

# Prey C:P ratio and phosphorus recycling by a planktivorous fish: advantages of fish selection towards pelagic cladocerans

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**Abstract** – Prey selection by the small planktivore *Galaxias maculatus* and phosphorus gain depending on diet has been examined related to predator–prey daily distribution. Prey elemental composition (C:P ratio) was analysed as a factor of selectivity. Field experiments were carried out to evaluate the effect of prey on phosphorus recycling. Results revealed that the pelagic cladoceran *Ceriodaphnia dubia* was a highly selected prey by all fish sizes at dawn and dusk, despite its low abundances in comparison with other zooplankters, while the littoral were ingested only during day. The high selectivity for *C. dubia* showed that *G. maculatus* moved to pelagic zone during night to consume this prey but returned to littoral areas during day due to the increase in predation risk. Analysis of elemental composition revealed that P content of *C. dubia* was significantly higher than other planktonic or littoral prey (*Bosmina longirostris*, *Boeckella gracilipes* and chironomids). Phosphorus recycling experiments showed that *G. maculatus* would transport and supply P in available forms for primary producers. Fish size was observed to influence P recycling as YOY had significant higher mass-specific P release in the morning and mid-day when they preyed mainly upon *C. dubia*. Low prey C:P ratio appears as a factor that would add benefits to the classical visual fish selection towards cladocerans, as this preference would imply a net phosphorus gain for fish.

**Key words:** C:P ratio; nutrient recycling; nutritional requirements; elemental composition; food selection

## Introduction

Phosphorus (P) is an essential element for all living organisms, and, in particular, it is necessary for making up the structure of bones and teeth in vertebrates due to the deposition of calcium phosphate in skeletons (Sterner & Elser 2002). In addition to the internal skeleton present in all vertebrates, fishes also possess calcified scales, with high P content, that contribute to stoichiometry of the organisms (Hendrixson et al. 2007). Therefore, fishes will sequester relatively more P in their bodies than invertebrates (Vanni et al. 2002) and will concentrate P within their biomass compared with what they eat (Elser et al. 1996; Sterner & George 2000). Fishes can be sources or sinks of phosphorus at the ecosystem (lake) level and at the habitat level

(benthic and water column habitats), depending on fish feeding habits (proportion of P consumed from the benthos and from the water column), migration patterns and especially the fate of carcass P (Vanni et al. 2013). In addition, nutrient release by fishes is an important standpoint of the trophic theory as they have been indicated as links between benthic and pelagic habitats (Shapiro & Carlson 1982). These links are established when fishes prey on benthic and littoral organisms and excrete ingested nutrients in the pelagic zone (or vice versa) in forms that can be incorporated by phytoplankton (e.g.,  $\text{PO}_4^{-3}$  and  $\text{NH}_4^+$ ) (Brabrand et al. 1990; Schaus & Vanni 2000). Therefore, through a combination of habitat differential use and excretory activities, fishes are effective nutrient transporters within a lake and can potentially affect primary production (Vanni &

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Layne 1997; Attayde & Hansson 2001; Vanni et al. 2006).

Zooplankton body size structure has a strong effect on predation selectivity of visual predators, but, at the same time, *Daphnia* and calanoid copepods have different P content despite roughly similar body size (Sterner & Elser 2002). Planktivorous fish prefer cladocera to copepods due to differential escape ability (Drenner & McComas 1980; Lampert & Sommer 2007), but P content of prey may constitute another factor on predator preference that remains poorly understood. However, elemental content on prey selection has been already shown in other organisms such as nanoflagellates (John & Davidson 2001; Shannon et al. 2007; Meunier et al. 2012) and copepods (Cowles et al. 1988).

Zooplankton community of North Andean Patagonian lakes is characterised by the dominance of small crustaceans (<1 mm). Cladocerans are dominated by *Ceriodaphnia dubia* (Richard) and *Bosmina longirostris* (O.F. Müller), while copepods are greatly represented by the calanoid *Boeckella gracilipes* Daday (Modenutti et al. 1998). The absence of *Daphnia* is remarkably in many of these lakes as an outcome of the extremely high sestonic C:P ratios (above 500 atomic) (Balseiro et al. 2004, 2007). Stoichiometric theory predicts that sestonic C:P ratios >300 would result in P limitation for *Daphnia* growth (DeMott & Gulati 1999; Urabe et al. 2002) as was also observed in Andean lakes (Balseiro et al. 2007; Laspoumaderes et al. 2013).

The small and scaleless native fish *Galaxias maculatus* (Jenyns) (Pisces: Galaxiidae) (7 cm of total length) has a widespread distribution in the Southern Hemisphere (McDowall 1971). In Patagonian lakes, this species is heavily preyed by exotic and autochthonous fish (salmonids and *Percichthys trucha* (Cuvier & Valenciennes), respectively) (Macchi et al. 2007). Therefore, littoral refuge and school formations are strategies of *G. maculatus* juveniles and adults to reduce mortality by these piscivores (Barriga et al. 2002). Previous studies have shown that fish larvae and early juveniles consumed mainly zooplankton, while late juveniles and adults combine planktonic and littoral prey (Cervellini et al. 1993; Reissig et al. 2003; Barriga et al. 2012). In particular, a shift was observed in prey electivity from copepod nauplii preferred by larvae to cladocerans by juveniles (Modenutti et al. 1993). Diel vertical and horizontal migrations by larvae and adults of *G. maculatus* revealed that this species display movements during dawn and dusk, with high fish densities in the surface pelagic waters at night (Rechencq et al. 2011). Therefore, *G. maculatus* could be an important link between habitats as they have an active role in nutrient recycling (Reissig et al. 2003).

As food quality may be as important as food quantity for animal growth and reproduction (Boersma & Kreutzer 2002), prey P content would become an important factor in selection; thus, fish habitat differential use (e.g., littoral vs. pelagic) may result from searching prey with high P content. While horizontal movements to littoral areas during day are forced by predator avoidance (Barriga et al. 2002), here, we hypothesise that night movement to pelagic areas is related with the chance to prey on high P content prey (*C. dubia*). Thus, the aims of this study were (i) to evaluate prey elemental composition (C:P ratio), in order to compare the different prey (littoral vs. pelagic), and (ii) to evaluate the effect of this selective predation on the P recycling by fish.

