



## Mesozooplankton (Crustacea: Copepoda and Cladocera) dynamics in a large, ultra-oligotrophic Andean lake

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**Abstract:** We studied mesozooplankton dynamics in the Tristeza arm of lake Nahuel Huapi. Mesozooplankton biomass, size and vertical distribution showed marked seasonal fluctuations. Mesozooplankton was represented by seven species. We document for the first time the existence of *Daphnia* cf. *pulex* in this lake.

**Keywords:** deep lakes, Nahuel Huapi, *Daphnia*, pristine, Patagonia

**Resumen:** Dinámica del mesozooplancton (Crustacea: Copepoda and Cladocera) en un gran lago ultra-oligotrófico de los Andes. Estudiamos la dinámica del mesozooplancton en el Brazo Tristeza del lago Nahuel Huapi. La biomasa, la talla y la distribución vertical del mesozooplancton fluctuaron de manera estacional. El mesozooplancton estuvo representado por 7 especies. Documentamos por primera vez la presencia de *Daphnia* cf. *pulex* en este lago.

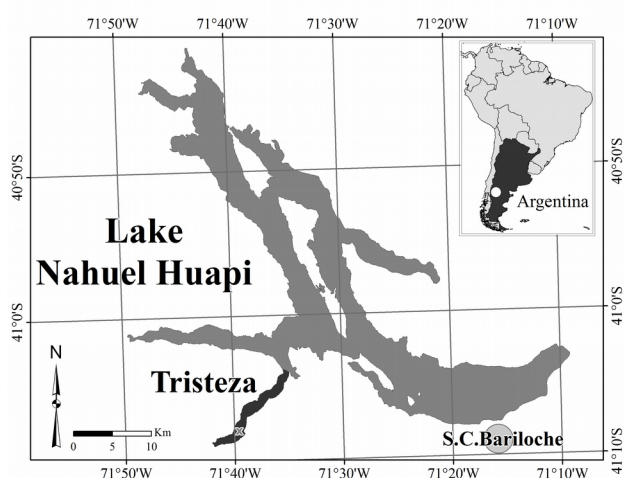
**Palabras clave:** lagos profundos, Nahuel Huapi, *Daphnia*, pristino, Patagonia.

The mesozooplankton community comprises copepods and cladocerans ranging in size between 0.2 mm and 5.0 mm. Its structure varies both in time and space. Mesozooplankton is dominated by small calanoid copepods of the genus *Boeckella* in deep Andean lakes. Small cladoceran species (*Bosmina longirostris*, *B. chilensis* and *Ceriodaphnia* cf. *dubia*) are also present (Modenutti *et al.* 2010), whereas large cladocerans (i.e. *Daphnia commutata*) (Marinone *et al.* 2006), the calanoid *Parabroteas sarsi* (Reissig *et al.* 2004), and cyclopoid copepods (Modenutti *et al.* 1998) are usually scarce in these lakes. In relation to its predators, *Galaxias maculatus* is the main planktivore in these lakes, its life cycle involves ontogenetic migrations from the littoral to the pelagic zone. *Galaxias maculatus* spawns in the littoral zone from early spring to early

summer (Barriga *et al.* 2002). Its larval stage migrates to the pelagic zone and feeds on zooplankton in summer (Barriga *et al.* 2012), and at the end of this larval period, mature individuals migrate from the pelagic to the littoral zone of lakes, changing their spatial and trophic niches (Cervellini *et al.* 1993). The objective of this work was to describe the mesozooplankton community dynamics in the Tristeza arm of lake Nahuel Huapi. Moreover, we report, for the first time, the presence of *Daphnia* cf. *pulex* in this lake.

