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# Diel patterns in space use, food and metabolic activity of *Galaxias maculatus* (Pisces: Galaxiidae) in the littoral zone of a shallow Patagonian lake

**Daniela Milano · Juana C. Aigo ·
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**Abstract** We detected that *Galaxias maculatus* exhibits a pattern where metabolic activity increases after sunrise and peaks between noon and sunset, but this species feeds in the afternoon, until several hours after sunset. Moreover, we showed that *G. maculatus* is observed in the littoral zone during the day, disappears completely from this zone after sunset and returns at sunrise. Littoral prey species are common in the diet of *G. maculatus*, but this study showed that pelagic prey is also present during twilight and night hours in smaller individuals (<50 mm), which is related to habitat use. These behavioural rhythms are especially important for *G. maculatus*, which runs a high predation risk when consuming prey that is widely available outside the littoral zone. This risk is ameliorated under the protection of low light intensity. Thus, *G. maculatus* is a key species linking lower trophic levels, such as the plankton community, to higher levels of native and exotic piscivores. These displacements of *G. maculatus* generate an active flow of energy and matter between habitats, with a potentially profound effect on the entire food network and energy dynamics of the lake.

**Keywords** Metabolic rate · Feeding · Habitat use · *Galaxias maculatus*

## Introduction

Community structure can be defined in part by the pattern of resource allocation among species in a community. Resource utilization and allocation are related to trophic patterns, both spatial and temporal (Matthews 1998). The time and energy spent performing an activity will have associated benefits and costs; for example, time spent feeding yields a benefit in terms of energy and nutrients acquired, and the costs may include an increased predation risk, or the energy expended on foraging (Wootton 1990). Commonly, as with many other taxa, fish need to balance energy requirements and food intake (growth) against the costs of foraging and exposure to predation risk (mortality) in order to maximize fitness (Beauchamp et al. 2007). The balance between growth and

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mortality is typically expressed via space–time budgets of habitat use (Fraser and Mecalfe 1997; Hartman and Hayward 2007). The patterns of fish distribution in littoral habitats may be highly diverse in response to food availability and predation risk (Werner and Hall 1988). As a consequence, some fish species may be found to migrate on a diel scale from the highly structured safe daytime littoral zone habitat with lower food density, towards the more hazardous, and also more profitable, open water habitat at night (Huryn 1998; David and Closs 2003; Gliwicz et al. 2006; Stuart-Smith et al. 2006).

*Galaxias maculatus* (Jenyns 1842) is a species with a wide circumpolar distribution, which shows diadromous or landlocked life history patterns (McDowall 2003). In South America, this species presents both styles of life history, constituting an important component of native aquatic communities (Pascual et al. 2002; Cussac et al. 2004). Patagonian landlocked populations show ontogenetic intralacustrine movements (Cussac et al. 1992). These populations spawn in the littoral area and approximately 28 days later the larvae hatch and move to the limnetic zone. The larvae stay for about 6 months in this area and then move to the coast in schools (Barriga et al. 2002). Juveniles and adults of these populations spend most of their lives in the littoral zone (Barriga et al. 2002). In their early stages, they feed on plankton in the pelagic zone, and as they grow, their diet switches to benthic-littoral prey (Battini 1997). In turn, *G. maculatus* is a common prey item for native perch (*Perchichthys* sp.) and introduced salmonids (Macchi et al. 2007). It has been suggested that predators take advantage of the high availability and the increased vulnerability of *G. maculatus* while they migrate from the pelagic to the littoral zone (Macchi et al. 1999; Barriga et al. 2011). However, in the stomach contents of predators, body sizes corresponding to the juvenile status are often found, which have already been recruited to the littoral zone (Juncos et al. 2013). Moreover, with hydroacoustic techniques, Rechencq et al. (2011) described the diel horizontal migrations of "fish larvae and small fish" (galaxiids) in deep Andean Patagonian lakes. This fish group is concentrated in near shore habitats during the day and performs a migration from the coast outward at sunset, when light intensity decreases, returning to the coast at dawn.

Evidence suggests that *G. maculatus* plays an important role in linking lower with superior trophic levels and is responsible for energy transfer from the plankton community to the piscivores, thus representing the union between pelagic and littoral zones. This role is well documented in the early life stages of *G. maculatus,* and this role could continue in later stages once recruited to the littoral zone. For this reason, we decided to study diel rhythmic patterns in *G. maculatus*, for which we propose to (a) determine the pattern of metabolic activity through consumption of oxygen, (b) find feeding pattern and type of prey consumed and (c) establish the spatial use of the littoral zone throughout the 24-h cycle. We believe that from this evidence, we can understand the importance of rhythmic behaviour in explaining how the opportunist, but extremely vulnerable, *G. maculatus* minimizes predation risk and maximizes foraging efficiency.

## Materials and methods

### Study site

Individuals of *G. maculatus* were collected from the shallow Lake Morenito (41°05′S, 71°32′W), which connects with Lake Moreno, belonging to the Nahuel Huapi drainage, in Río Negro, Argentina. The whole system is of origen glacial and drains into the Atlantic. It is located near the city of San Carlos de Bariloche, in an area characterized by the presence of mixed forests of *Austrocedrus chilensis* and *Nothofagus* species. Lake Morenito has an area of 0.83 km$^2$ and a maximum depth of 15 m. It is a cold polymictic lake, and its surface may freeze partially in winter. The water temperature is homogeneously distributed throughout the water column for most of the year. In spring and autumn, temperatures range between 10 and 15 °C, reaching 22 °C in the summer (Modenutti et al. 2000). It has a highly developed littoral area with an abundance of macrophytes (*Schoenoplectus californicus*, Potamogeton *sp*. and *Myriophyllum linguatus elatinoides*).

