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Exploring bioenergetics of diadromous *Galaxias maculatus* in the southernmost extreme of its distribution: Summer is not always the better season

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ABSTRACT

The puyen *Galaxias maculatus* is a fast-growing species, and the studied population at the southernmost extreme of the species' distribution (54°S) has the shortest growing season among the South American populations (least daylight hours and lowest temperatures), as well as the largest size. Thus, it offers an opportunity to study the effects of strong seasonal variation. The energy allocation pattern on a diadromous population of the species and the influence of 'Winter' (4 °C, light:dark photoperiod of 7:17) and 'Summer' (10 °C, light:dark photoperiod of 17:7) experimental conditions on the bioenergetics of the species were studied using both physiological and biochemical indicators. Somatic growth, energy density, food consumption, oxidative metabolism and oxygen consumption were measured.

In wild conditions, summer and winter were the more 'energy demanding' seasons, leading to the lowest energy density of individuals. Same levels of food consumption were found both in wild and 'Summer' and 'Winter' experimental conditions. Moreover, basal metabolism did not differ under experimental conditions, however, under 'Winter' experimental conditions individuals did not grow and diminished their somatic condition and energy reserves. Therefore, during winter this population would not lower energy demands (as expected by temperature) with no changes in metabolic rates, but with the consequent lower gross conversion efficiency, the complete absence of growth in length, mass and muscle, and the depletion of energy reserves. These findings suggest a mechanism for metabolic demands, i.e. postprandial increase in O_2 consumption (SDA) independent of the amount of ingested food, relocating energy reserves to fuel winter costs in *G. maculatus*.

These results provide evidence of different energy allocation strategies for winter and summer. A higher proportion of energy could be directed to growth and reproduction in summer, and to activity support in winter.

1. Introduction

The ecological success of an organism depends critically on its ability to acquire and allocate resources to reproduction and other life functions in order to maximize fitness. Combination and interaction of environmental factors lead to constraints with far-reaching influences upon the physiological performance of living organisms, ultimately affecting their ability to grow, survive and reproduce (Claireaux and Lefrançois, 2007). High latitude environments are characterized by strong seasonal variations in climatic conditions and energy availability (food resources), which impose strong seasonal constraints and lead to the development of several strategies for energy acquisition and allocation (Drent and Daan, 1980; Stearns, 1989). As a consequence, seasonal growth and energy storage cycles are common among cold and temperate fish (Callow, 1985; Stearns, 1992; Aristizabal, 2007). When the available energy intake is low during winter, depletion of energy is regarded as a major cause of mortality in freshwater fishes of cold and cold-temperate environments (Post and Evans, 1989; Finstad et al., 2004). Many fish lose energy reserves throughout the winter, relying upon stored lipid reserves to fuel metabolism during that season (Toneys and Coble, 1980; Schultz and Conover, 1997).

Measures of energy content such as energy density could provide insight into the physiological status of a fish, which may reflect its condition for wintering, migrating, spawning (Rottiers and Tucker, 1982; Shearer, 1994; Ludsin and DeVries, 1997), and/or may evaluate its sensibility to overcome habitat changes (Simčič et al., 2015). A decline in the nutritional status of a fish verifies that the energy derived from feeding was not enough to meet metabolic needs. For instance, lower average temperatures at higher latitudes lead to a reduction in nearly all biological rates, including growth rate (Gillooly et al., 2001). The metabolic rate of ectothermic organisms depends at least on temperature and body size (Clarke and Johnston, 1999; Gillooly et al., 2001). The metabolic response that accompanies meal digestion is labeled specific dynamic action (SDA) and represents the energy expended on ingestion, digestion, absorption, and assimilation of a meal (see review of Secor, 2009). This physiological phenome-

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non implies even an enhancement of oxygen consumption, gastrointestinal blood flow and heart rate (Eliason et al., 2008; Sandblom and Axelsson, 2011). Reliance on lipid reserves is believed to be necessary because food consumption and the ability to digest food at low temperatures are severely limited, such as the time demanded for SDA, that could be extended (Pérez-Casanova et al., 2010; Vanella et al., 2010). Low food consumption rates by overwintering fish may also be limited by food availability (Cunjak and Power, 1987; Foy and Paul, 1999). Also, seasonal variations in metabolic rate are assumed to entail alterations in reactive oxygen species (ROS) formation (Abele and Puntarulo, 2004) and total antioxidant capacity (TAC) in steady state (Da Rocha et al., 2009).