Lake Nahuel Huapi is Northern Patagonia's largest natural lake with a surface area of 557 km<sup>2</sup> and maximum depth of 464 m (Quirós 1988). The basin includes seven branching arms with 357 km of shoreline. This lake can be classified as ultra-oligotrophic to oligotrophic, with mean total

phosphorus concentrations of  $3.8 \mu\text{g L}^{-1}$ , mean chlorophyll-a concentrations of  $0.41 \mu\text{g L}^{-1}$  and a summer stratification period (Baigún & Marinone 1995). This study was carried out in Tristeza arm (Fig. 1), which belongs to the less studied western zone of the lake. It was sampled once per season from winter 2007 to autumn 2008 between 13:00 and 16:00 h. We selected one offshore site (depth > 100 m) and one near-shore site (ca. 35 m depth). At the offshore station we measured temperature profiles, except in summer when we could measure only deep and surface temperatures. We collected mesozooplankton samples by vertical tows using a closing-conical net (105  $\mu\text{m}$  mesh). Stratified samples of 10-m strata were taken down to 70 m at the offshore station, and down to 30 m at the near-shore station. One sample was taken per stratum, and all samples were preserved in 4 % sucrose-formalin. Horizontal trawls were taken with an ichthyoplankton net (380  $\mu\text{m}$  mesh) in order to detect presence/absence of galaxiid larvae. These trawls followed a straight line in a pelagic area between the near-shore and offshore sites and were collected at 20-30 m depth.



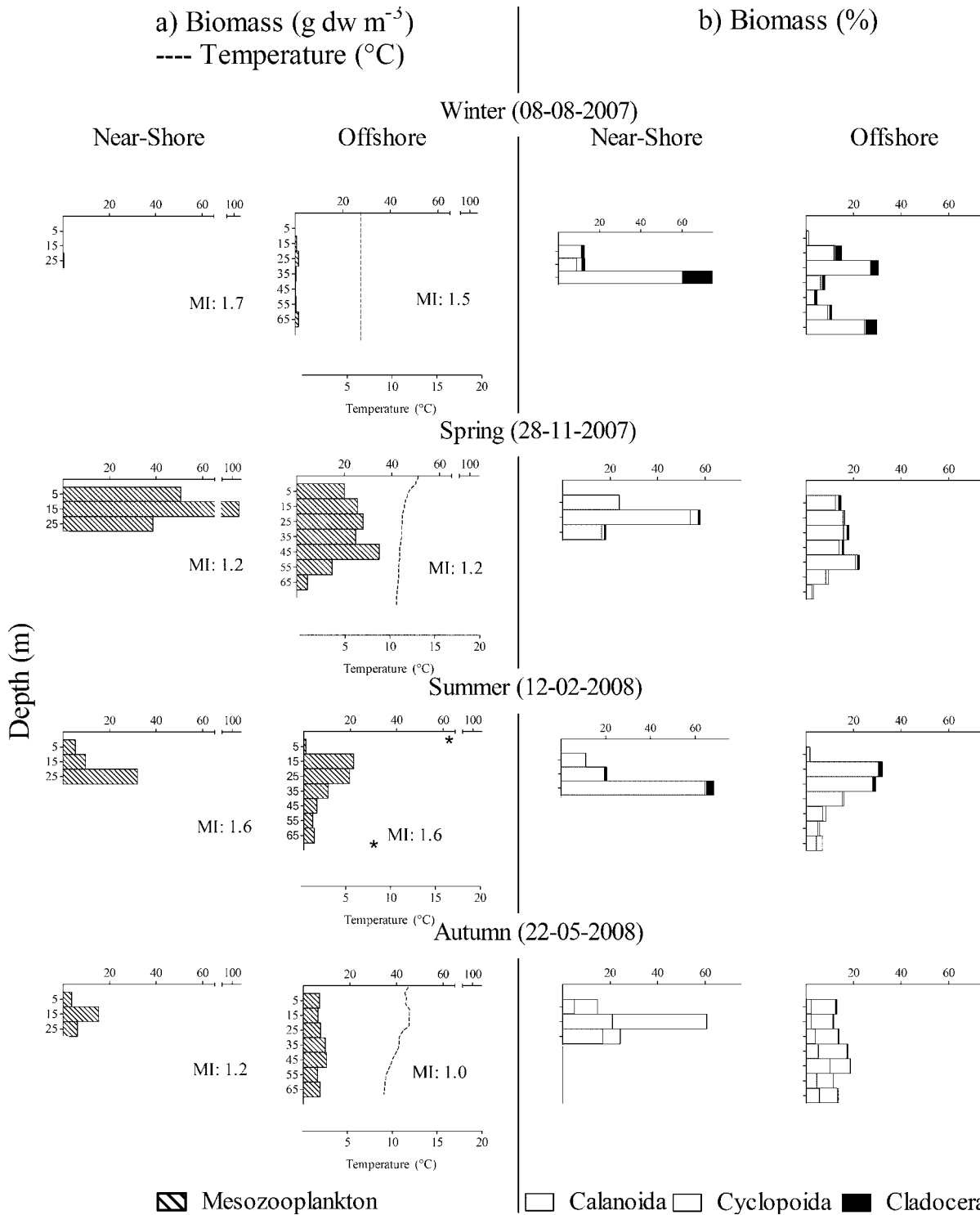
**Figure 1:** Lake Nahuel Huapi: the study arm is highlighted in grey and x represents the location of the survey. San Carlos de Bariloche (S.C. Bariloche) city is shown.