## Methods

### Study site

The study was conducted in Lake Moreno West (41°5'S, 71°33'W; 758 m a.s.l.) included in the Nahuel Huapi National Park located in Patagonia, Argentina (Fig. 1a,b). The lake area is of 6.1 km<sup>2</sup>, and its maximum depth is of 90 m. An extended region of its littoral zone is characterised by the presence of the emergent macrophyte *Schoenoplectus californicus* (Meyer) Soják. The lake has a warm monomictic thermal regime remaining stratified from late November through April (spring–summer months). The lake is ultraoligotrophic and has high transparency with very low nutrient concentrations (Queimaliños et al. 1999). Crustacean zooplankton is dominated by *Ceriodaphnia dubia* and *Boeckella gracilipes* (Balseiro et al. 2001).

This lake has five native fish species: *Galaxias maculatus*, *G. platei* Steindachner, *Percichthys trucha*, *Odontesthes hatcheri* (Eigenmann) and *Diplo-mystes viedmensis* Mac Donagh, and three introduced ones: *Salmo trutta* (L.), *Salvelinus fontinalis* (Mitchill) and *Oncorhynchus mykiss* (Walbaum). Salmonids and *P. trucha* prey on *G. maculatus* (Macchi et al. 2007). All predators are found in benthic and pelagic habitats, being *O. mykiss* the most efficient predator of *G. maculatus* and the predator with the highest prevalence in the pelagic habitat (Macchi et al. 2007).

### Field study

#### *Zooplankton horizontal distribution*

Zooplankton horizontal distribution was studied along a transect between the littoral and pelagic zone. Three sampling points were defined along the horizontal transect: *littoral zone* with *S. californicus* (2.5 m maximum depth), *near shore* (10 m maximum depth) and *offshore* (Fig. 1b). Samples were

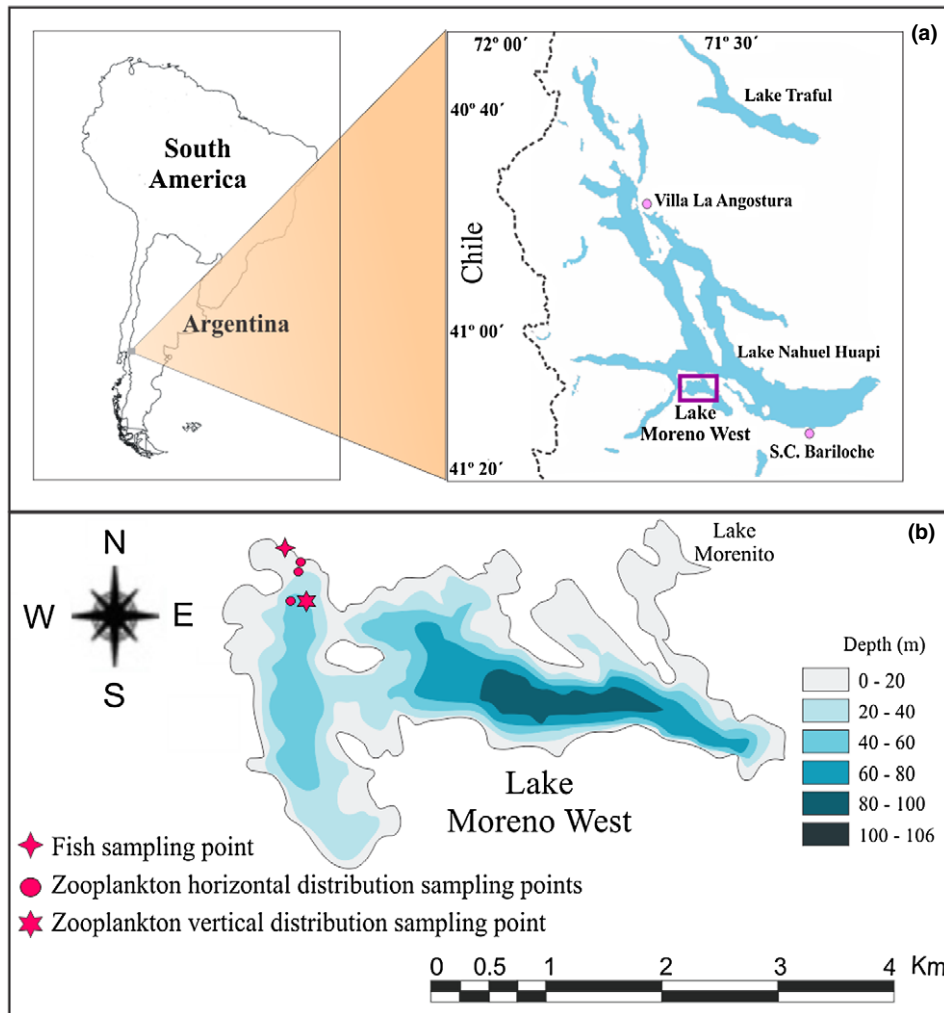


Fig. 1. (a) Geographical location of Lake Moreno West (b) Lake Moreno West showing sampling points [map adapted from Rechencq et al. (2011)].

obtained during spring at 6:30 am (sunrise), 16:00 (afternoon) and 23:00 (night), to assess horizontal daily movements of zooplankton. Vertical tows with 40-cm-diameter conical plankton net of 202- $\mu$ m mesh were collected in each site to obtain depth-integrated zooplankton proportions at different distances from the coast. Sampling was performed during a clear sunny day and on a crescent-moon night. The crustacean species were quantified at the laboratory under a stereomicroscope in 5-ml Bogorov chambers.

