling becomes intense in the spring-summer (Sobarzo *et al*., 2007), although copepods dominate the zooplankton community year-round over the continental shelf (Escribano *et al*., 2007). During our experiments, *in situ* data showed that the water column in Mejillones Bay in May 2010 (Fig. 2a) was relatively mixed, with temperatures in the range of 15.5 to 13.3°C, with a weak thermocline. Salinity had little variation with depth and ranged from 34.9 to 34.8, while dissolved oxygen decreased gradually with depth, with values from 3.5 mL O_2 L^{-1} at the surface to 0.6 mL O_2 L^{-1} at 60 m depth. In October 2010 (spring) off Concepcion (Fig. 2b), temperature varied with depth from 12.8 to 10.5°C, with strong stratification and a shallow thermocline within 10 m. Salinity varied with depth from 34.57 to 34.84, with a shallow halocline (<10 m) and dissolved oxygen varied from 5.8 mL O_2 L^{-1} at the surface to 0.2 mL O_2 L^{-1} at 50 m. In August 2014 conditions off Concepción (Fig. 2c) were typical of winter, with very low surface salinity (<31) and a much deeper thermocline (>30 m) and a well-mixed and oxygenated water column.

Phytoplankton biomass (Chl-*a*) during winter in Mejillones Bay (Table 1) was rather low, in the range of 0.12-0.33 mg m^{-3} and POC between 272 mg C m^{-3} in surface water and 1218 mg C m^{-3} at the deepest layer (75 m), with a C/Chl-*a* ratio between 913-10,000. Concepción (Table 1) had a typical spring condition in October 2010, with a Chl-*a* concentration of 11.8 mg m^{-3} in surface water and 4.8 mg m^{-3}at 25 m and POC was 348 with a C/Chl-*a* ratio 89.5. In the winter 2014 Chl-*a* was in the range of 0.2-0.4 mg m^{-3} in the upper 10-m layer, although no POC data were available. The lowest values of Chl-*a* and highest C/Chl-*a* ratio found in Mejillones Bay in May 2010 suggested that much of the food available for zooplankton did not contain Chl-*a*. By contrast, the area off Concepción exhibited a highly productive environment with high values of Chl-*a* and low (<150) C/Chl-*a* ratios in spring, whereas in winter 2014 in this site the Chl-*a* condition was similar to that of Mejillones Bay.

Copepod sizes for the species considered in the study, in terms of body length and C-content, were obtained from previous works and are summarized in Table 2. For these species, evacuation and ingestion rates in Mejillones Bay were estimated for four species (Table 3). Of these, two species are the calanoid copepods, *Calanus chilensis* and *Paracalanus* cf. *indicus*, and two are the cyclopoid copepods, *Corycaeus* spp. and *Oncaea* spp. Off Concepción, evacuation and ingestion rates were estimated for two small calanoids, *Acartia tonsa* and *Paracalanus* cf. *indicus* and three larger-sized calanoids *Calanoides patagoniensis*, *Pleuromamma gracilis* and *Rhyncalanus nasutus*.

Temporal variation of copepod gut content (*GC*), and exponential decay fitted for both locations are presented in Fig. 3. Some data points, usually those observed at the end of the incubation period, were considered as outliers and thus excluded from fitted equations (as shown in Fig. 3).