We counted mesozooplankton under a dissecting microscope in a 5 ml Bogorov chamber. Mesozooplanktonic organisms were identified at species level. Copepods included both adults and copepodites. Taxonomic identifications followed Reid (1985) and Bayly (1992) for copepods; Paggi (1979, 1995), and Benzie (2005) for cladocerans. In order to estimate the mesozooplankton biomass we measured the lengths of at least 100 organisms (or

entire samples) from each taxonomical group (Calanoida, Cyclopoida and Cladocera) in each sample site. Dry weight was calculated using standard length-weight regressions (Dumont *et al.* 1975, Bottrell *et al.* 1976). As a measure of mesozooplankton vertical distribution we calculated the Morisita Index (MI) (Hurlbert 1990). Finally, in order to evaluate the homogeneity of mesozooplankton taxonomical groups (and homogeneity within these groups) in relation to time, Chi-square tests of Homogeneity were performed. Temporal and spatial differences in mesozooplankton size were analyzed by means of a two-way with interaction linear model with heterogeneous variance (Verbeke & Molenberghs 2000) with main effects of season (4 levels: winter, spring, summer and autumn) and site (2 levels: near-shore and offshore). Heterogeneity of variance was modeled by setting one error parameter to each of the eight levels of the interaction. Data were first transformed to attain normality using Box-Cox transformations, as discussed by (Peltier *et al.* 1998). The model was fitted using PROC MIXED (SAS Institute Inc. 2013) with option UN(1), i.e. a diagonal covariance matrix of error terms with heteroscedastic interaction levels. Therefore, all test of hypothesis had their error degrees of freedom corrected by the procedure suggested by (Kenward & Roger 1997). All contrasts among levels within the significant interaction were tested by using Tukey's test.

The thermal regime was typical of warm monomictic lakes, with complete mixing from autumn to spring, a substantial increase in the temperature of surface layers during the summer, and lowest temperatures during the winter (Fig. 2a). Mesozooplankton stratification was associated with summer, when the Morisita Index reached high values (Fig. 2a). Worthy of note is the depth at which cyclopoid copepods were found during this season (Fig. 2b), 61% of their biomass being found at depths greater than 50 m. Considering adult cyclopoid copepods alone, this percentage reached 80%. Trawling for ichthyoplankton produced positive results during this season alone. In winter mesozooplankton abundance in many of the sampled strata was practically null. This led to relatively high values of the Morisita index, but did not imply a marked stratification pattern.

Of the total mesozooplankton biomass, 85% consisted of calanoids throughout the year, while cyclopoids accounted for approximately 13% of the total. Cladoceran biomass was very low in



**Figure 2:** (a) Mesozooplankton biomass distribution and temperature profiles during the study period, with sample dates shown between brackets. The biomass x-axis is divided into 2 parts and the temperature x-axis lies below the corresponding diagram. In summer the deep and surface water temperatures are shown (\*). MI: Morisita index. (b) Mesozooplankton biomass proportion.

comparison with that of copepods. The mesozooplankton were represented by seven species: four cladocerans, (*Daphnia* cf. *pulex* Leydig, 1860; *Ceriodaphnia* cf. *dubia* Richard, 1894; *Bosmina chilensis* Daday, 1902 and *Bosmina*