#### Zooplankton vertical distribution

Zooplankton vertical distribution was studied in the *offshore* sampling station on mid-summer (January) (Fig. 1b). Sampling session was performed during a clear sunny day and on a new moon night. Vertical tows from 0–5 m, 5–10 m, 10–15 m, 15–20 m, 20–25 m, 25–30 m, 30–35 m and 35–40 m were carried out with a closing conical plankton net of 202- $\mu$ m mesh size. Samples were obtained at 12:00, 16:00, 20:00 and 24:00 h. Temperature and light

[photosynthetically active radiation (PAR) 400–700 nm] profiles were quantified from 0 to 40 m with a PUV 500B submersible radiometer (Biospherical Instruments Inc., San Diego, CA, USA) at 12:00, 16:00 and 20:00 h.

At the laboratory, crustacean species were identified and quantified under a stereomicroscope in 5-ml Bogorov chambers.

#### C:N:P of main *G. maculatus* prey

We compared carbon, nitrogen and phosphorus contents, and C:N and C:P atomic ratios of the more abundant prey in the guts. For this purpose, *Ceriodaphnia dubia*, *Boeckella gracilipes*, *Bosmina longirostris* and one selected morphotype of chironomid larvae (Insecta: Diptera) were collected from the lake, and body elemental composition was analysed. Chironomid larvae were collected from the littoral zone, at the same fish sampling point (Fig. 1) with an Ekman drag. At the same time, zooplankton was collected from the pelagic zone, in the same sampling

point used to study the vertical distribution of zooplankton (Fig. 1b), by vertical trawls from 30 to 0 m with 40-cm-diameter conical plankton net with 202- $\mu$ m mesh. Individuals were rinsed with distilled water for ulterior analyses of particulate organic phosphorus (POP), particulate organic nitrogen and particulate organic carbon. Seven replicates of each analysis were performed. To ensure that the chironomids belonged to the same morphotype, each individual was inspected under a stereomicroscope and then measured. POP contents were determined using ascorbate-reduced molybdenum blue technique after digestion with potassium persulphate following APHA (1989).

For C and N analyses, individuals of each species were transferred to prewashed, precombusted (450 °C) and preweighed fibre glass GF/F filters. The filters were dried at 60 °C for at least 3 days, to constant weight. Dried samples were placed in tin capsules and then combusted in the Thermo Finnigan Elemental Analyzer EA 1112. The number of animals in each sample was 30 *C. dubia* adults (~0.7-mm body length), 30 *B. gracilipes* adults (~0.9-mm body length), 120 *B. longirostris* (~0.35-mm body length) and 3 chironomid larvae (~8-mm body length).

#### Prey selectivity

Individuals of *G. maculatus* for gut content analysis were captured using a seine net (20-m long, 1.6-m deep, 1-mm mesh size) in a coastal shallow area of the lake (Fig. 1b). Samples were taken on January 2003 and October 2006 at *morning* (8 h), *mid-day* (13 h) and *sunset* (20 h). Fish collected in the littoral zone during the morning were assumed to have migrated there after foraging in the pelagic. Fish were anaesthetised with a CO<sub>2</sub>-saturated solution to prevent regurgitation. After weighing and measuring, fish were fixed with 4% (final volume) formaldehyde solution for posterior analysis of stomach. In the laboratory, the gut content was analysed by identifying and counting each prey item under a stereomicroscope.

Chesson's selectivity coefficient (Chesson 1983) was calculated for each prey item found in the pelagic zone (*Ceriodaphnia dubia*, *Bosmina longirostris*, *Boeckella gracilipes* and cyclopoid copepods) as:

$$\alpha_i = (r_i/n_i) / \sum_{i=1}^m (r_i/n_i)$$

where  $r_i$  is the proportion of prey type  $i$  in the gut of *G. maculatus*, and  $n_i$  is the proportion of prey type  $i$  in the environment for  $m$  different prey types. The  $n_i$  was calculated as depth-integrated zooplankton density for a given species obtained at different sampling times (morning, midday and sunset), from data of zooplankton vertical distribution (see Results). Chesson's coefficient varies from 0 to 1, and a value  $>1/m$

(in this case, 0.25) indicates positive selective feeding on that group.

#### Fish phosphorus excretion

To evaluate the relationship between *G. maculatus* size and phosphorus excretion rates and the variations over a daily cycle, field experiments were performed. Three rounds of excretion measurements were conducted during 23 January 2003 and 24 January 2003: *morning* (at 8 h), *mid-day* (at 13 h) and *sunset* (at 20 h). In each experiment, we used three different size classes of *G. maculatus* based on the total length, which according to Macchi (2004) correspond to the following ages: youth of the year ( $3.59 \pm 0.03$  cm total length;  $0.17 \pm 0.01$  g), adults of about 2 years old ( $4.47 \pm 0.07$  cm total length;  $0.51 \pm 0.03$  g) and adults of about 5 years old ( $6.10 \pm 0.13$  cm total length;  $1.66 \pm 0.13$  g). Hereinafter, these size classes will be referred to: *YOY*, *small adults* and *large adults*, respectively. Five replicates of each size class were conducted in the morning and sunset measurements, and three replicates were carried out at the mid-day measurement.

Water for all excretion trials was collected from the lake and filtered to remove suspended particles using sterile membrane filters of 0.45- $\mu$ m pore size. The experimental units (1-l acid-washed polycarbonate bottles) were filled with the filtered water. Fish were collected by seine and were placed immediately into an acclimation container holding clean lake water. After 1 h, fish were carefully rinsed with filtered lake water and transferred to the experimental units (one individual/bottle). Immediately after the addition of *G. maculatus*, a sample of 250 ml water was collected to assess the initial nutrient condition (initial time). The bottles were incubated in the lake for 1 h. During incubations, bottles were kept in shallow water of the lake margins to maintain constant temperature and to minimise stress. After 1 h, water samples were collected from each bottle and filtered through sterile membrane filters (0.45- $\mu$ m) to remove faeces and other particles. Water samples were also collected from five control bottles (incubated for 1 h with no fish) for every round of measurements.