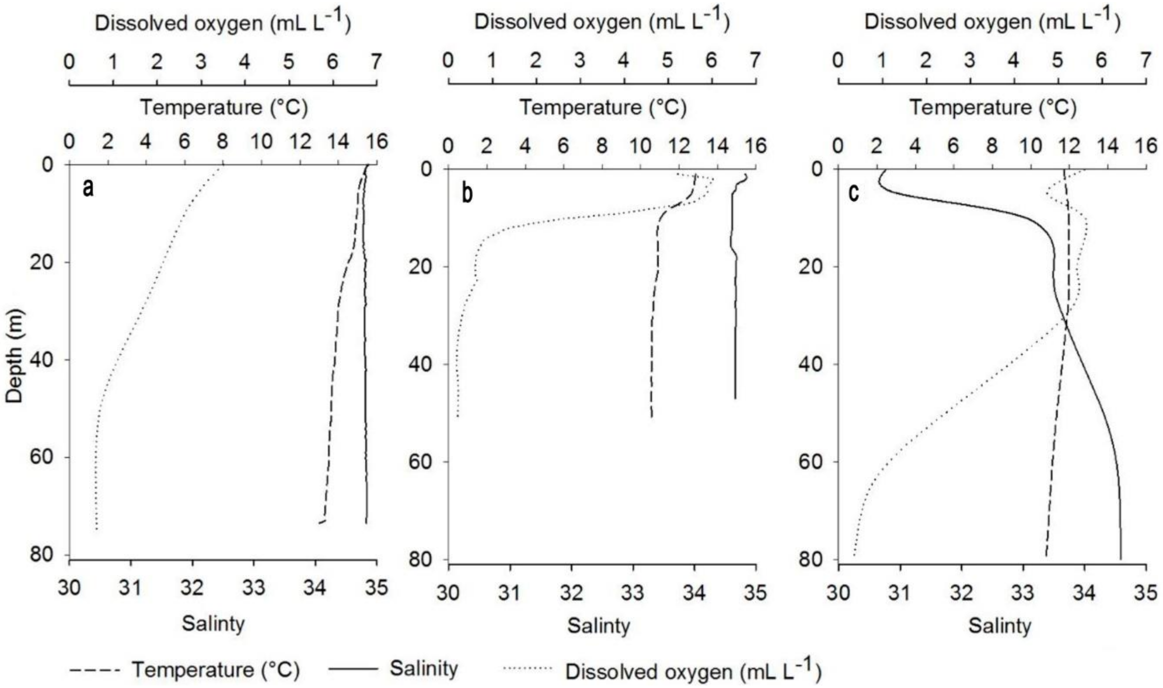

Figure 2. Vertical profiles of temperature, salinity and dissolved oxygen in May 2010 in coastal waters of a) Mejillones Bay, and b) off Concepción in October 2010 and c) August 2014. Profiles were obtained from CTD casts equipped with an oxygen sensor.

Table 1. Concentration of chlorophyll-*a*, phaeopigments, particulate organic carbon (POC) and the C/Chl-*a* ratio at different depths in coastal waters of Mejillones Bay and off Concepción. Phytoplankton pigments were measured by the fluorometric method and POC with mass spectrometry. nd: no data available.

Location	Season	Depth (m)	Chlorophyll-*a* (mg m^{-3})	Phaeopigments (mg m^{-3})	POC (mg C m^{-3})	C/Chl-*a* Ratio
Mejillones Bay	May 2010	0	0.31	0.64	272	913
		10	0.33	0.40	338	1035
		30	0.29	0.74	593	1905
		50	0.20	1.00	520	3153
		75	0.12	0.54	1218	10062
Concepción	October 2010	0	11.8	3.80	nd	nd
		10	7.30	3.71	348	89.48
		25	4.80	2.23	nd	nd
	August 2014	0	0.26	0.18	nd	nd
		5	0.24	0.19	nd	nd
		10	0.38	0.30	nd	nd
		20	0.29	0.28	nd	nd
		30	0.17	0.13	nd	nd

The decrease of *GC* over time showed that complete evacuation times varied among species, from 0.5 h for *Oncaea* spp. and *C. patagoniensis* and 2 h for *A. tonsa*, *C. chilensis* and *P.* cf. *indicus*. From the decay gut pigment content over time were estimated evacuation and ingestion rates for all species (Table 3). Evacuation rates obtained in Mejillones Bay ranged between 1.16 and 4.70 h^{-1} both corresponding to *C. chilensis,* under day and night conditions, respectively. Evacuation rates estimated off Concepción were in the range of 0.41 and 4.38 h^{-1}, corresponding to *A. tonsa* and *P.* cf. *indicus*, respectively. No significant differences in evacuation rates were found between regions, seasons, day/night conditions or species (ANOVA, $P > 0.05$; Table 4).

The highest ingestion rate (Table 3) was for *P.* cf. *indicus* off Concepción in the spring, with 9.41 ng Chl-*a*

Table 2. Copepod size ranges (total length) and their mean carbon content from literature for the species under study. These estimates were used to relate copepod grazing with copepod sizes. Mean C content has been derived from length-weight regressions, usually using dry weight and then converted into C with the factor = 0.4.

Genera or species	Size range (mm)	Carbon content (µg C)	References
Calanus chilensis	3.10 - 3.22	58.06	Escribano *et al.* (1997)
Corycaeus spp.	1.20 - 0.70	0.728	Chisholm & Roff (1990)
Oncaea spp.	1.10 - 0.57	0.788	Chisholm & Roff (1990)
Acartia tonsa	1.34 - 0.77	5.320	Bergreen *et al.* (1988)
Paracalanus cf. *indicus*	1.40 - 0.74	1.196	Chisholm & Roff (1990)
Calanoides patagoniensis	2.75 - 2.08	58.06	Escribano *et al.* (1997)
Pleuromamma gracilis	2.55 - 1.50	4.70	Bennet & Hopkins (1989)
Rhyncalanus nasutus	6.10 - 2.82	33.30	Hopcroft *et al.* (2002)

Table 3. Estimates of grazing by copepod species sampled from two locations (Mejillones Bay and off Concepción) in the upwelling zone of Chile. Sampling was done in daytime and nighttime conditions. Grazing by copepods was estimated by the gut fluorescence method. GC: gut content, N: individual number, K: evacuation rate, I: ingestion rate, I´: C-specific ingestion rate, K´: C-specific evacuation rate, and DR: daily ration. Mean ± SD values are provided.