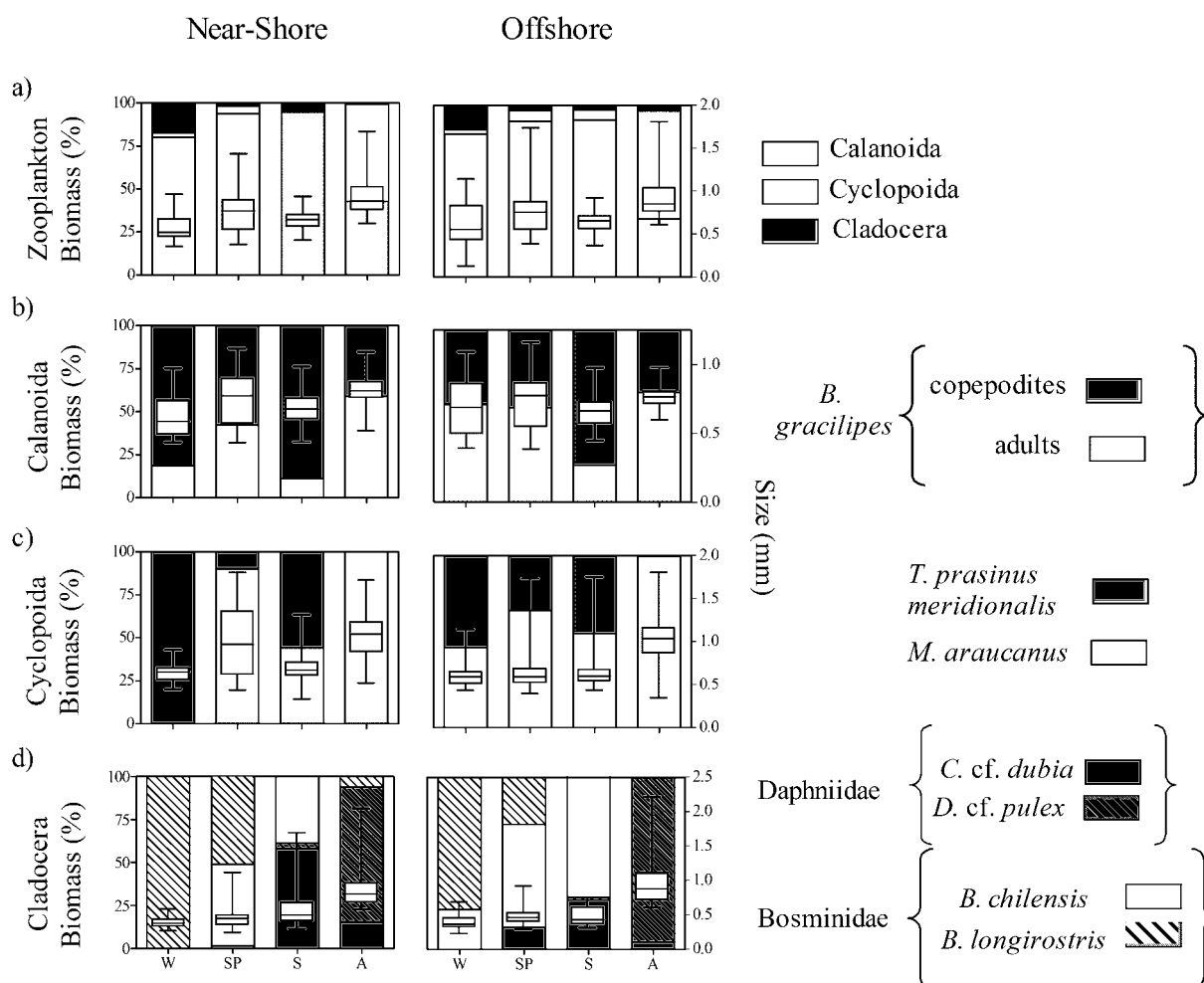
*longirostris* (O.F.Müller, 1776)), two cyclopoids, (*Tropocyclops prasinus meridionalis* (Kiefer, 1931) and *Mesocyclops araucanus* Löffler, 1962) and a calanoid (*Boeckella gracilipes* Daday, 1901). Mesozooplankton biomass varied widely between

seasons, showing a prominent peak in spring and the lowest values in winter (Fig. 2a). Differences were also observed in the proportions of Cladocera, Calanoida and Cyclopoida during the year (Fig. 3a). The community was composed mainly of *B. gracilipes*, except during autumn, when the biomass consisted mostly of *M. araucanus*.