### Diel metabolic activity

We set experiments to measure oxygen consumption over 24 h in *G. maculatus*. Fish were collected with baited traps in October 2008 and transported alive in containers equipped with an aerator. To allow them to

acclimatize, *G. maculatus* individuals were kept 1 week before each test in an incubation chamber at constant temperature (in accordance with experimental temperature) and photoperiod (13L:11D; light/dark) and fed a natural diet. We used a closed respirometric chamber (diameter = 9 cm, length = 18 cm, volume = 1.1 L) to monitor oxygen consumption. Oxygen concentration and temperature were recorded continuously during the experiments at 1-s intervals using a digital oxygen meter (YSI Model 5000) connected to a computer. The respirometric chamber was completely filled with water from the same source and under identical temperature conditions to that used in the acclimation aquarium. Each test was conducted with a single specimen of *G. maculatus* which had been deprived of food for 24 h; in this way, the concentration of dissolved oxygen did not exceed 5 mg/L. To reduce the effect of fish manipulation in each test, we measured oxygen consumption for 25 h, and then, data from the first hour were eliminated before analysis (Milano et al. 2010).

Metabolic rate was estimated from the slope of a plot of oxygen concentration versus time and expressed as mg $(O_2)$ g $(\text{fish})^{-1}$ $\text{min}^{-1}$. To test the internal origin of the rhythm of metabolic activity and light effect (as external cues), fish were exposed to different light conditions at 17 °C: continuous darkness (DD), continuous lighting (LL) and light–dark periods (LD) (Reebs 2002). LL was achieved with 120 cm Philips TLT, 40W/54RS daylight tubes. LD was obtained by alternating 2 h twilight (0600–0800 h), 11 h light, 2 h twilight (1900–2100 h) and 9 h darkness, simulating an October photoperiod. Two hours of twilight were achieved with an electronic device controlled by a timer, which increases or decreases light intensity gradually. In addition, to test metabolic activity at different temperatures in light–dark conditions, we measured oxygen consumption at 5, 9, 13 and 17 °C. After each experiment, the fish were measured: total length, TL, and total weight, TW. Seven individuals were used for DD, six individuals for LL and eleven individuals for LD of 0.96 ± 0.53 g TW (mean ± SD) and 60.54 ± 10.17 mm TL (mean ± SD) for 17 °C. We used seven individuals for 5 °C, seven individuals for 9 °C, eight individuals for 13 °C and six individuals for 17 °C of 0.97 ± 0.23 g TW (mean ± SD) and 61.52 ± 4.87 mm TL (mean ± SD).

Because oxygen consumption was calculated for each fish every hour, the data violated the assumption of independence. Time measurements were grouped into 6 four-hour categories. The criterion used to start the grouping of hours was the time simulated sunrise began. Then, repeated-measures ANOVA (RM ANOVA) was used to determine the effect of light conditions and temperature (fixed factors) and time (repeated measurements) in the diel metabolic pattern of *G. maculatus*. Fisher's least-significant difference test (Fisher's LSD) was performed to test all pair-wise comparisons for significant differences.

Diel feeding pattern and resource use

Fish were collected with a seine net (25 m long, 1.5 m high and 5 mm mesh size) in an area between the coastline and the vegetated area (depth of area sampled ranged between 30 cm and 1.8 m). Catches were made in January, March, July, September and November 2008, as many as possible over 24 h, at intervals of 4 h each. Fish were immediately anaesthetized in the field with $CO_2$ to prevent regurgitation and then fixed with 4 % formaldehyde for posterior analysis of stomach contents. Stomachs were removed, and stomach contents were examined using a stereomicroscope to identify each food category. Prey items were sorted into taxonomic groups down to species level when possible. We considered undigested prey items as food recently eaten (whole) by fish, and we calculated the proportion of undigested prey at each hour of the day in order to reconstruct their periods of feeding activity (Beauchamp et al. 2007; Chipps and Garvey 2007). For each fish, the weight contribution of each category of food (*i*) was estimated, determined as $P = P_i P_t^{-1}$, where $P_i$ is the total weight of the undigested prey and $P_t$ is the total weight of prey in stomach contents. Because normality and homogeneity of variance assumptions were violated, we used nonparametric Kruskal–Wallis test (K–W) to evaluate differences between mean proportions of undigested prey and time. Dunn's Method was used to examine all pair-wise comparisons for significant differences. Each month's data were analysed separately.

To evaluate *G. maculatus* food resource use, prey items were grouped according to their most common habitat (Table 1): littoral (prey associated with littoral zone), pelagic (prey occurring outside the littoral zone) and allochthonous (prey of terrestrial origin). For each prey category, total weight of almost intact undigested prey items in the stomach contents was

**Table 1** Clustering of prey according to its most common habitat

| Littoral | | Pelagic | Allochthonous |
|---|---|---|---|
| Chironomidae L | *Alona* sp. | Copepoda Cyclopoidea | Terrestrial insects |
| Chironomidae P | Ostracodae | Copedoda Calanoidea | Arachnida |
| Ceratopogonidae L | Hirudinea | *Ceriodaphnia* sp. | |
| Trichoptera L | Adult Diptera | *Bosmina* sp. | |
| Trichoptera P | Insect eggs | | |
| Ephemeroptera L | Galaxias eggs | | |
| Other insects L | *Chidorus* sp. | | |
| Amphipoda | Acari | | |

*L* larvae, *P* pupae

used, and fish were grouped into five size groups for each time of the day and for each month. Using the statistical package PRIMER (version 5.2.9), a multi-variate analysis was performed to determine how *G. maculatus* uses resources. Pair-wise Bray–Curtis similarity indices were calculated, which provide a measure of diet of different size and time of the day (Marshall and Elliot 1997). The resulting similarity matrix was then used to perform a hierarchical agglomerative clustering (using group-average linking) to define trophic groups. A similarity coefficient >60 % was used as a criterion to determine the trophic groups. An ANOSIM (analysis of similarities) test was used to search for differences in resources used among these groups. This permutation test analyses differences between replicates within groups, in contrast to differences between groups, computing an *R* statistic under the null hypothesis (Ho): no differences between trophic groups in resources used. *R* may fall between −1 and 1, so *R* is close to 0 if the null hypothesis is true and $R = |1|$ if all replicates within groups are more similar to each other than any replicates from different groups. Importance of prey category in different trophic groups was assessed by the SIMPER (percentage of similarity analysis) procedure, which determines the prey categories responsible for the resulting grouping in terms of Bray–Curtis mean similarity.