Location	Season	Species	GC ± SD (ng Chl-*a* ind^{1})	N	K (h^{-1})	I ± SD (ng Chl-*a* ind^{-1} h^{-1})	K specific (µg C^{-1} h^{-1})	I specific ± SD (ng C µg C^{-1} h^{-1})	DR ± SD (%)
Mejillones Bay	May 2010	*Calanus chilensis* (day)	0.16 ± 0.04	16	1.16	0.18 ± 0.05	0.02	0.14 ± 0.04	0.0002 ± 0.00
		Calanus chilensis (night)	0.90 ± 0.34	15	4.70	4.23 ± 1.62	0.08	3.28 ± 1.25	0.005 ± 0.00
		Corycaeus spp.	0.51 ± 0.25	20	1.52	0.78 ± 0.38	2.09	47.92 ± 23.76	6.58 ± 3.26
		Paracalanus cf. *indicus*	0.10 ± 0.01	20	2.37	0.24 ± 0.02	1.98	8.97 ± 0.62	0.75 ± 0.05
		Oncaea spp.	0.08 ± 0.00	20	2.09	0.17 ± 0.01	2.65	9.49 ± 0.37	1.20 ± 0.05
Concepción	October 2010	*Acartia tonsa*	1.92 ± 0.12	20	0.41	0.79 ± 0.05	0.08	6.69 ± 0.41	0.13 ± 0.01
		Paracalanus cf. *indicus*	2.15 ± 0.37	20	4.38	9.41 ± 1.63	3.67	353.97 ± 61.15	29.60 ± 5.11
	August 2014	*Calaniodes patagoniensis*	0.73 ± 0.68	10	2.34	1.71 ± 1.58	0.04	1.33 ± 1.22	0.002 ± 0.00
		Rhyncalanus nasutus	1.57	4	3.28	5.14	0.10	6.94	0.02
		Pleuromamma gracilis	3.15 ± 2.46	5	2.19	6.88 ± 5.37	1.16	164.61 ± 128.50	8.76 ± 6.83

ind^{-1} h^{-1}, and the lowest rate was for *Oncaea* spp. with 0.17 ng Chl-*a* ind^{-1} h^{-1} in Mejillones Bay during winter. The maximum daily ration was 29% for *P.* cf. *indicus* during spring and the minimum was 0.0002% for *C. chilensis* in Mejillones Bay during winter (Table 3). Significant differences in ingestion rates were found between regions (ANOVA $P < 0.05$). We did not find significant differences among species, or did not observe differences between day and night conditions, although for the latter there were too few data to make statistical comparisons (Table 4). In any case, the initial gut content of *C. chilensis* was ~4 times higher at night as that during the day, while in the first hour of incubation *C. chilensis* had evacuated 87% of the initial *GC* (Fig. 3).

C-Specific evacuation and specific ingestion rates tended to decrease with body mass of individuals (Fig. 4). *C. chilensis*, with a size of ~3.1 mm, had the lowest specific ingestion rate of 0.14 ng C x µg C^{-1} h^{-1} and *P. cf. indicus* in Concepción the highest with specific ingestion rate 353 ng C × µg C^{-1} h^{-1} (Table 3). C-specific rates and the carbon content of copepods was used to verify the relationship between the specific ingestion rates of copepods and body mass.

The results showed a strong relationship between the variables specific ingestion rate and specific evacuation rate (Regressions, $R^2 = 0.54$, $P < 0.001$; $R^2 = 0.86$, $P < 0.001$). We thus obtained the ng allometric model for herbivorous copepods:

$$I = 0.27BMc^{-0.89} \qquad (8)$$

where I is the specific ingestion rate and BMc is the mean biomass of the size class. Changes in size structure of the zooplankton community should be reflected in the slope of the normalized size spectrum. For example, dominance of small-sized classes has a spectrum with a steeper slope (>1.0), while a higher presence of larger-sized classes results in a less steep slope (<1.0). Fig. 5 shows the shape of zooplankton size spectra that depend on slope magnitudes (s = 0.5, s = 1.0, s = 2.0) for a size range of 0.5 to 74 µg C. Therefore, we can consider the slope of the size spectrum as a suitable index of the community size structure.