The size and proportion of the different species or stages within each group showed seasonal variations (Fig. 3). This was observed at both near-shore and offshore sites. The Season X Site interaction was highly significant in all 3 ANOVA tests (Calanoida  $F = 7.18$ ,  $P < 0.001$ , d.f. = 3; Cyclopoida  $F = 7.27$ ,  $P < 0.001$ , d.f. = 3; Cladocera  $F = 6.73$ ,  $P < 0.001$ , d.f. = 3). Size differences within any group were generally more marked in

relation to time than to space. The largest sizes of cyclopoid copepods were found in autumn (Fig. 3c, Table I). In accordance with this, *M. Araucanus* made up 100% of the biomass. In summer and winter the smallest cyclopoid sizes were observed (Fig. 3c, Table I), with *T. prasinus meridionalis* making up most of the biomass. As observed with the cyclopoids, the cladocerans presented the largest sizes in autumn (Fig. 3d, Table II). During this season *D. cf. pulex* clearly dominated. In contrast, the smallest sizes were observed during winter (Fig. 3d, Table II) and the cladoceran biomass belonged exclusively to organisms of the Bosminidae family.

This is the first seasonal study of the mesozooplanktonic community in lake Nahuel



**Figure 3:** Mesozooplankton biomass proportion. Box-plots indicate mesozooplankton size. The y-axes on the right are on different scales. W, Winter, SP, Spring, S, Summer and A, Autumn. The mesozooplanktonic community is not seasonally homogeneous with respect to the proportions of: a) taxonomical groups, b) stages, c) and d) species composition. In all cases for Chi-square test of Homogeneity,  $P < 0.05$ .

**Table I:** Summary of statistical tests displaying the effects of the interaction between Season and Site on Cyclopoida size. NS indicates non significance at the 0.05 level. W, Winter, SP, Spring, S, Summer and A, Autumn.

Site	Season	df	t-value	p-value	Site	Season	df	t-value	p-value
Near-Shore	W - SP	35	-4.80	$P < 0.001$	Offshore	W - SP	40.4	-1.48	NS
	W - S	21.5	-1.20	NS		W - S	29.8	-1.47	NS
	W - A	21.2	-8.16	$P < 0.001$		W - A	28.1	-9.81	$P < 0.001$
	SP - S	148	5.92	$P < 0.001$		SP - S	180	0.19	NS
	SP - A	146	-3.71	$P < 0.01$		SP - A	171	-11.15	$P < 0.001$
	S - A	193	-14.85	$P < 0.001$		S - A	196	-14.04	$P < 0.001$

**Table II:** Summary of statistical tests displaying the effects of the interaction between Season and Site on Cladocera size. NS indicates non significance at the 0.05 level. W, Winter, SP, Spring, S, Summer and A, Autumn.

Site	Season	df	t-value	p-value	Site	Season	df	t-value	p-value
Near-Shore	W - SP	78.5	-3.25	$P < 0.05$	Offshore	W - SP	197	-6.37	$P < 0.001$
	W - S	91.2	-7.56	$P < 0.001$		W - S	199	-5.58	$P < 0.001$
	W - A	55.2	-17.95	$P < 0.001$		W - A	174	-23.92	$P < 0.001$
	SP - S	194	-5.27	$P < 0.001$		SP - S	197	0.50	NS
	SP - A	167	-18.19	$P < 0.001$		SP - A	178	-17.62	$P < 0.001$
	S - A	155	-10.33	$P < 0.001$		S - A	174	-17.10	$P < 0.001$

Huapi. The near-shore and offshore sites were relatively homogeneous in terms of composition and size of the different taxonomical groups, whereas on a temporal scale greater heterogeneity was observed at each site. The mesozooplankton community was dominated by *B. gracilipes*, the most important zooplankton in terms of density and frequency of occurrence in Araucanian lakes on both sides of the Andes (Modenutti *et al.* 1998). Cyclopod copepods were represented by two species: a large one, *M. araucanus*, and a small one, *T. prasinus meridionalis*. In autumn *M. araucanus* dominated the mesozooplankton community in terms of biomass. Similarly to cyclopoid copepods, we can assign the herbivorous zooplankton found to two groups, according to size. In this way we can distinguish between the bosminids which dominated during winter and spring, and the daphnids which dominated during autumn.