Growth itself imposes a significant energy cost, and any trade-off associated with high growth rates will lead organisms to grow below their physiological maximum (Nylin and Gotthard, 1998). Rapid growth evolves when a minimum size must be reached rapidly or to compensate for slowed growth due to environmental conditions (Arendt, 1997). The puyen or inanga, *Galaxias maculatus*, has a wide distribution in the Southern Hemisphere (McDowall, 1968; Waters and Burridge, 1999; Cussac et al., 2004) and is a fast-growing species, living in cold-temperate waters in Patagonia (Baigún and Ferriz, 2003; Pascual et al., 2007; Carrea et al., 2013). The species exhibits high life history plasticity along its extensive geographical distribution, including landlocked and diadromous populations. Populations from southern Tierra del Fuego (54°S), given its geographical location, have the shortest growing season (Shuter and Post, 1990) of all South American populations but also the largest size reported (Boy et al., 2007, 2013). Previous studies suggest that winter is the season with strongest energy demands by the *G. maculatus*' diadromous population at Arroyo Negro, and that it relies on energy stores (perivisceral fat) built up during spring-summer to overwinter (Boy et al., 2007, 2009), as the mosquitofish *Gambusia affinis*, Colorado pikeminnow *Ptychocheilus lucius* and largemouth bass *Micropterus salmoides* (Reznick and Braun, 1987; Thompson et al., 1991; Miranda and Hubbard, 1994; Fullerton et al., 2000). Short-term fasting restrains growth, having a negative impact on its condition and decreasing about 7% the energy density of the individuals (Boy et al., 2013). During the short favorable season (summer months) — in terms of environmental variables — the population concentrates growth and reproduction. Mature gonads represent up to 30% of body mass (Boy et al., 2007), which is supplied not only by liver (common energy reservoir in fish) but also by muscle, which reduces its energy density (Boy et al., 2009). This population displays a seasonal growth strategy that shifts from increases in length in the early growing season to increases in mass, and finally to energy reserves toward the end of the growing season (Boy et al., 2007, 2009), a similar strategy to that found in striped bass, *Morone saxatilis*, during the first year of life (Hurst and Conover, 2003). Thus, it offers an opportunity to study the effects of strong seasonal variations in water temperature and daylight hours (photoperiod), the two main environmental factors which have been identified to affect the biology of this diadromous population from Tierra del Fuego (Boy et al., 2007). The experiments of summer/winter temperature and daylight conditions allow us to discriminate the effect of these factors from others influencing fish bioenergetics, such as food availability or the costs of foraging, and those of reproduction, among others. Therefore the study of the effects of these factors on the bioenergetics would enhance the understanding of the seasonality of energy allocation of this species.

It can be hypothesized that energy allocation to somatic growth, food consumption and maintenance in a southernmost population of *G. maculatus* varies depending on summer/winter conditions of temperature and daylight. Furthermore, the negative effects of the extreme environmental conditions on its energy allocation pattern (among different organs/tissues) decrease the energy density of its individuals. In this context, physiological and biochemical indicators have been combined to a) explore the seasonal variation of energy reserves (energy density of individuals and somatic indexes) in a diadromous population of puyen, and b) study the influence of summer/winter experimental conditions of temperature and daylight hours on growth, and on the energy allocation pattern between different processes such as growth, basal metabolism, food consumption and food assimilation. The information provided herein will not only contribute to the comprehension of the bioenergetics of this species, but will also provide a knowledge base to evaluate the effects of the susceptibility of *G. maculatus* populations to variations in environmental conditions.

2. Materials and methods

2.1. Wild population

2.1.1. Study area and sampling

The study was conducted in Arroyo Negro (AN), in the Parque Nacional Tierra del Fuego (54°50′S, 68°34′W). Mean monthly photoperiod fluctuates between 17 daylight hours (L):7 dark hours (D) during summer and 7L:17D during winter. Water temperature was recorded with dataloggers (iButton-TMEX, Dallas semiconductor) every 60 min during 2010–2013, then mean daily water temperature was calculated. Four seasonal samplings were made during 2011 in January (summer), April (autumn), July (winter) and October (spring). Fish were captured with a seine net (10 m long, 1 m deep, 0.7 mm mesh), and 157 individuals in total of 64.17 ± 3.48 mm total length were anesthetized to death with MS-222, and the rest of the captured individuals were immediately returned to the water. Total length, mass, condition and somatic indexes were determined (see '**Total length, mass, condition and somatic indexes**'). Once dissected and organs/tissues weighed, the whole individuals were dried at 60 °C to a constant mass and stored in sealed bags at − 20 °C until calorimetric determinations (see '**Energy density of individuals**').