Diel habitat use

To evaluate the occurrence of *G. maculatus* in the littoral zone, fish were collected with baited traps during November 2008 and January, February, March and April 2009. Baited traps operated at six time points, covering a 24-h cycle, and at two depths: surface (50–90 cm) and deep (5 m). To predict the presence/absence patterns of *G. maculatus* in relation to time and

depth, nonlinear logistic regression (LR) analysis was chosen because it can take into account both null samples (score 0) and samples containing fish (score 1) at the level of individual sampling points. Each month was treated separately, where hours of day (Time) and depth were used to build the model as far as they improved the fit. A stepwise backward procedure using LR statistics was used to select significant variables. Probability of the dependent variable predicted by the model was calculated using $\chi^2$ test, and Hosmer–Lemeshow test was used to assess goodness of fit for the model. If the fit is good, a high value of probability is associated with the presence of *G. maculatus* in the littoral zone, and vice versa; if the probability value is low, absence is expected.

Results

Diel metabolic activity

The interaction of time of day and light conditions had a significant effect on the metabolic rate of *G. maculatus* (Table 2). Under constant light (LL), *G. maculatus* showed no significant differences in metabolic response between different daylight hours (Fisher's LSD, $P > 0.05$), while under constant darkness (DD), metabolic activity was significantly greater between 1000 and 2100 hours (day-sunset) than between 0200 and 0500 hours (night) (Fisher's LSD, $P > 0.05$). Under LD, metabolic rate was significantly (Fisher's LSD; $P < 0.05$) higher between 1000 and 0100 hours (Fig. 1 top panel). Metabolic activity increased significantly (Fisher's LSD; $P < 0.05$) in LD compared to LL and DD conditions (Fig. 1 top panel).

Furthermore, time of day interaction with temperature had a significant effect on the metabolic rate of

**Table 2** Repeated-measures ANOVA results showing the effect of light conditions and temperature (fixed factors) and time (repeated measurements) in the diel metabolic pattern of *G. maculatus*

|  | $df_{f, r}$ | $F$ | $P$ |
|---|---|---|---|
| Time | 5, 105 | 15.17 | 0.0001 |
| Light condition | 2, 21 | 2.86 | 0.079 |
| Time × light conditions | 10, 105 | 2.91 | 0.003 |
| Time | 5, 120 | 13.634 | 0.0001 |
| Temperature | 3, 24 | 6.326 | 0.0026 |
| Time × temperature | 15, 120 | 3.26 | 0.0002 |

Degrees of freedom of factors and residuals ($df_{f, r}$), $F$ values and significance level between groups

**Fig. 1** *Galaxias maculatus* diel rhythms of oxygen consumption in three light conditions at 17 °C, and four temperatures in *light–dark* period, during a 24 h period. *Circles* and *vertical bars* indicate mean and standard deviation
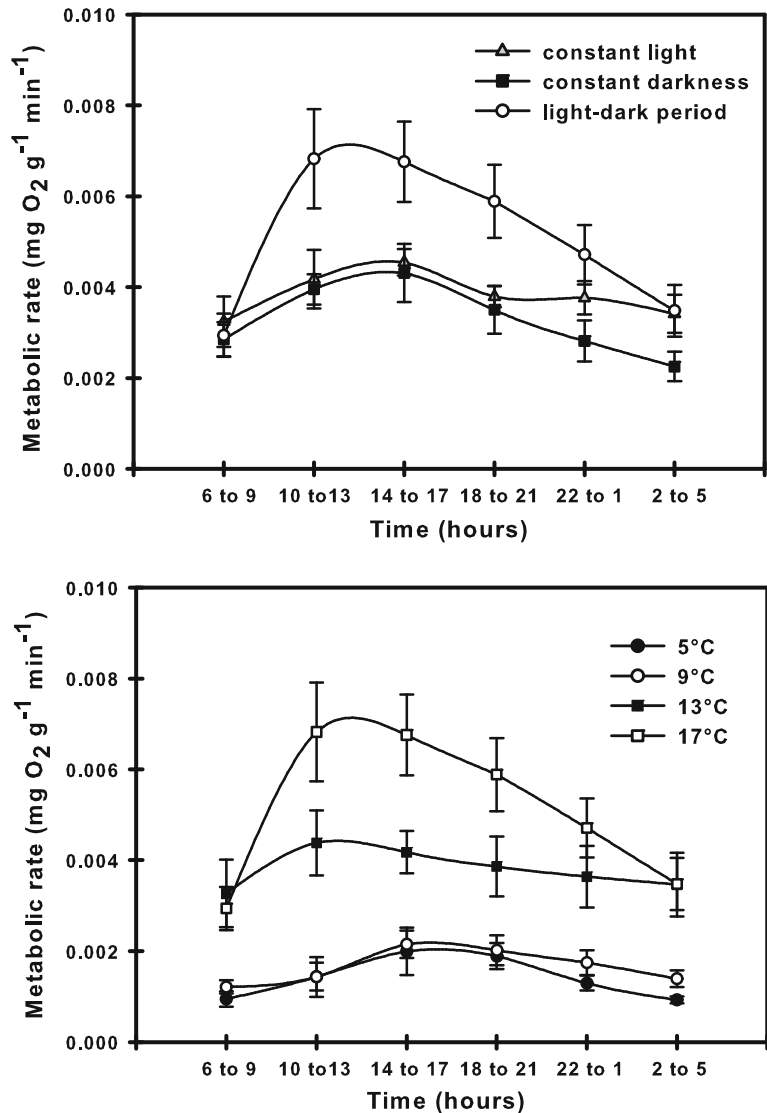
*G. maculatus* (Table 2). Metabolic activity at 5–9 °C was significantly lower than at 13–17 °C (Fisher's LSD; $P < 0.05$). While at low temperatures (5–9 °C), metabolic activity was higher between 1400 and 2100 hours (Fisher's LSD; $P < 0.05$); at higher temperatures (13–17 °C), metabolic activity was higher between 1000 and 2100 hours (Fisher's LSD; $P < 0.05$) (Fig. 1 bottom panel).