Fish were collected from each experimental unit, anaesthetised with a CO<sub>2</sub>-saturated solution and preserved for measuring, weighing and evaluating gut content to assess the food consumption at different times of day (before phosphorus excretion measurements).

Water samples were transported to the laboratory immediately after collection in thermally insulated containers. Soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) were measured using the ascorbate-reduced molybdenum blue technique (APHA 1989), with the purpose of verify the propor-

tion of released P in dissolved forms that is rapidly available for primary production (SRP) in food web.

Excretion rates were determined as the difference between initial and final nutrient concentrations and were expressed as  $\mu\text{g P (SRP or TDP) (g fish wet mass)}^{-1}\cdot\text{h}^{-1}$  to standardise excretion in relation to size.

Statistical analyses

Statistical differences in nutrient recycling between different size classes of *G. maculatus* (YOY, small adults and large adults) at different time of day (morning, mid-day and sunset) were determined through two-way ANOVA. POP contents, and C:N and C:P ratios in *Ceriodaphnia*, *Boeckella* and chironomid larva were compared statistically through one-way ANOVA. Data were log-transformed to meet

normality in mass-specific SRP released by *G. maculatus*. Tukey’s test was used for multiple comparisons of means. All ANOVAS analyses were carried out with SigmaStat.

Results

Zooplankton horizontal distribution

Zooplankton species showed a differential distribution among horizontal sampling stations (Fig. 2). Harpacticoid copepods and chydorid cladocerans were found only in the littoral zone and together with cyclopoid copepods dominated the zooplankton in this sector (Fig. 2).

The pelagic zooplankton in both near and offshore sampling points were mainly constituted by *Cerio-*

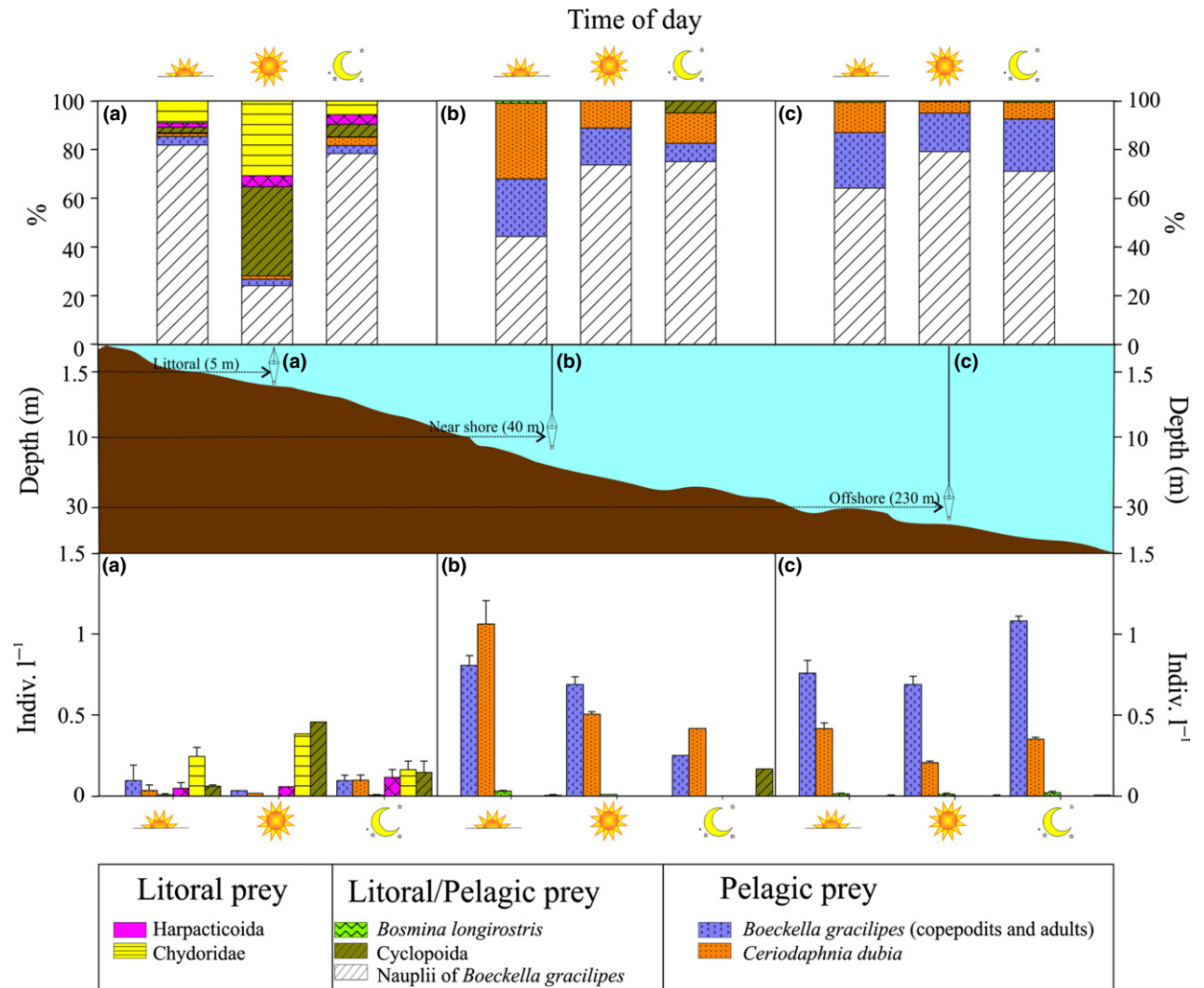


Fig. 2. Zooplankton horizontal distribution at different times of day: sunrise (sun dawn), afternoon (sun), night (moon), and at different distances from the coastal: (a) vegetated littoral zone (5 m); (b) near shore (40 m); and (c) offshore (230 m). Upper panel shows depth-integrated zooplankton proportions. Lower panel shows depth-integrated zooplankton densities (nauplii are not shown for better visualisation of the densities). Data were obtained by vertical tows with conical plankton net.