2.2. Experimental approach

2.2.1. Holding conditions

Adult fish captured as mentioned above of homogeneous sizes (62.58 ± 0.52 mm total length (TL) and 0.83 ± 0.06 g total mass (TM)) were selected. All the fish used in the trials were captured from the AN population, and at a water temperature of about 5 °C. The fish were placed in aquaria and left to acclimate for 2 weeks to captivity conditions, being fed ad libitum every 3 days with hake (*Merluccius hubbsi*), following Vanella et al. (2012) and Boy et al. (2013). Proximate composition of *M. hubbsi* was reported by Vanella et al. (2012), and the energy content of the muscle used along the experiments was determined (see below). Oxygen was maintained near saturation conditions (11.12 ± 0.14 mg/l) in each aquarium. Temperature, photoperiod and pH were selected to reproduce the average autumn conditions (6 °C; light:dark photoperiod of 8L:16D; pH 7.5) of the population under study (Boy, *pers. obs.*). No visual signs of stress were observed during acclimation.

Two experimental treatments were performed, which were conducted simultaneously: 'Summer' conditions (10 ± 1 °C, photoperiod of 17L:7D) and 'Winter' conditions (4 ± 1 °C, photoperiod of 7L:17D). After two weeks of acclimation to experimental conditions, the temperature and photoperiod of each treatment (day 0 of the experiment) were reached at a rate of 1 °C/day and 1 h/day, respectively. For experiments 1 and 2 (see below) each treatment was replicated in 8 aquaria with 10 individuals per aquarium. For experiment 3

(see below), fish were kept in two aquaria, one under 'Winter' conditions and the other under 'Summer' conditions, before being introduced in the individual respirometry chambers.

2.2.2. Experiment 1. Growth and food consumption

One individual per aquarium was sacrificed following deep anesthetization (with MS-222) for the somatic growth study and a second one to measure muscle fiber morphometrics (see '**Muscle fibers**') at 0, 32 and 59 days of experiment (0 day, 32 days and 59 days, respectively). A third individual per aquarium was sacrificed for measurements of energy density (see '**Energy density of individuals**') at 0 day and 59 days. Measurements of total length and total wet mass were taken at 0 day, 32 days and 59 days (see '**Total length, mass, condition and somatic indexes**'). Both groups ('Winter' and 'Summer' treatments) were fed twice a week with known quantities of hake muscle until satiation. The excess of food was removed from the aquarium after 1 h, dried in an oven at 60 °C, and weighed. Dried mass was converted to wet mass (80.84% of water content). This percentage of water content of *M. hubbsi* in the experimental conditions was previously determined in six samples of hake offered as food in the experiments. Samples were weighed (wet mass), then submerged in water for 1 h simulating the time they spent in the aquaria during experimental feeding, and finally dried and weighed again (dry mass). The difference between dry and wet mass was expressed as percentage of total wet mass.

2.2.3. Experiment 2. Energy allocation and oxidative metabolism parameters

At the beginning and at the end of the experiment (0 day and 32 days, respectively) two individuals per aquarium were sacrificed with deep anesthesia, measured and dissected (see '**Total length, mass, condition and somatic indexes**'). Once dissected and organs/tissues weighed, a piece of muscle of one individual was stored at − 80 °C until oxidative balance determinations were done (see '**Oxidative metabolism parameters**'), and the other individual was dried for energy density determinations (see '**Energy density**').