Metabolic activity of *G. maculatus* exhibited a 24-h rhythm under the different lighting conditions and temperature used; metabolic rate was higher during daylight than during night hours. An endogenous mechanism (DD) seems to be present in the metabolic activity of *G. maculatus*, besides an exogenous

mechanism related to light and temperature, both following a 24-h cycle. Diel metabolic pattern showed greater activity between 1000 and 2100 hours at 17 °C. Under light–dark periods (LD), metabolic activity was greater between 1000 and 0100 hours; at low temperatures, metabolic activity was greater between 1400 and 0100 hours.

Diel feeding pattern and resource use

*Galaxias maculatus* showed significant differences between mean proportion of undigested prey and time of day in January (K–W; H = 16.03; $df = 5$; $P = 0.007$), and September (K–W; $H = 71.80$; $df = 5$; $P = 0.001$) (Fig. 2), whereas there were no significant differences in March (K–W; $H = 7.43$; $df = 3$; $P = 0.059$), July (K–W; $H = 8.28$; $df = 4$; $P = 0.082$) and November (K–W; $H = 9.97$; $df = 5$; $P = 0.076$). In January, the average proportion of undigested prey was significantly higher at 2100 hours than at 0500 hours (Dunn's Method; $P < 0.05$). September showed a clearer diel feeding pattern, where the proportion of undigested prey was significantly higher between 1500 and 2400 hours than between 0400 and 1100 hours (Dunn's Method; $P < 0.05$). Even though no statistically significant differences were observed in the other months, in March there appeared to be a higher consumption of food at 1000 hours, in July at 1900 hours and November at 1200 and 2400 hours.

Stomach content analysis by prey habitat reveals that *G. maculatus* feeds on littoral, pelagic and allochthonous prey items. The littoral prey consumed by *G. maculatus* is associated with vegetation and the littoral bottom. However, *G. maculatus* made differential use of prey categories according to time of day and season (Fig. 3). In January, four trophic groups were distinguished to a significant level (ANOSIM, $R = 0.99$, $n = 18$, $P = 0.001$). One trophic group (similarity = 97.31) is formed by individuals of less than 50 mm that feed in the evening (1700 and 2100 hours) and at night (0100 hours), eating pelagic prey. A second trophic group is comprised of individuals exceeding 50 mm (similarity = 90.41), with a diet characterized by littoral prey, at several times of the day. Individuals of 41–50 mm, at 0900 and 1300 hours, formed the third trophic group (similarity = 90.01); this similarity was given by the contribution of littoral; allochthonous and pelagic prey. Finally, the fourth trophic group includes fish of

41–50 mm at 1700 hours and 51–60 mm at 0900 hours (similarity = 77.26), eating allochthonous prey. Thus, *G. maculatus* measuring less than 50 mm used mainly planktonic resources at sunset and night, while larger sizes fed on the littoral-benthic resource during daytime (Fig. 3).

In March, two significant trophic groups were recognized (ANOSIM, $R = 1.000$, $n = 8$, $P = 0.018$). One trophic group showed highest similarity (95.02) in diet composition and consisted of fish of 31–50 mm at 1000 hours, 41–50 mm at 1800 hours, 51–60 mm at 2100 hours and 41–50 mm at 2400 hours that fed on littoral prey. A second trophic group comprised fish of 31–40 and 41–50 mm at 2100 hours, and 31–40 mm at 2400 hours (similarity = 83.87), which eat pelagic prey. Again, smaller size *G. maculatus* made use of planktonic resources in the hours after sunset (Fig. 3).

In July, three significant trophic groups were recognized (ANOSIM, $R = 1$, $n = 10$, $P = 0.003$). The first trophic group, which showed the highest similarity (100) in food composition, is made up of fish of 41–50 mm at 1000 hours, and 31–40, 41–50 and 51–60 mm at 1500 hours, all of which ate pelagic prey. The second trophic group was composed of fish of 31–40 mm at 1000 hours, 41–50 mm at 1900 hours and 31–60 mm at 2300 hours (similarity = 86.61), associated with a diet of littoral prey. For the third group, we could not calculate average similarity or contribution to the group since there were only two samples in the group. However, the data denote that this group consisted of fish of 51–60 mm at 1900 hours and 31–40 mm at 2300 hours, and had a diet composed of littoral and allochthonous prey. In winter (July), *G. maculatus* presented pelagic prey in the stomach from the morning until the afternoon (1500 hours) and made use of the littoral zone from sunset onwards. This pattern was different from the other months, probably as a result of sunrise occurring at around 0900 hours, in conjunction with the lower temperatures which could have decreased digestion rate (Fig. 3).

Two significant trophic groups were established in September (ANOSIM, $R = 0.999$, $n = 18$, $P = 0.007$). One group showed the highest similarity (97.81) in diet and contained fish belonging to most of the size ranges at different times, accounted for by littoral-benthic prey. Another group included fish of 31–40 mm at 0400 and 1500 hours (similarity = 88.71), with a diet consisting of littoral-benthic and pelagic prey. We