The presence of *Daphnia* in Tristeza arm is a novel finding. According to the key by Benzie (2005), the studied *Daphnia* belong to the "pulex group", and were determined as *D. cf. pulex* since they present "surface reticulation between the

ocellus and the ventral margin of the rostrum with polygons whose length and width are similar". Some time ago, Balseiro *et al.* (2007) did not find *Daphnia* in the eastern zone of lake Nahuel Huapi. These authors suggested the unfavorable stoichiometric ratios of the seston and fish predation as the reason for the absence of this keystone genus. Arcagni *et al.* (2015) have recently mentioned the presence of *Daphnia sp.* in Rincón arm. This arm is located on the north-western zone the lake. The *Daphnia* species found is probably the same as the one we found in Tristeza arm. However, the true identity of this *Daphnia* remains problematic since very similar polyploid populations of hybrid origin (probably *D. pulex x D. pulicaria*) have been reported in southern Patagonia (Adamowicz *et al.* 2004). Thus, the next step should be the confirmation of this species by molecular studies.

The distribution of mesozooplankton was stratified in the water column during summer. For most zooplankton species, patterns of dispersion are affected by the onset and breakdown of thermal stratification in a lake. The degree of aggregation in the vertical plane increased when the lake became

thermally stratified (Thackeray *et al.* 2006). As long ago as 1961 (Löffler), studies carried out to the west of the Andes indicated the presence of copepods (adults of *T. prasinus meridionalis* and *M. araucanus*, and copepodites of Cyclopoida and Calanoida) at depths below 50 m. Paradoxically, only one study on planktonic communities to the east of the Andes included sampling of deep lake strata (Queimaliños *et al.* 2012). The presence of mesozooplankton in deep layers during the day is associated with predation (Reissig *et al.* 2004) or with the intensity of UV radiation (Alonso *et al.* 2004). For whatever reason, the presence of *T. prasinus meridionalis* and *M. araucanus* in such deep strata of the water column is compatible with the high level of transparency of lake Nahuel Huapi. Thus, in future research studies, samples should be taken below 50 m depth, since in summer, 80% of the adult cyclopoid copepods were located beneath this depth.

Bottom-up and Top-down effects may shape the mesozooplankton community dynamic in the pelagic zone of lake Nahuel Huapi. Zooplankton size presented a bimodal distribution; small in summer and winter and larger in spring and autumn. Food limitation and low temperatures are likely to depress zooplankton size and biomass during winter. The spring peak in zooplankton biomass probably indicates favorable conditions for this community. For instance, the increase in temperature is reflected by an increase in the abundance of nanoplanktonic flagellated cells such as *Crysochromulina parva* Lackey (Díaz *et al.* 1998). Furthermore, horizontal migration by fish may shape the size of the mesozooplanktonic community. The ontogenetic migration pattern of *G. maculatus* (Barriga *et al.* 2002) coincides with our data on presence/absence of galaxiid larvae and the dynamics of zooplankton size. The presence of *G. maculatus* larvae in the pelagic zone of the lake could account for the decrease in zooplankton size in summer, whereas their return as mature individuals to the littoral zone of the lake may explain the increase in zooplankton size in the pelagic zone in autumn.

This study highlights the heterogeneity of the mesozooplanktonic community in lake Nahuel Huapi. Moreover, the presence of *Daphnia cf. pulex*, a large phytoplankton grazer, adds a new player to the food web of this ultra-oligotrophic lake. However, knowledge of mesozooplankton ecology and its relationship with time and space heterogeneity is still incomplete in the lake, e.g., the deep vertical distribution of cyclopoid copepods in

summer and the high proportion of *Mesocyclops* biomass in autumn deserve further research.

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