2.2.4. Experiment 3. Oxygen (O_2) consumption

Oxygen consumption was determined by closed respirometry, a technique already employed by Urbina and Glover (2013) in *G. maculatus*. Fish (n = 12 for 'Summer' conditions and n = 11 for 'Winter' conditions) were transferred to individual chambers (517.4 ml each, a volume large enough to allow spontaneous fish movements), made of translucent plastic material. Before each assay, the chambers were deeply cleaned to discard the influence of the biofilm on the measurements. Control chambers (i.e. lacking fish) were used to discount background oxygen consumption. In order to measure O_2 consumption, the chambers were sequentially closed (2.5 h) and refilled (0.5 h) with O_2 saturated water. This allowed maintaining O_2 saturation levels above 80% at any given treatment and fish size. After incubation, the PO_2 inside the chambers was, in average, 10.86 mg O_2/l; which represents about 96% of saturation. Each chamber was equipped with a Strathkelvin 1302 Clark-type polarographic O_2 electrode, connected to a Strathkelvin 928 6-channel O_2 system. Measurements were made continuously. Feeding was suspended 10 days before the fish were transferred to the respirometric chambers. Once the O_2 consumption rate reached stable values (about 72 h), baseline (BL) was calculated (see '**Oxygen consumption measurements**'). This fasting time was found enough to induce significant food consumption, given that *G. maculatus* was fasted for a month and fish were observed in good health and with no changes at muscle level (Boy et al., 2013). After BL was measured (five days later to their introduction into the chambers), fish were fed ad libitum (which represented about 8–11% of body mass) with hake muscle inside the chambers. The food was weighed before its introduction, and, after 1 h, the remaining food was removed from the chambers and weighed again in order to quantify the ingested food, as described in '**Experiment 1. Growth and food consumption**'.

2.2.5. Variables measured

2.2.5.1. Total length, mass, condition and somatic indexes

Individuals were measured (total length, TL, 0.01 mm), weighed (total wet mass, TM, 0.01 g) (wild population, experiments 1, 2, 3), and sexed. The specimens (wild population, experiment 2) were dissected and the wet mass of the gonads (MG), liver (ML), perivisceral fat (MF) and stomach and gut (MD) obtained (0.0001 g). Somatic indexes were calculated as follows: Fulton condition index (K) = TM / (TL [cm] ^ 3) ∗ 100; gonadosomatic index (GI) = MG ∗ 100 / TM; hepatosomatic index (HI) = ML ∗ 100 / TM; perivisceral fat index (FI) = MF ∗ 100 / TM; digestive index (DI) = MD ∗ 100 / TM.

2.2.5.2. Food intake

The ingested food per aquarium after each feeding was calculated for experiment 1 as: IFD (%) = wet mass of ingested food in the aquarium (MIF, g) / aquarium fish biomass (ABI, g) ∗ 100, where ABI at each feeding was estimated through the regression between TM of individuals sacrificed at 0 day and M of individuals sacrificed at 32 days; and through the regression between M of individuals sacrificed at 32 days and those sacrificed at 59 days. Four regression lines were used: between 0–32 days and 32–59 days for winter treatment, and between 0–32 days and 32–59 days for summer treatment. Along the experimental period there were 16 feedings, the mean IFD (MIFD, %) per feeding was calculated to each treatment, averaging the IFD (%) of the 8 aquaria from each treatment. Ingested food was also expressed on a daily basis, on mass units ($MIFD_m$, g/g day) and on energy units ($MIFD_e$, kJ/g day).

2.2.5.3. Energy assimilation to biomass

The energy assimilation to biomass was calculated from experiment 1 as the relation between the energy gained in biomass and the energy ingested along the experimental period, as gross conversion efficiency: GCE = gain aquarium fish biomass (kJ) / food consumed in the aquaria (kJ).

2.2.5.4. Energy density

Individuals (wild population, experiments 1, 2) and muscle of *M. hubbsi* used as experimental food were dried at 60 °C to constant mass, and the calorific content was obtained by burning pellets in a micro-bomb calorimeter (Parr 1425) following Boy et al. (2009). The values obtained were corrected for ash and acid content and expressed as energy density ED (kJ/g ash free dry mass, AFDM) and as EDW (J/g ash free wet mass, AFWM). Benzoic acid calibrations were carried out periodically.

2.2.5.5. Muscle fibers

A cross section of the axial muscle of individuals from experiment 1 was cut at 2/3 of total length (following Boy et al., 2013) and stained with Hematoxilin-Eosin. The number (F) and mean diameter of white/fast fibers were quantified on a quarter of histological sections (cross sectional area, CA, mm^2) using image-analysis software (ImageProPlus). The percentage of new fibers (NF, fibers smaller than 20 μm) was calculated for each individual, as

NF(%) = NF ∗ 100 / F. The mean values of F, NF (%) and CA (mm^2) were calculated for each experimental group.