**Fig. 2** Diel undigested prey proportion (*black circle*) and water temperature (*white triangle*) by months (March temperature data of Aigo 2010). At the top of *each graph*: Darkness hours (*black bars*), sunrise and sunset hours (*grey bars*) and daylight hours (*white bar*)
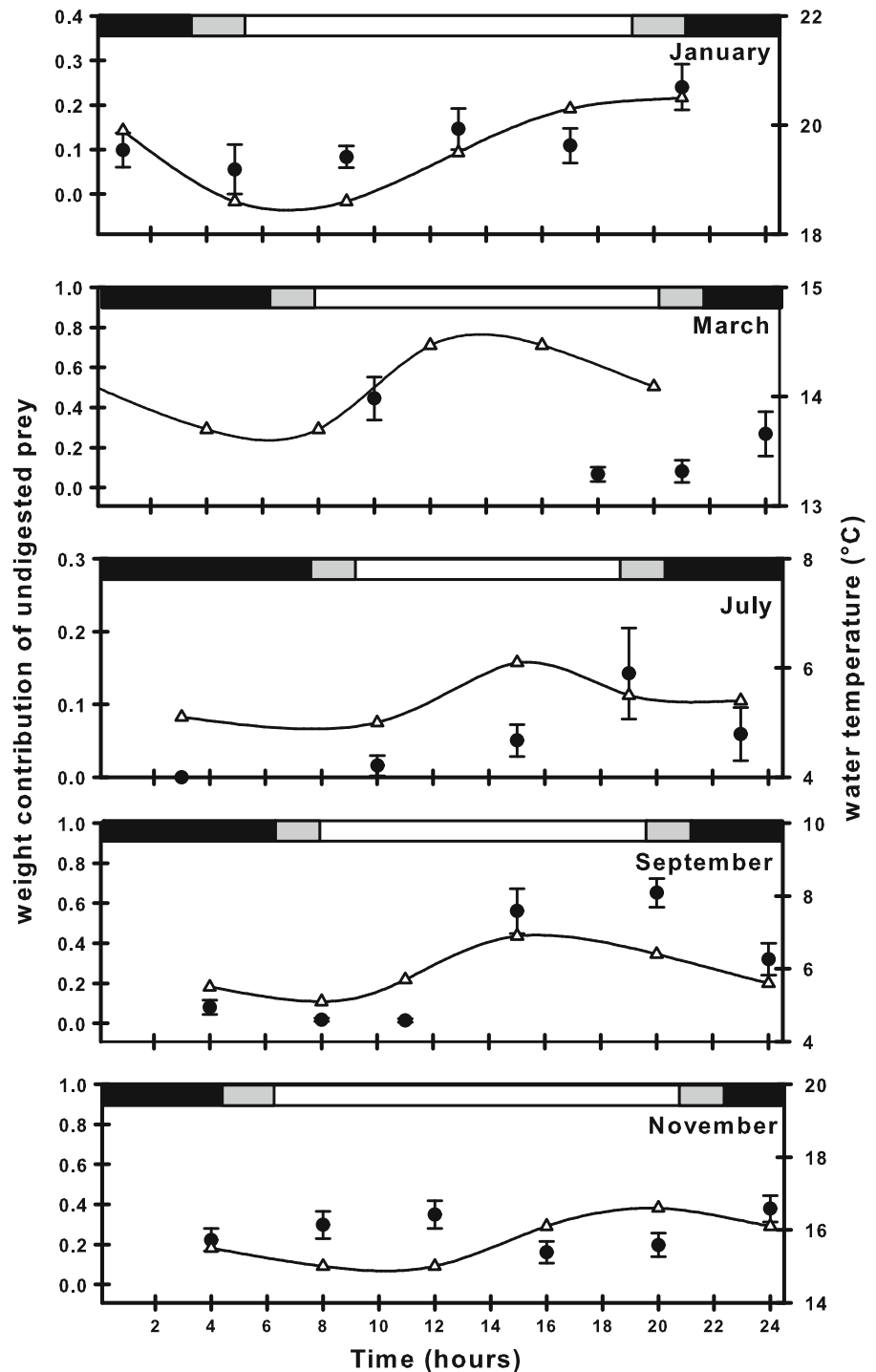


observed a minimum consumption of pelagic prey during this month, the diet of *G. maculatus* being composed mainly of prey belonging to the littoral zone (Fig. 3).

Finally, in November, three trophic groups were significantly recognized (ANOSIM; $R = 1$, $n = 17$, $P = 0.001$). The highest similarity (91.97) belonged to fish of 61–70 mm at 0400 hours and to fish of
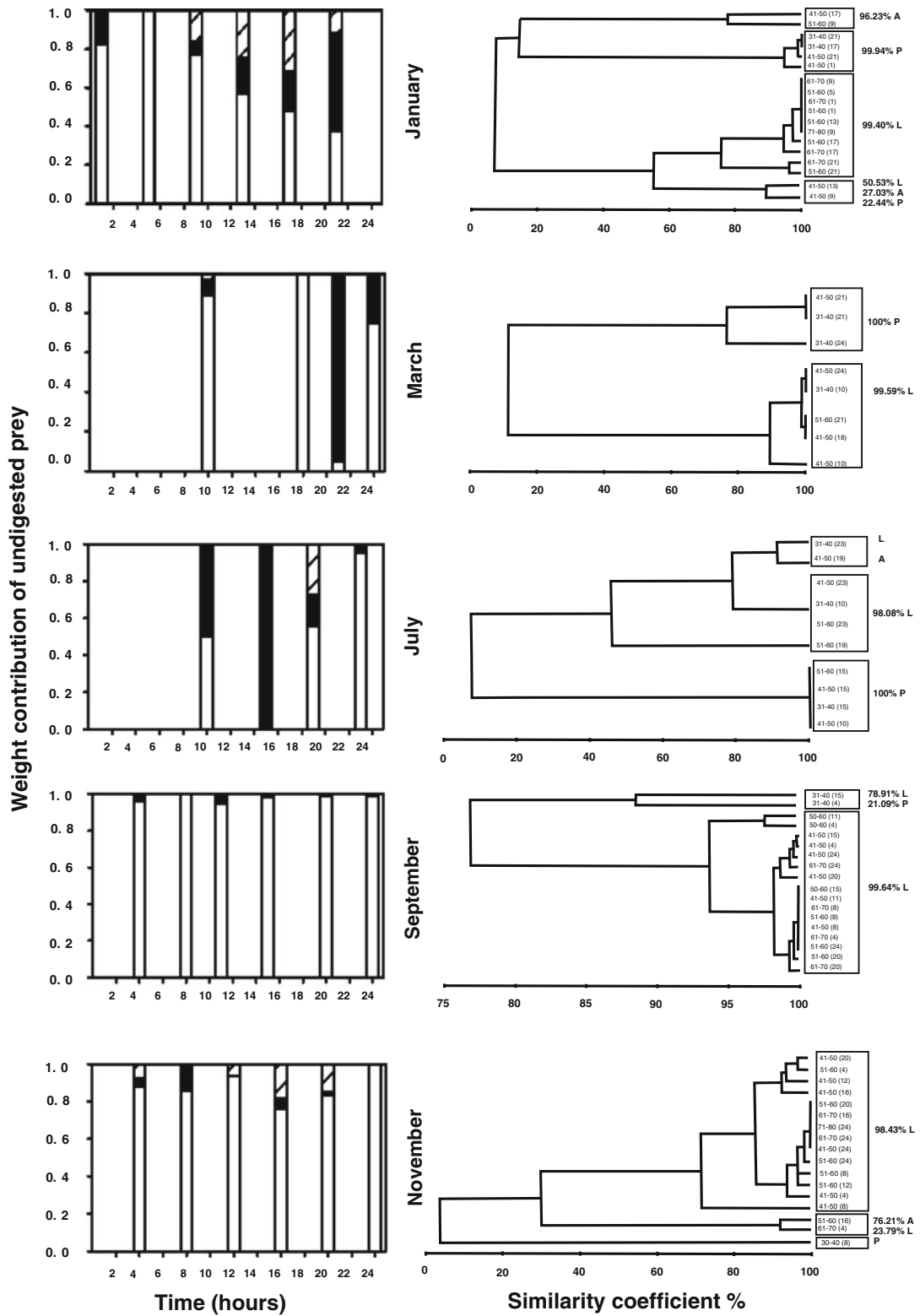
◄ **Fig. 3** Diet composition (expressed as weight of undigested prey) in relation to time (*left panels*). Littoral prey (*white bar*), pelagic prey (*black bar*) and allochthonous prey (*grey bar*). Dendrograms for hierarchical clustering based on the Bray-Curtis similarity matrix constructed on the diet composition and contribution percentage (*right panels*). Each *box* represents the trophic group defined by hierarchical agglomeration clustering, inside the *box* shows the range of sizes of trophic groups and brackets the time of day