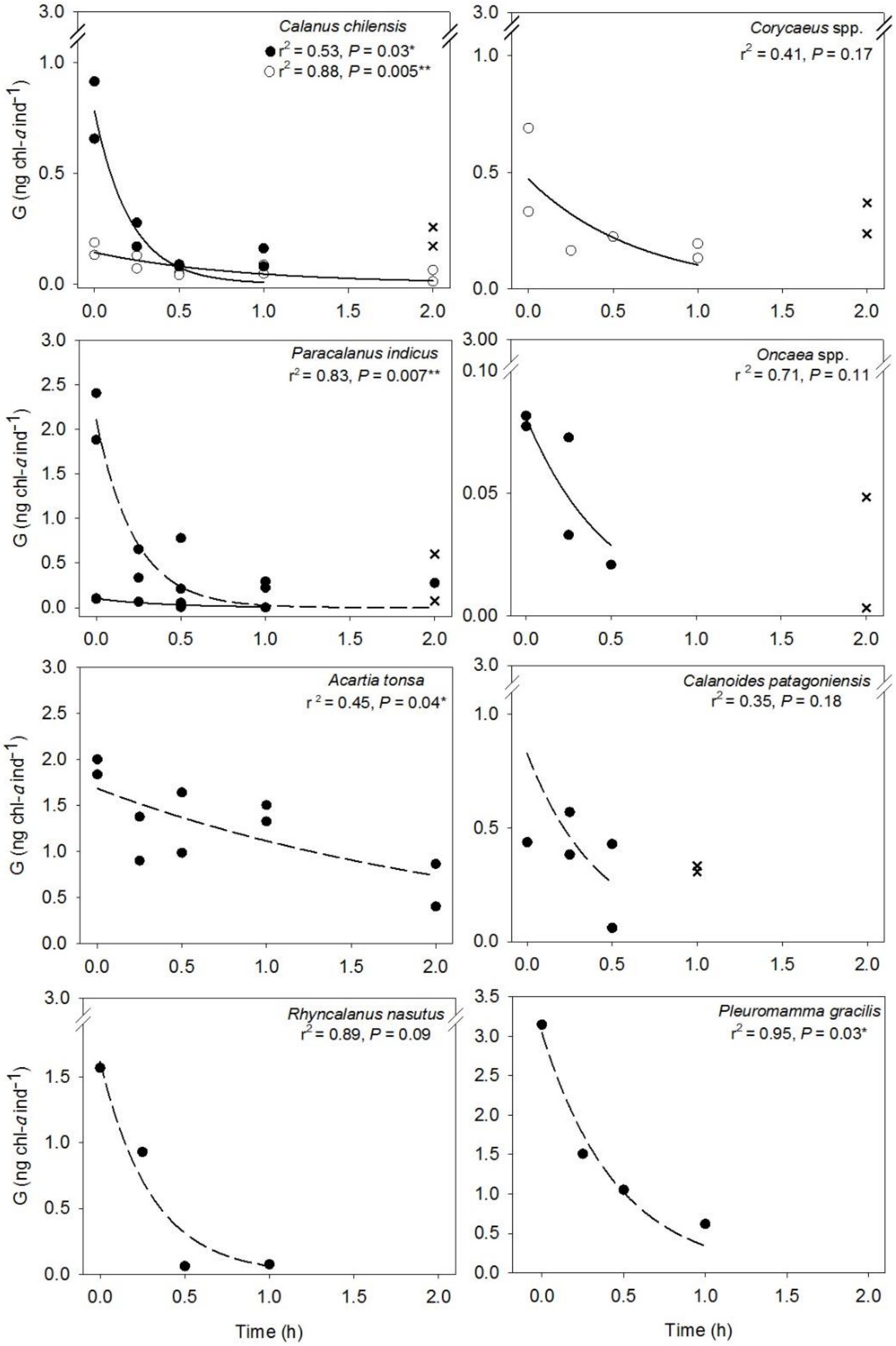

Figure 3. Gut content (GC) as a function of time for eight dominant copepods species from two upwelling sites off Chile. Data were fitted by non-linear exponential decay regressions. Filled circles represent estimates under daylight conditions and open circle in nighttime conditions, continuous and broken lines are fitted curves for Mejillones Bay and off Concepción, respectively.

Since grazing is size dependent in accordance with Eq. (8), changes in the slope of the size spectrum should reflect in the total grazing impact (GI) of the copepod community. However, application of Eq. (4) results in a gradual decrease in the total number and biomass of the community when the absolute value of the slope increases (more negative). For that reason, GI was standardized by total biomass (TB) to assess the effect of changing slope (s). Therefore, biomass-standardized GI represents an index of copepod grazing efficiency. After application of Eq. (7), the changes in total biomass and GI as a function of s are shown in Fig. 6.

An initial, perfectly uniform distribution of copepod size classes (s = 0) results in a very low GI with a high

Table 4. ANOVA to test the effect of location, light condition and species on the evacuation rate and ingestion rate of dominant copepods species from two locations (Mejillones Bay and off Concepción) in the coastal upwelling region of Chile. Data were log-normalized prior to ANOVA application. df: degrees of freedom, F: F-ratio, *P*: probability, *Significant effect.