*daphnia dubia* and *Boeckella gracilipes* (nauplii, copepodits and adults), followed by *B. longirostris* and cyclopoid copepods (Fig. 2). The highest abundances of *C. dubia* were found during sunrise in both near and offshore sampling points. *C. dubia* and *B. gracilipes* appeared in the littoral zone at very low abundances ( $<0.1 \text{ indiv.}\cdot\text{l}^{-1}$ ). Finally, nauplii of *Boeckella gracilipes* were the most abundant zooplankton and were widely distributed along the horizontal and vertical sampling transects, showing the highest abundance in zooplankton (Fig. 2 upper panel).

#### Zooplankton vertical distribution

On mid-summer (January), Lake Moreno West presented a thermal stratification. We observed a thermocline at 30 m depth although within the epilimnion, a secondary thermocline at 13-m depth was also observed (Figure S1a). This secondary thermocline was observed to change its position along the day. Surface PAR irradiance varied along the day from darkness during night to  $\sim 2000 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at noon, with a mean of  $1400 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  between 11 and 17 h. The euphotic zone (1% of surface PAR irradiance) was extended up to 30 m depth.

Crustacean zooplankton was dominated by *Boeckella gracilipes* followed, in very low abundances, by *Bosmina longirostris*, *Ceriodaphnia dubia* and cyclopoid copepods. Differences in vertical distribution were observed between day and night. During the period of high surface PAR irradiance, zooplankton maximum abundance was found below 20 m. On the contrary, at mid-night (24 h), zooplankton was distributed between 5 and 20 m depth (Figure S1a). In day samples, more than 81% of crustacean zooplankton remained at depth with irradiances  $\leq 19.8 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (1% of surface irradiance at mid-day).

#### C:N:P of main *G. maculatus* prey

Phosphorus content of *C. dubia* was significantly higher than those of *B. longirostris*, *B. gracilipes* and chironomid larvae (One-Way ANOVA,  $P < 0.001$ ), and its C:P atomic ratio was significantly lower (One-Way ANOVA:  $P < 0.001$ ; Fig. 3). In turn, the littoral chironomid larvae had significantly higher phosphorus content and lower C:P atomic ratio than *B. gracilipes* and *B. longirostris* (Fig. 3). *Ceriodaphnia* body C:P atomic ratio averaged  $76.47 \pm 4.45$  (mean  $\pm$  SE), whereas chironomid body C:P averaged  $110.94 \pm 6.21$ , *B. gracilipes*  $181.87 \pm 4.62$  and *B. longirostris*  $262.28 \pm 7.33$  (mean  $\pm$  SE). Consequently, *C. dubia* was the most phosphorus-rich prey of *G. maculatus*. The C:N atomic ratio was

#### Prey C:P ratio and prey selection by fish

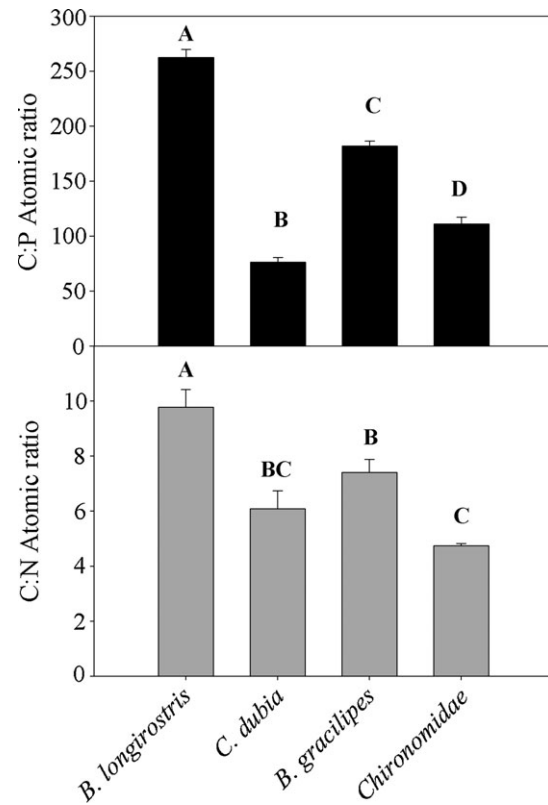


Fig. 3. C:N and C:P atomic ratio of *Bosmina longirostris*, *Ceriodaphnia dubia*, *Boeckella gracilipes* and chironomid larvae. The letters A, B, C and D represent groups with significant differences between them when assessed with a *posteriori* test (Tukey,  $P < 0.001$ ). BC belong to both groups.

significantly lower in chironomid larvae than in planktonic prey such as *C. dubia*, *B. gracilipes* and *B. longirostris* (one-way ANOVA:  $P < 0.05$ ; Fig. 3).

#### Fish gut content analysis

Inspections of gut contents at different times of day revealed that *G. maculatus* switched between pelagic and littoral prey. Pelagic prey was dominant in the morning and midday in all fish sizes while littoral prey dominated only at sunset (Fig. 4). The most consumed pelagic prey was the cladoceran *C. dubia* which dominated in all fish sizes followed by *B. longirostris* and the calanoid *B. gracilipes*. *C. dubia* was always dominant in the morning and mid-day, while *B. longirostris* codominated in biomass in YOY in the morning. Fish captured in the sunset showed different gut content depending on size. YOY remained consuming cladoceran prey of the water column (*Bosmina* and small chydorids), with the inclusion of some small benthic chironomids. Small adults resembled YOY, but with an increase in the importance of benthic prey. Finally, large adults preyed mainly on chironomids and adults of insects (Fig. 4).

Values of Chesson's index clearly indicated that *C. dubia* was strongly preferred by all fish sizes in

morning and midday, whereas the copepods, *B. gracilipes* and cyclopoids, were avoided in the pelagic zone (Fig. 5).