51–60 mm at 1600 hours, showing diet composition consisting of allochthonous and littoral-benthic prey. The second group (similarity = 89.01) was represented by all size groups, at different times of the day, with only littoral prey in their stomachs. And finally, a group for which we did not calculate average similarity or contribution to the group, since it had fewer than two samples, was composed of fish of 31–40 mm at 0800 hours, which feed on pelagic prey. During this month, *G. maculatus* was limited mainly to littoral resource use, adding the allochthonous resource to the diet. Small individuals made use of the planktonic resource in the early hours of the morning (Fig. 3).

Diel habitat use

The logistic regression predicted a nonuniform distribution of *G. maculatus* in the littoral zone (Table 3). The results indicate that the occurrence of *G. maculatus* in the littoral zone was explained significantly by hours of day (time) in November, March and April; by the time and by depth in February; and both depth and had significant effects in January (Table 4).

In general, during the daytime, *G. maculatus* was more likely to occur in the littoral zone, mainly between 1200 and 2000 hours, whereas between 0000 and 0400 hours *G. maculatus* completely disappeared from this zone (Fig. 4). The Hosmer–Lemeshow statistic indicates that the models were able to predict

the presence in November and February and absence in January, March and April of *G. maculatus* in the littoral zone (Table 3).

## Discussion

*Galaxias maculatus* exhibited a 24-h metabolic activity rhythm under the different lighting conditions and temperature. An endogenous mechanism (DD) seems to be present as well as the exogenous mechanism related to light and temperature, both following a 24-h rhythm. A cyclic pattern of approximately 24 h in constant light conditions (DD or LL) suggests the existence of a metabolic endogenous mechanism in an organism (Reebs 2002). When an endogenous mechanism is involved, exogenous factors can have an influence as synchronizers of the endogenous mechanisms (Boujard and Leatherland 1992; Merrow et al. 1999). *G. maculatus* showed increased metabolic activity between 1000 and 2100 hours, both in constant darkness and at 13–17 °C. When temperatures were lower (5–9 °C), activity began at 1400 hours, and when the effect of light (LD) was incorporated, metabolic activity extended until 2100 hours. In fish, it is shown that the activity level is directly related to oxygen consumption, and for various fish species, diel rhythms in activity and oxygen consumption are similar (Kaufmann 1990; Haijin et al. 1997; Thetmeyer 1997; Porter 2001) and mostly, the fluctuation in metabolic rate is the result of the activity (Sims et al. 1993). In our experimental tests, individuals could swim freely due to the volume of the respirometric chamber, which allows us to think that fluctuations in oxygen consumption are due to the cost of maintaining bodily functions and in part to the swimming that individual can perform in the experimental

**Table 3** Logistic regression results to predict occurrence of *G. maculatus* in littoral zone

| | Lt mm (*n*) | Model | | | Hosmer and Lemeshow test | | | Nagelkerke $R^2$ | Model correct classification of cases (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | $\chi^2$ | *df* | *P* | $\chi^2$ | *df* | *P* | | |
| November | 36.3–70.7 (117) | 6.914 | 1 | 0.009 | 8.291 | 4 | 0.081 | 0.180 | 70.8 |
| January | 33.1–66 (25) | 6.647 | 1 | 0.010 | 10.770 | 4 | 0.029 | 0.209 | 89.6 |
| February | 33.5–75.9 (153) | 24.781 | 2 | 0.001 | 11.827 | 8 | 0.159 | 0.159 | 85.4 |
| March | 25.2–90.4 (426) | 16.250 | 2 | 0.001 | 17.567 | 8 | 0.025 | 0.383 | 72.9 |
| April | 33.5–76.9 (299) | 17.011 | 1 | 0.001 | 17.018 | 4 | 0.002 | 0.404 | 89.6 |