		Evacuation rate		Ingestion rate	
Source of variation	df	F	*P*	F	*P*
Location	1	0.016	0.902	10.603	0.004*
Season	1	0.840	0.384	1.837	0.193
Day/night	1	0.914	0.367	3.022	0.100
Species	8	2.870	0.429	2.027	0.446

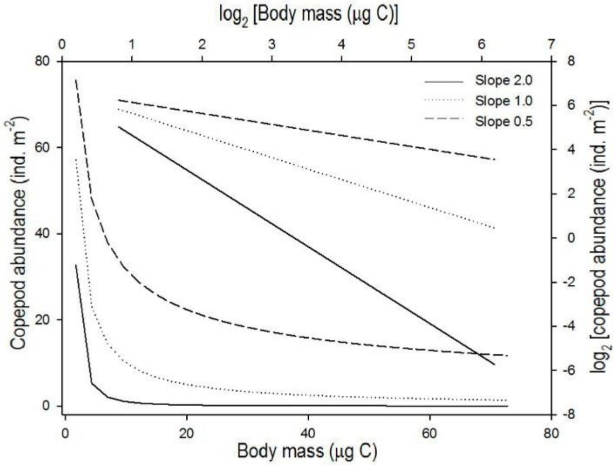

Figure 5. Simulated non-normalized and normalized size spectra of the copepod community from the coastal upwelling zone off Chile with three different slopes to assess the effect of changing the proportion of size classes (species). Slopes were in the range of values obtained in previous studies from the same region (Manríquez *et al*., 2009).

biomass. This hypothetical condition requires the presence and high abundance of large-sized grazers. In contrast, a system dominated by small size classes (s > 1.0) implies a high C-specific GI. This positive relationship between s and GI indicates a greater (more efficient) phytoplankton C flux through the food web in systems dominated by small-sized organisms.

From the time series data at Sta.18, the estimated slope of the copepod size spectrum ranged between -2.04 and 0.08, with a mean of -0.88. Monthly variation shows a clear pattern between upwelling *vs* non-upwelling periods, characterized by highly negative values during non-upwelling conditions (April to August) *vs* more positive values in the upwelling season (September to March). This seasonal pattern basically reveals the predominance of small sized classes during the autumn-winter season. There was also a negative trend in the slope through the years (Fig. 7).

The highly significant relationship between grazing efficiency (C-specific grazing) and the slope of the size spectra is shown in Fig. 8a, clearly indicating that copepods can graze more efficiently when their community is dominated by small-sized species. Meantime, total grazing impact of the phytoplankton biomass did not show such relationship (Fig. 8b), and the relationship between the fraction of phytoplankton C being removed by copepod grazing and the slope of the size spectrum exhibited much scattering, but clearly high levels of C removal are only achieved when this slope reached strongly negative values (Fig. 8c). The variability in the relationship between the slope of the

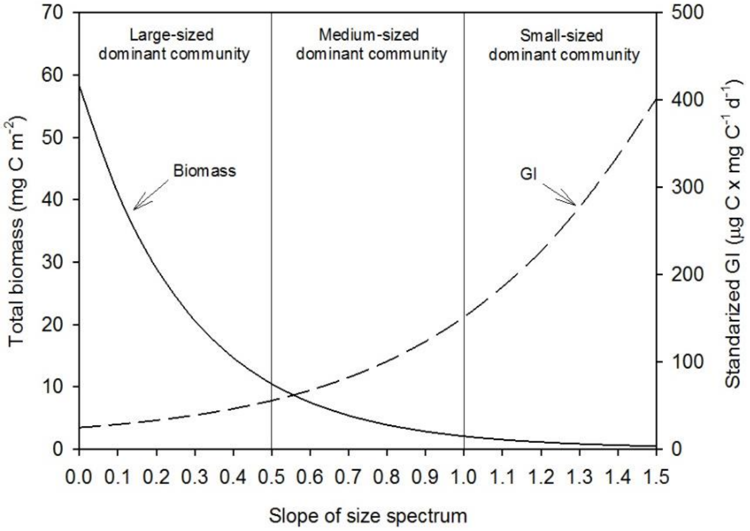

Figure 6. The relationship between the slope of the size spectrum, biomass of the copepod community, and biomass-standardized grazing impact of copepods on phytoplankton as derived from simulated size spectra, and assuming a size-dependent grazing rate of copepods. The importance of different copepod communities dominated by small, medium, or large copepods on grazing impact (GI) is illustrated.