#### Nutrient excretion

*Galaxias maculatus* phosphorus excretion increased with body size, but mass-specific excretion rates were inversely correlated with fish size (Fig. 6; Table 1). However, this trend did not remain equal along the day, as statistical analysis showed that YOY had significantly higher mass-specific P release in the morning and mid-day, but these differences were not significant at sunset due to an increase in P release of the large-sized fish (Table 2). Small adults had a TDP and SRP release with similar values to large adults at the morning and to YOY at midday (Fig. 6, Table 2).

The SRP/TDP ratio was higher than 0.5 in all fish sizes (YOY:  $0.70 \pm 0.04$ ; small adults  $0.74 \pm 0.04$  and large adults:  $0.77 \pm 0.03$ ) showing that released P was largely constituted by SRP. Thus, the relationship between SRP/TDP ratios and fish body mass showed a rather constant value indicating that this ratio was not size dependent ( $R^2 = 0.09$ , regression slope  $P > 0.05$ ).

Changes in food items seemed to alter phosphorus recycling in *G. maculatus* (Figs 4 and 6), but it is difficult to assign a general trend because a delay might occur between prey ingestion and nutrient recycling. Nevertheless, YOY released more P in the morning and mid-day when they have been preying mainly upon *C. dubia* (Figs 4 and 6).

#### Discussion

Pelagic zone is a profitable habitat for fishes because zooplankton prey are easier to be seen and more abundant than benthic prey (Gliwicz 2003). Fishes are highly selective on certain food items that differ in body size and have dissimilar behaviour (Drenner & McComas 1980; Lampert & Sommer 2007). Our field study in Lake Moreno West showed a selection of *G. maculatus* towards *C. dubia*. This cladoceran species has low C:P ratio and was preferred by all fish sizes over the calanoid copepod *Boeckella gracilipes*, in spite of their lower abundance and similar body size. In this sense, copepods have relatively low P concentration (Andersen & Hessen 1991; Laspoumaderes et al. 2013; this paper), and the low abundance observed in the guts may suggest that prey elemental ratios could be also an explanation of *G. maculatus* preference for *C. dubia*. Consequently, planktonic populations with low C:P ratio could have an additional risk of been selected by *G. maculatus* than species of high C:P

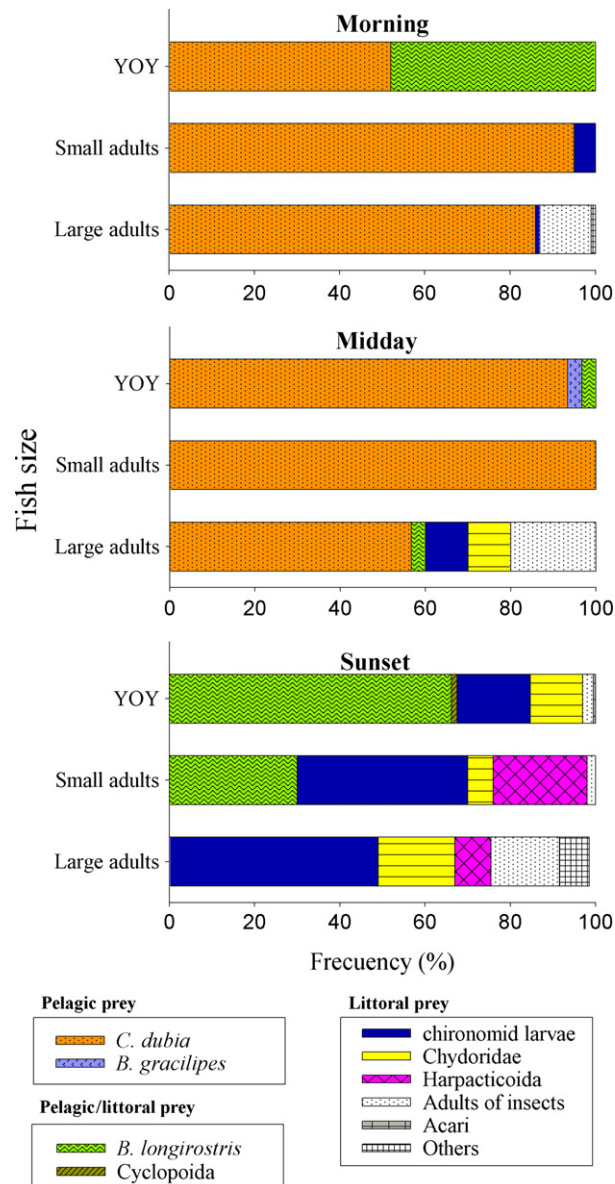


Fig. 4. Percentage of different prey observed in the stomachs of *G. maculatus* caught at different times of day on summer 2003 and spring 2006: morning (8 h), mid-day (13 h) and sunset (20 h). Three different size classes of *G. maculatus* were analysed: YOY ( $3.59 \pm 0.03$  cm total length;  $0.17 \pm 0.01$  g), small adults ( $4.47 \pm 0.07$  cm total length;  $0.51 \pm 0.03$  g) and large adults ( $6.10 \pm 0.13$  cm total length;  $1.66 \pm 0.13$  g).

ratio (e.g., *Bosmina longirostris* and *B. gracilipes*). Furthermore, in previous studies, it was shown that juveniles of *G. maculatus* were capable of detecting and preying on *C. dubia* although it was undetectable in the plankton samples (Modenutti et al. 1993) indicating that this high P prey could be positively selected. In addition, it has been demonstrated that planktonic organisms are able to discriminate between different foods on the basis of nutritional quality, that is, dinoflagellates and copepods can select prey cells based on their nutrient content