**Table 4** Summary of parameters in the logistic regression model

| Variable | $\beta$ | SE | Wald | $df$ | $P$ | Exp($\beta$) |
|---|---|---|---|---|---|---|
| **November** | | | | | | |
| Time | 0.121 | 0.049 | 6.021 | 1 | 0.014 | 1.128 |
| Constant | −1.599 | 0.622 | 6.616 | 1 | 0.010 | 0.202 |
| **January** | | | | | | |
| Depth by time | 0.130 | 0.052 | 6.153 | 1 | 0.013 | 1.139 |
| Constant | −2.359 | 0.603 | 15.321 | 1 | 0.001 | 0.094 |
| **February** | | | | | | |
| Time | 0.266 | 0.077 | 12.056 | 1 | 0.001 | 1.304 |
| Depth | −2.058 | 0.885 | 5.407 | 1 | 0.020 | 0.128 |
| Constant | −1.177 | 0.731 | 2.594 | 1 | 0.107 | 0.308 |
| **March** | | | | | | |
| Time | 0.257 | 0.083 | 9.603 | 1 | 0.002 | 1.294 |
| Depth by time | −0.135 | 0.070 | 3.688 | 1 | 0.055 | 0.874 |
| Constant | −1.660 | 0.649 | 6.549 | 1 | 0.010 | 0.190 |
| **April** | | | | | | |
| Time | 0.217 | 0.064 | 11.561 | 1 | 0.001 | 1.242 |
| Constant | −1.535 | 0.636 | 5.831 | 1 | 0.016 | 0.215 |

$\beta$ is the estimated coefficient, with standard error (SE); Wald statistic; degrees of freedom ($df$) and Exp($\beta$) is the predicted change in odds for a unit increase in the predictor

chamber. Under these criteria of evaluation, we can indirectly interpret the diel metabolic pattern, influenced by light and temperature, as a possible diel pattern of activity.

Time spent searching for food is part of an animal's overall activity. However, it must be emphasized that even if feeding does take place during the active phases, fish do not eat during the entire period of activity (Boujard and Leatherland 1992). Mean undigested prey in the gut contents of *G. maculatus* exhibited seasonal differences. In early spring (September), there was higher prey consumption at 2000 hours, and in summer (January) at 2100 hours, in the hours around dusk. Even though we do not have statistically valid data to support it, in late summer (March) high food intake seems to occur at 1000 hours and also at 2400 hours; while in winter (July), this seems to occur at 1900 hours. In spring (November), there is no clear pattern. As shown by the results of metabolic activity, feeding activity can also be regulated by the photoperiod and temperature, as both factors modify foraging activity, feeding rates and digestion rate (Bush 2003; Aguzzi et al. 2005), and also influence the quality and abundance of food (Modenutti et al. 1993). In general, we observe that most feeding activity takes place around dusk, while responding to variations in the photoperiod, and

extends even into the night when the temperature is higher. These results correspond to those found for metabolic activity in relation to these variables.

Our results show that *G. maculatus* exhibits seasonal changes in diet, probably associated with prey availability. While littoral prey is consumed throughout the year, consumption of this prey increases during the spring, probably because the supply of littoral prey also increases during this season (Añon Suarez 1991, 1997, 2002; Añon Suarez and Albariño 2001). Moreover, evaluation of the consumption of different prey categories suggests that *G. maculatus* uses pelagic resources in evening and night hours, and this pelagic prey consumption begins to be marked in summer (January) and continues until winter (July). This exploitation of the pelagic resource is observed smaller *G. maculatus* individuals (<50 mm) in association with hours of twilight. From late summer to early winter (larval-juvenile transition; 28 mm), ontogenetic migration takes place, moving from the pelagic to the littoral zone (Barriga et al. 2002), and is also accompanied by a gradual change of diet (Cussac et al. 1992; Cervellini et al. 1993). However, our results show that those individuals feeding on pelagic resources are larger than the size corresponding to migration, allowing us to ensure that they are juveniles (28–40 mm, Barriga et al. 2011) that

**Fig. 4** Diel occurrence in surface (*black bars*) and depth (*bars*) of *G. maculatus* in the littoral zone. Photosynthetically active radiation (PAR) data (*line with black circle*), recorded by a radiometer GUV 511 (Biospherical Instruments) located in the Laboratorio de Fotobiología—*INIBIOMA-CONICET, Universidad Nacional del Comahue*
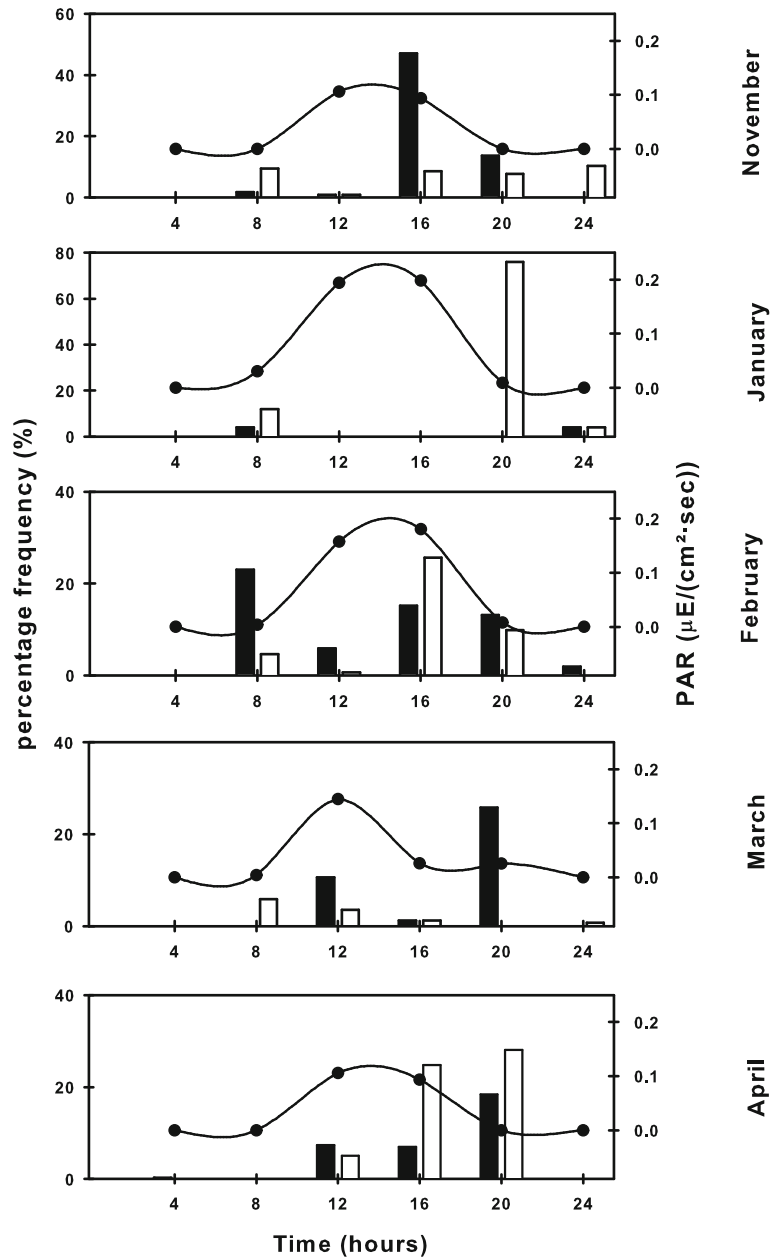