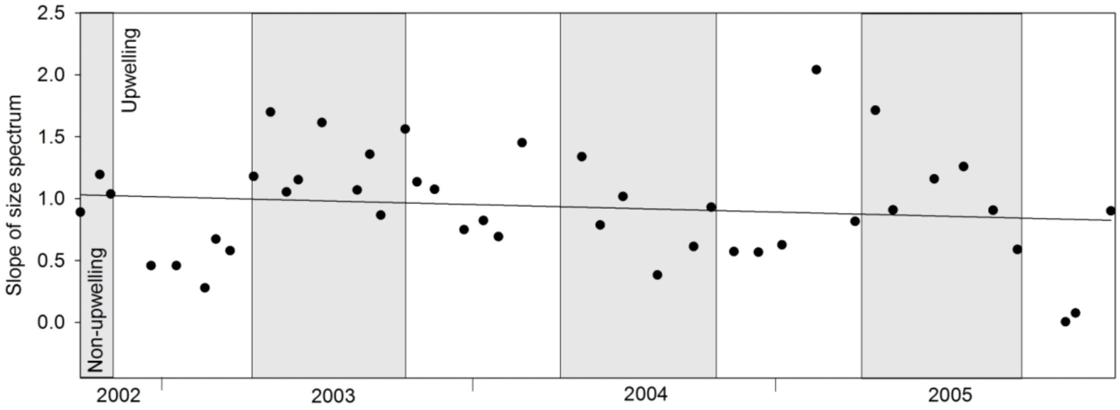

Figure 7. Monthly variation in the slope of the normalized size spectra of the copepod community at Station 18 in the coastal upwelling system off central/southern Chile between August 2002 and December 2005. Absolute values of the slope are plotted such that greater values illustrate more negative slopes. Shaded areas illustrate non-upwelling (April to August) conditions. The continuous line shows a significant negative trend ($P < 0.05$) through years.

copepod size spectra may be associated with a strong effect caused by seasonal changes in phytoplankton C. Phytoplankton peaks during the upwelling season when copepods exhibit a flatter slope of their size spectra (Fig. 7), and become depleted during non-upwelling upon dominance of small-sized classes (more negative slopes).

Therefore, a greater grazing impact would take place during non-upwelling (low phytoplankton) compared to a condition of active upwelling (high phytoplankton). This seasonal pattern of grazing impact is illustrated in Fig. 9.

DISCUSSION

This analysis is based on empirical results from estimates of copepod feeding using the gut fluorescence method. This approach to assessing copepod grazing has been widely applied in a variety of environments and conditions, despite criticism related to invalid assumptions (Båmstedt *et al.*, 2000). A reassessment of the method by Durbin & Campbell (2007) demonstrated that assumptions could be sustained. For instance, it was early suggested that some Chl-*a* pigment could be lost from the gut after degradation, affecting estimates of gut content. However, it was shown that such potential loss is not significant, and corrections are inappropriate (Durbin & Campbell, 2007). Therefore, this method has continued to be a highly valuable tool for studies on zooplankton feeding (Saiz & Calbet, 2011). Several studies based on the application of the gut fluorescence method have substantially contributed to understanding the role of zooplankton feeding for C flux and trophodynamics in various marine ecosystems (Calbet, 2001; Fielding *et al.*, 2007; Vargas *et al.*, 2007; Saiz & Calbet, 2011).

Our gut-content measurements reflect short-term (<24 h) *in situ* feeding and cannot tell us about diurnal feeding rhythms, as demonstrated by Head & Harris (1987). Therefore, scaling ingestion rates (I) from h to days can yield questionable results. Indeed, the very high I of *Paracalanus* cf. *indicus* (Table 3) scaled to days suggests a daily ingestion of about 8 times its body C without considering a potential diurnal feeding rhythm. However, we still decided to assess GI on a daily basis to make our study applicable to estimates of phytoplankton production and biomass standing stock, usually integrated over 24 h. On the other hand, diurnal rhythms may not be so important in these systems given that copepods are aggregated in the upper layer with restricted diurnal migration owing to the shallowness of the oxygen minimum zone in this region (Escribano *et al.*, 2009), and also given that we did not observe day-night differences in evacuation and ingestion rates.

We assessed the feeding of copepod species during particular conditions in both upwelling sites. However, these species represent the dominant copepod community in the Chilean upwelling zone, both in the northern and central/southern regions. At Mejillones Bay, the small *Paracalanus* cf. *indicus* dominate most of the year and numerically represents up to 80% of the copepod community (Hidalgo *et al*., 2010). At times of the year, *A. tonsa* can also account for more than 80% of the community (Escribano *et al*., 2012). The cyclopoid *Corycaeus* spp. and *Oncaea* spp. are two of several small-sized species always present in high abundance in northern Chile (Hidalgo *et al*., 2010). Larger-sized copepods are less abundant and in