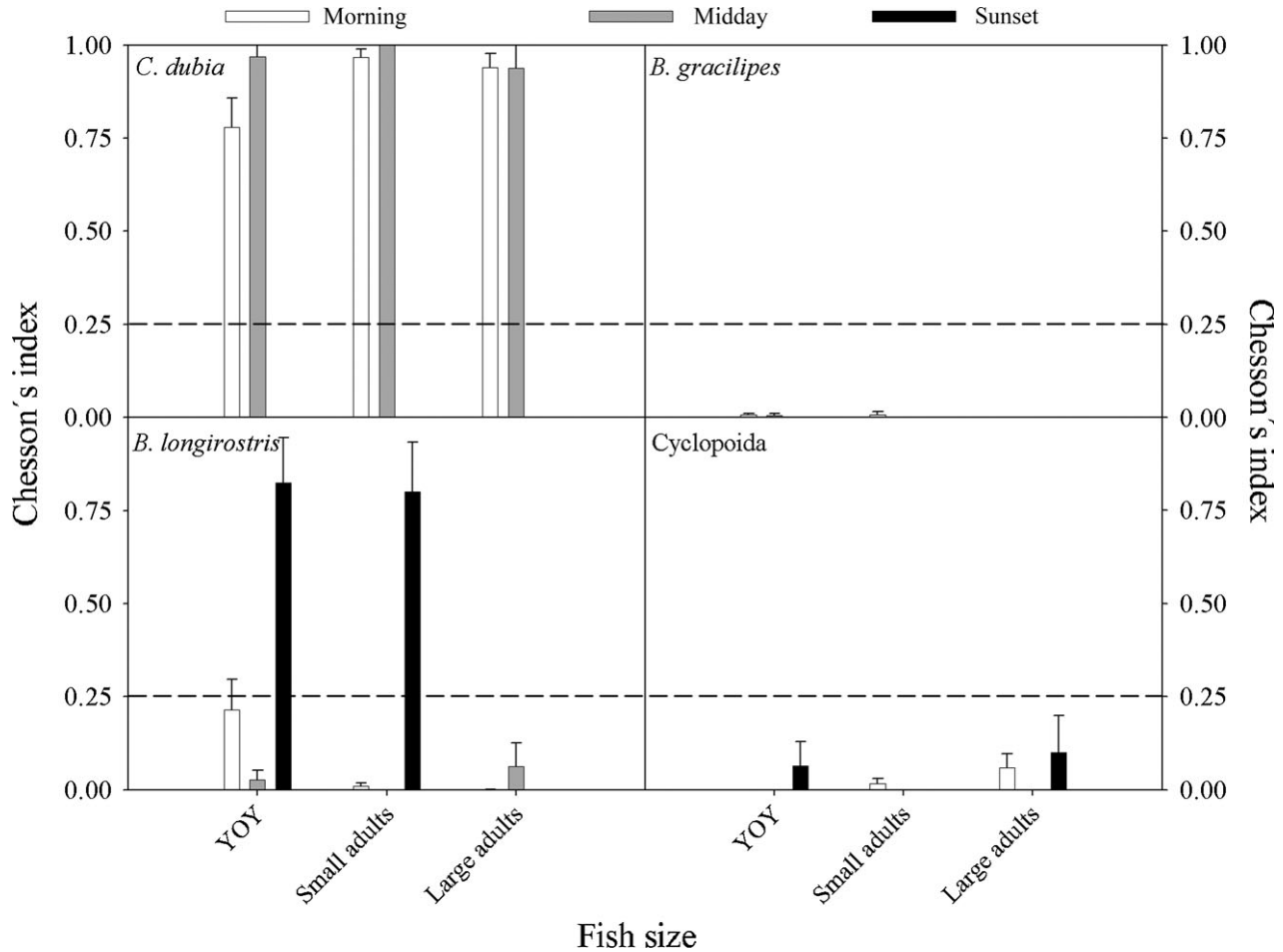


Fig. 5. Chesson's index of feeding selectivity for pelagic prey by *G. maculatus*. Values above dashed lines indicate selective feeding on that group.

Table 1. Phosphorus excretion rates of different fish size independently of time of day.

Nutrients	Fish size	Minimum value	Maximum value	Mean ± SE
TDP	YOY	8.3	22.0	12.54 ± 1.12
	Small adults	3.5	14.5	8.47 ± 0.91
	Large adults	2.5	14.0	4.80 ± 0.85
SRP	YOY	5.6	16.9	9.03 ± 0.95
	Small adults	2.8	10.8	6.21 ± 0.73
	Large adults	1.9	9.8	3.62 ± 0.57

Values are expressed as  $\mu\text{g}$  of P (SRP or TDP)  $(\text{g fish wet mass})^{-1}\cdot\text{h}^{-1}$ .

(Cowles et al. 1988; Meunier et al. 2012). Different groups and species of zooplankton differ significantly in their average nutrient ratios (Andersen & Hessen 1991; Laspoumaderes et al. 2013, this study) implying that predators can fulfil their stoichiometric requirements and elemental content by selecting different zooplanktonic prey.

According to the growth rate hypothesis (Elsner et al. 1996), juveniles should require more P than adults (Laspoumaderes et al. 2010). As food quality

may be at least as important as food quantity for animal growth and reproduction (Boersma & Kreutzer 2002; Hessen 2008), the preference of fish towards the consumption of cladocerans could favour growth of fishes due to the acquisition of higher amount of phosphorus. Hence, fish P requirements would be satisfied with the selection towards high P cladoceran prey. Thus, fish habitat differential use (e.g., littoral vs. pelagic) could result from searching higher food quality despite cladoceran's low relative abundance. Thus, this selection may result in a great advantage when fish are investing in growth.

The profitability of an offshore area cannot be evaluated without assessing whether the risk of feeding offshore is indeed rewarded by an increased energy gain (Gliwicz et al. 2006) or quality gain. In particular, pelagic zones are dangerous for small planktivorous *G. maculatus* because of the presence of large predator such as *P. trucha* and introduced salmonids (Barriga et al. 2012). The observed pelagic distribution of *C. dubia* and the high selection of *G. maculatus* towards them indicate that this fish species need to move to the pelagic zone to feed on



Table 2. Two-way ANOVA summary (F values) of mass-specific TDP and SRP release by *G. maculatus*. Tukey's tests are also shown.