were recruited to littoral zone. This first indication leads us to think that *G. maculatus* makes a horizontal movement at dusk-night in search of pelagic prey that maximizes their foraging efficiency using pelagic resources at low light intensities. Regarding space use analysis, presence of *G. maculatus* in littoral zone shows significant overall patterns in relation to time and depth of capture, and clearly showed diurnal behaviour with higher catches mainly during daylight hours and with low or zero catches during the night

hours, again there is a horizontal movement. Presence of *G. maculatus* on the surface is more frequent during the morning and part of the afternoon (1200–1600 h). Towards the evening (2000 h), *G. maculatus* moves into deeper areas until it disappears from the littoral zone at night. An opposite movement is seen at sunrise, to deeper areas in early morning (0800 h) and then back to more superficial areas during the day. Through these results, it clearly shows that *G. maculatus* makes a diel horizontal movement offshore

from the coast area at dusk and returns at dawn. Besides, to habitat shifts during its early life history, a third littoral-pelagic movement in Lake Morenito has been registered in relation to the absence of juveniles in the littoral zone and the high density of pelagic fish recorded by echosounder (Cussac et al. 1992). Additionally, Reissig (2005) observed the presence of pelagic prey in the stomachs of small individuals of *G. maculatus* in a deep lake, Moreno Lake. Finally, by using hydroacoustic techniques in deep lakes in the nearby, there have been detected, simultaneously to diel vertical migration of plankton, a horizontal displacement of fishes to areas near the coast occurs (Rechencq et al. 2011; Lindegren et al. 2012) The movements of the planktonic community and *G. maculatus* to near shore areas may facilitate the encounter between both groups of organisms. And this may be even more marked in a shallow lake like Lake Morenito, where the distances between pelagic and littoral habitats are closer. Alonso et al. (2004) observed differences in the abundance of zooplankton on surface waters in three interconnected lakes that differs in their transparency; one of them was Lake Morenito that showed the lower transparency (kdPAR: $0.55 \, m^{-1}$) and the higher zooplankton abundance ($396.86 \pm 48.43 \, ind \, L^{-1}$, Garcia 2012). Interestingly in this lake, these authors showed no trend in the seasonal vertical migration zooplankton. More specifically, the proportion of individuals occupying the surface layer did not differ significantly between the peak radiation (spring-summer) and low (autumn–winter). Regarding predators, their presence in the littoral zone in Lake Morenito is associated with water temperature. Relative abundance of *Percichthys trucha* is higher in spring–summer (70–90 % of the catch) and lower in winter (0–40 % of the catch), while *Oncorhynchus mykiss* shows a reverse pattern, with 70–100 % of the catch in winter and 0–20 % of the catch in summer (Aigo 2010). In addition, larvae were more preyed on than juveniles and adults in deep lakes, while the reverse pattern was found in Lake Morenito, where predation risk is low (Barriga et al. 2011). Thus, we may think that *G. maculatus* makes a horizontal movement at dusk-night to consume pelagic prey, using the complexity of the littoral zone for their protection against large piscivorous predators, and maximizing their foraging efficiency by using pelagic resources at low light intensities. Through diel horizontal migration of *G. maculatus*

between the coast and the open water area may maximize fitness by taking advantage of the high availability of zooplankton in open waters at night. This seems to be an efficient foraging strategy, driven by a trade-off between the use of food resources and predator avoidance in relation to light intensity (antipredatory window; Clark and Levy 1988; Gliwicz 2002; Gliwicz et al. 2006). This type of foraging strategy is often usually associated with fish that detect predators and prey visually. The particular characteristics of the retinal structure of *G. maculatus,* which allows them a good vision in the dark (Ali et al. 1990) and the ability to detect predators chemically (Milano et al. 2010), may be important for simultaneously avoid predators and detection and eventually capture prey in low light condition (McDowall 1997).

Maximum adult size attained by *G. maculatus* may be not large enough to dissuade predators; therefore, this species suffers high mortality by predation throughout its entire life cycle (Vigliano et al. 2009; Juncos et al. 2011; Barriga et al. 2011). This species must therefore use several antipredator strategies, and so, these behavioural rhythms are especially important for the opportunistic, but extremely vulnerable, *G. maculatus*. In highly transparent environments, such as Patagonian lakes (Díaz et al. 2007), *G. maculatus* is highly exposed to visual predators. Predation risk would be higher if it fed during daylight hours on the more available prey outside the littoral zone. However, predation risk is reduced by feeding during hours of low light intensity. This strategy is of great importance, particularly in the juvenile stage, which presents rapid growth and reaches sexual maturity in a few months (the following summer; Macchi 2004). Within these Patagonian freshwater ecosystems, *G. maculatus* is a key species, linking lower trophic levels, such as the plankton community, to the upper assembly of native and exotic piscivorous fishes, so they are responsible for energy transfer between the pelagic and littoral zones. Besides, *G. maculatus* modifies nutrient dynamics by their own nutrient release as by altering the nutrient by reducing zooplankton biomass (Reissig et al. 2003). Consequently, recognition of the duality of pelagic and littoral production pathways, which are closely linked by mobile consumers such as *G. maculatus*, will provide an enhanced energetic template for understanding the trophic dynamics of Patagonian lakes.

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