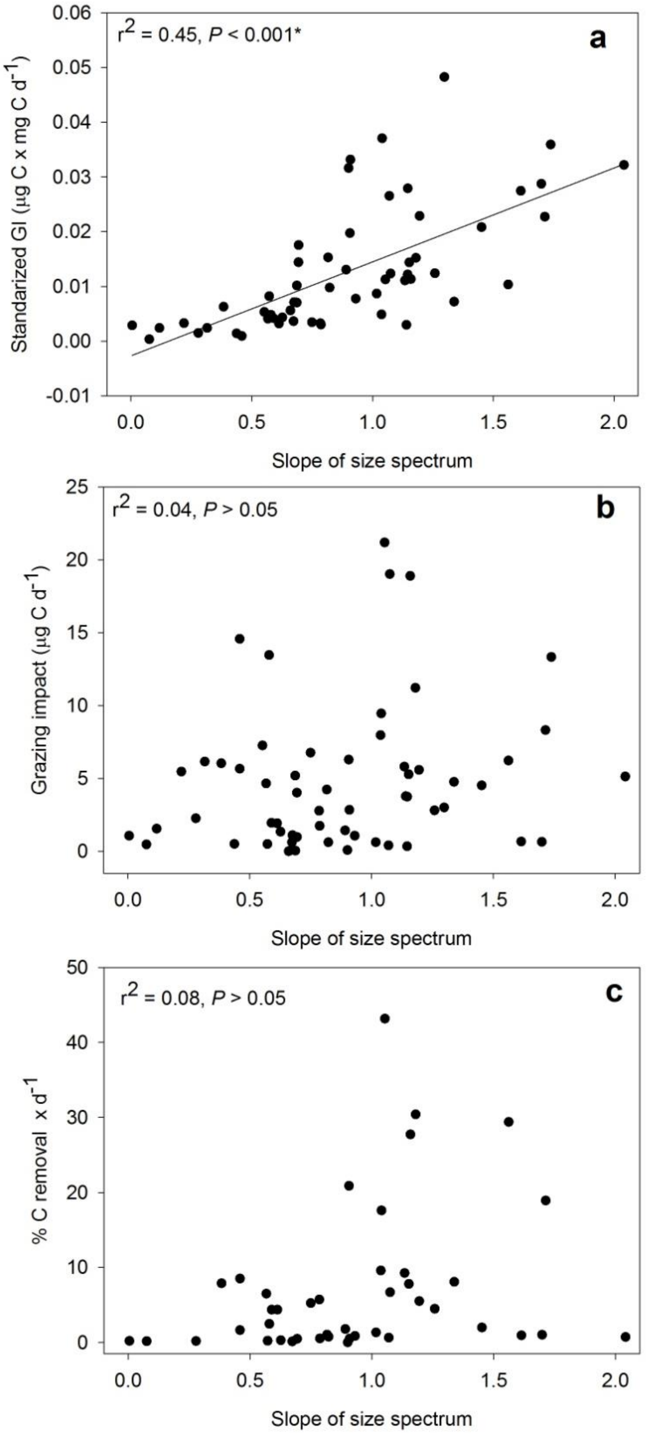

Figure 8. The relationship between the slope of the normalized size spectra of the copepod community at Station 18 in the coastal upwelling system off central/southern Chile between August 2002 and December 2005 and copepod grazing on phytoplankton biomass on a daily basis. a) the relationship between the slope and C-specific grazing impact (standardized GI), b) The relationship between the slope and total daily grazing impact, and c) the relationship between the slope and the daily percentage of phytoplankton C removal via copepod grazing.

Mejillones Bay *C. chilensis* become abundant occasionally during the annual cycle (Escribano *et al*., 1997), whereas other large calanoids, such as *Eucalanus* spp. and *R. nasutus* are usually distributed offshore or in deeper water (Hidalgo *et al*., 2010). A similar copepod community is found off Concepción and again *P.* cf.