Dependent variable	Treatment			Tukey's test
	Size	Time of day	Interaction Size × Time of day	
TDP	18.78***	0.14	3.29*	Morning: YOY > SA = LA Midday: YOY = SA > LA Sunset: YOY = SA = LA
SRP	28.16***	6.57**	2.83*	Morning: YOY > SA = LA Midday: YOY = SA > LA Sunset: YOY = SA = LA

YOY, youth of the year; SA, small adults; LA, large adults.

Significant differences are indicated with asterisks (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

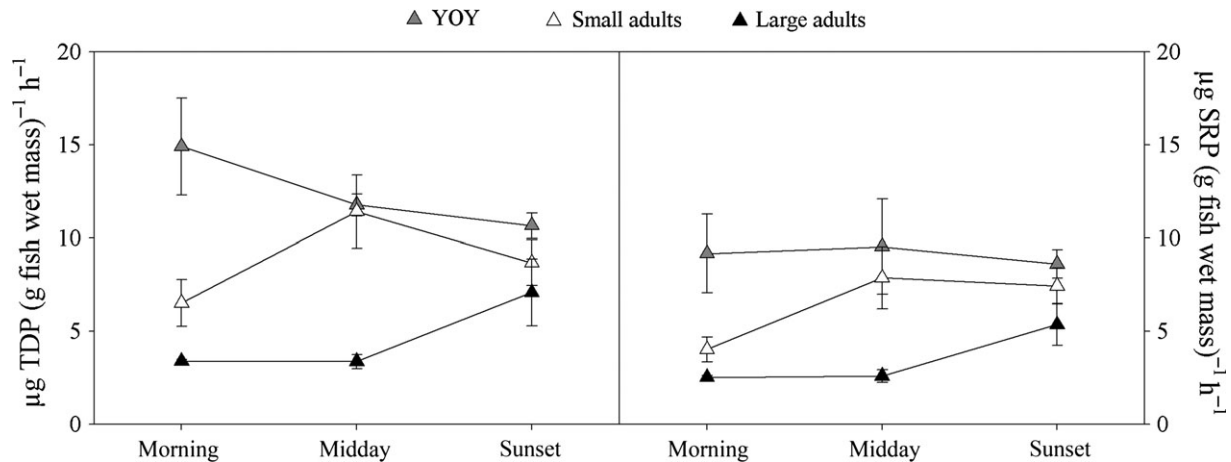


Fig. 6. Mass-specific TDP and SRP released by different sizes of *G. maculatus* in different times of day.

this prey. As pelagic habitat is dangerous for fish during day, fishes move to littoral habitats, but return to pelagic areas during dusk for feeding upon high-quality food (low C:P). This seems to be true for all *G. maculatus* size classes because all stomachs were full of *C. dubia* during morning and mid-day of spring and summer samplings. In the bluegill sunfish, plankton was also the most profitable food in every fish stage, and they grew better in cages suspended in the open water than in the littoral, where they are normally found during day (Werner & Hall 1988). Similarly, higher growth rates were observed in American dace allowed migrating and feeding offshore than those restricted to the littoral zone (Gauthier & Boisclair 1997). Indeed, here, we showed that P contents are significantly higher in pelagic prey than in littoral ones implying that also elemental composition may compensate for the energy cost of horizontal migration for feeding offshore during night.

Predator avoidance and foraging opportunity are two phenomena that explain habitat differential use of fishes and zooplankton (Gliwicz 2003). This study revealed significant shifts in gut content of *G. maculatus* with a clear dominance of pelagic prey during

morning and mid-day and a dominance of littoral prey during sunset. Because digestion processes take same hours (Fänge & Grove 1979), we assumed that gut contents of those fish captured during morning and mid-day would revealed the prey effectively consumed earlier (during night or dawn). Consequently, the dominance of the pelagic prey *C. dubia* in the guts during morning and mid-day would indicate that those prey were ingested during night when zooplankters increase their abundances at surface layers (Fig. 2 and Figure S1) and the predation risk by piscivores remains low due to darkness or low light at dawn. On the contrary, the gut contents of fish captured around sunset showed a dominance of chironomid larvae suggesting that littoral prey would be ingested during day in the structured habitat used as refuge from piscivores. Light regime was observed to be important for habitat choice and vertical or horizontal migration in both freshwater (Scheuerell & Schindler 2003; Gliwicz et al. 2006) and marine (Aksnes 2007) environments. Diel vertical and horizontal migrations suggest the use of an 'antipredation window' (Clark & Levy 1988; Gliwicz et al. 2006) to exploit high zooplankton prey density or quality in the pelagic zone at dusk or dawn. Transparent

environments such as Andean Patagonian lakes would be disadvantageous for planktivorous fish where *G. maculatus* clearly needs a low light 'window' to exploit high quality pelagic resources.

Fish mineral requirements may influence the cycling of nutrients in ecosystems (Sterner & Elser 2002). Diel migration patterns and changes in food origin (pelagic or littoral) may contribute significantly to the metabolism of lake system because fishes would transport nutrients. Phosphorus released by *G. maculatus* was largely constituted by SRP, providing a source of P in available forms for primary producers. Thus, habitat differential use (i.e., littoral vs. pelagic) of *G. maculatus* could act as effective nutrient transport within a lake. Dissimilar fish sizes exhibited differential nutrient recycling (Reissig et al. 2003; Pilati & Vanni 2007), and according to this, small *G. maculatus* recycled more P (mass specific) than large ones in the morning and mid-day when they all preyed mainly upon the high P content prey *C. dubia*. The role of consumers in supplying nutrients may vary depending on their own somatic elemental ratio and the nutrient content of the prey consumed.

Summarising, cladoceran species, which have a low C:P ratio, are selected by fish spite of their low abundances and the risk of small planktivore feeding offshore. Therefore, the pelagic zone seems to be a profitable habitat for fishes with a consequent net phosphorus gain for growth investment. This suggests that prey elemental composition could be an additional factor to classical fish selection towards cladocerans.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Zooplankton vertical distribution on summer (January). Note differences in x axis scales.