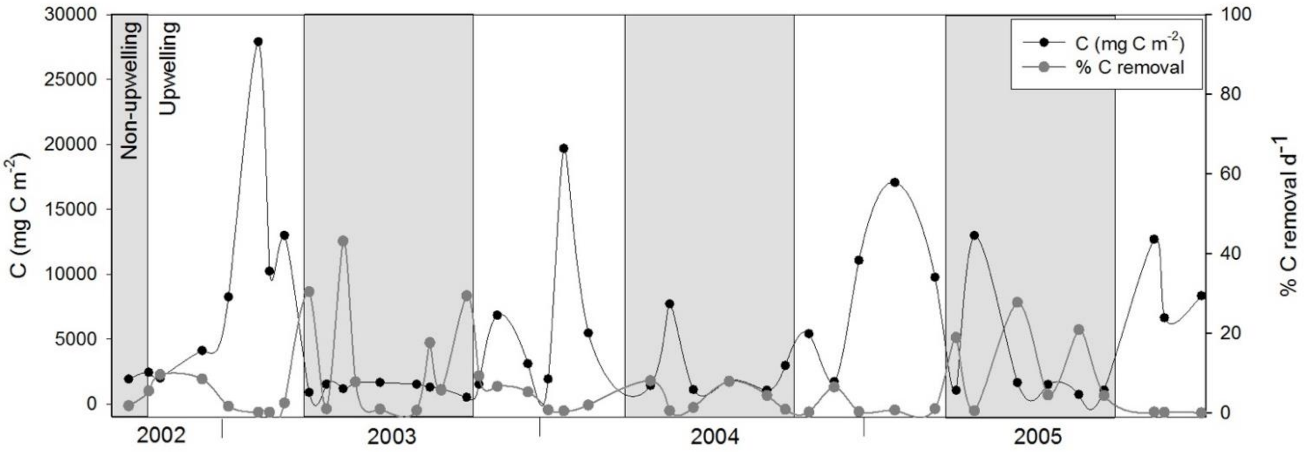

Figure 9. Monthly variation in total phytoplankton C at Station 18 in the coastal upwelling system off central/southern Chile between August 2002 and December 2005, and the percentage of daily phytoplankton C removal via copepod grazing. Shaded areas illustrate non-upwelling conditions (April to August).

indicus is often the dominant small-sized species (Escribano *et al*., 2007), although at times the cyclopoid *Oithona similis* (not found in our study) is highly abundant (Hidalgo *et al*., 2010). In this area, larger-sized copepods are represented by *C. patagoniensis* and *R. nasutus*, although they become abundant on very few occasions during the year and usually by early spring (Escribano *et al*., 2007).

Our results make clear the strong differences in feeding rates among species, although most of the variance is well explained by copepod size. Body size has been considered an important factor influencing copepod feeding (Peter & Downing, 1984; Saiz & Calbet, 2007). There are also theoretical and ecological bases to explain size effects on physiological rates of organisms in nature (Peters, 1983; Platt, 1985; Fenchel, 1988). Thus, scaling copepod feeding could be a useful approach to identify general patterns of copepod grazing (Saiz & Calbet, 2011). Our findings indeed reflect the size-dependence of both evacuation and ingestion rates of pigments, and such dependence may go beyond species-dependent attributes, at least in the dominant copepod community in our study region.

There is an interesting relationship between the impact of grazing and the size spectrum slope. For our simulated copepod size spectrum, both numerical copepod abundance and biomass were used to distribute the size classes. Size distribution follows a decreasing logarithmic function that may not be adequately represented by the mean biomass of each size class (Savage, 2004). This potential bias however does not affect the function for narrow size intervals and a limited size range of the spectrum, as we used in our study. The effect of arbitrarily assigning size class abundances may be more critical, since this has a direct effect on the slope. In this respect, our analysis with the natural community of copepods using the zooplankton time series from the upwelling zone, clearly showed that such size classes can indeed well represent a natural community, and most important, that the slope of the size spectrum indeed strongly relates to copepod grazing. This analysis also showed that seasonal effects are important to determine grazing impact. Such seasonal variation takes place after shifts of copepod feeding to a more heterotrophic diet during conditions of low Chl-*a* (Vargas *et al*., 2006). Thus, transfer of phytoplankton through the pelagic food web via copepod grazing is not only more efficient during low Chl-*a* (autumn-winter), but it can also transfer more C through a heterotrophic pathway.

Our study suggests that zooplankton communities dominated by small-sized copepods exert a greater impact on phytoplankton than those dominated by larger-sized copepods (slope <1.0). Small copepods in the upwelling zone are mainly omnivorous, although as stated above, they can switch their diet to diatoms when the latter are abundant (Kleppel, 1993; Vargas *et al*., 2006), whereas large calanoid copepods prefer a more herbivore diet (diatoms) (Vargas *et al*., 2007). Therefore, phytoplankton C can efficiently be transferred via grazing in highly productive upwelling systems, when small copepods are dominant, as in the coastal upwelling zone off Chile.

The effect on grazing of changing community size-structure may have further implications. It has been reported that upwelling intensity has increased in the last two to three decades due to stronger southerly winds in the coastal upwelling zone off Chile (Garreaud & Falvey, 2008). Increased upwelling may substantially modify the physical and chemical environment in the coastal zone by expanding the minimum oxygen zone, lowering temperature, altering water column stratification, increasing CO_2 outgassing and lowering pH (Bakun *et al*., 2010). Zooplankton composition can also change with climate-driven oceanographic variation. Species and size structure have indeed been subject to significant changes in the upwelling zone off Concepción in the last decade (Pino-Pinuer *et al*., 2014). The copepod community and copepod size structure (Ulloa *et al*., 2001) has also changed significantly owing to conditions related to the 1997-1998 El Niño phenomenon (Hidalgo & Escribano, 2001). Changes in the size structure of the zooplankton community, caused by ongoing climate change and other processes, are of crucial importance for phytoplankton C-flux and hence for the production of higher trophic levels, as suggested by our findings.

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