2.2.5.6. Oxidative metabolism parameters

Total antioxidant capacity against peroxyl radical (TAC) was evaluated through formation of reactive oxygen species (ROS) determination in tissue samples treated or not with a peroxyl radical generator (Amado et al., 2009; Pérez et al., 2015) and detected by fluorometer (Fluoroskan Ascent FL, Thermo Scientific) at λ_{ex} = 488 nm and λ_{em} = 525 nm. Total fluorescence production was calculated by integrating the fluorescence units (FU) along the time of the measurement. The results were expressed as area difference of FU/min/wet weight (WW) in the same sample with and without ABAP (2.2′-azobis (2-methylpropionamidine) dihydrochloride) addition and standardized to the ROS area without ABAP (background area). The relative difference between ROS area with and without ABAP was considered as a measure of antioxidant capacity, which was calculated as: TAC = (ROS area ABAP − ROS area background) / ROS area background, and expressed as a relative area/WW. High TAC values mean low antioxidant capacity, since high fluorescence levels were obtained after adding ABAP, meaning low competence to neutralize peroxyl radicals.

2.2.5.7. Oxygen consumption measurements

In experiment 3, and after obtaining the baseline level (BL), the postprandial increase in O_2 consumption (SDA) was evaluated. The O_2 consumption rate was measured from 3 h after feeding until its value returned to baseline level for 24 h (following Vanella et al., 2010). The magnitude of the SDA response was determined from individual plots of the O_2 consumption for each animal against time (following e.g. Johnston and Battram, 1993; Brodeur et al., 2003; Romero et al., 2006). Definitions of the variables measured were taken mainly from Secor (2009) and Vanella et al. (2010): baseline (BL, mg O_2/h/g wet mass); peak (P, mg O_2/h/g wet mass); factorial scope (FS, P / BL); duration (Dur, h); duration based on meal size (Dur'): time from feeding when metabolic rate is no longer greater than baseline values, relative to kJ g of ingested food (h/kJ g food); food consumed (FC): wet mass of ingested food (g); meal size (MS, %): ingested food as percentage of body mass; meal energy (ME): meal energy determined by bomb calorimetry, relative to mass (kJ/g); specific dynamic action (SDA, kJ/g); SDA coefficient (SDA Coeff); Q_{10}': $(\text{Variable}_{10\,°C\,|\,17hs\ light} / \text{Variable}_{4°C\,|\,7hs\ light})^{10\,°C\,/\,(10\,°C\,-\,4\,°C)}$, where Q_{10}' quantifies the effect of the temperature and day length on biological processes, and it was calculated only for the variables which showed significant differences between treatments (here experimental 'Winter' and 'Summer' treatments differed also on daylight hours).

2.2.6. Statistical analyses

All analyses were performed using the language R 2.15.1 (R Development Core Team, 2012).

2.2.6.1. Wild population

Assumptions of normality (Kolmogorov–Smirnov test, $p < 0.05$) and homogeneity of variances (Levene test, $p < 0.05$) were previously verified for each variable (Sokal and Rohlf, 1995). Given that they were reached in ED, differences between seasons were studied using a one-way ANOVA ($p < 0.05$), and post-hoc Tukey HSD multiple comparisons were used (Sokal and Rohlf, 1995). Otherwise, like in K, GI, HI, FI, DI differences were analyzed with non-parametric Kruskal-Wallis tests.

Single relationships between the ED and different somatic indexes on each season, and also single relationship among GI and DI between mature and maturing individuals (IG > 5%), were studied through Pearson correlations (*t*-test, Zar, 1996).

To explore the influence of the fat reserves, reproduction, food consumption and season in the ED of the individuals, generalized linear models (GLMs) were fitted, considering GI, FI, DI and Season (spring, summer, autumn, winter) as explanatory variables. The heuristic, marginal and random intercept models were built as follows:

heuristic model as ED ~ IG ∗ FI ∗ DI ∗ Season;

random intercept model as ED ~ GI ∗ FI ∗ DI ∗ Season, random = ~ 1 | Season;

and marginal model as ED ~ GI ∗ FI ∗ DI ∗ Season.

The first model is the only one which assumes that seasonal samples are independent. The model with the lowest Akaike's Information Criterion (AIC) was selected as the global model to do selection for the optimal model. Model selection was performed by dropping non-significant terms by one and comparing the short new model to the global model. Residual plots were evaluated for violations of model assumptions.

2.2.6.2. Experiment 1

Differences between treatments and times in TL, TM, K, ED were studied using two-way ANOVA ($p < 0.05$), given that assumptions of normality and homogeneity of variances were reached, followed by post-hoc Tukey HSD multiple comparisons. Differences in MIFD were analyzed through a Mann-Whitney test ($p < 0.05$) because homogeneity of variances was not reached; whereas $MIFD_m$, $MIFD_e$ and GCE were studied with *t*-tests ($p < 0.05$). Comparisons of F, NF and CA between treatments and during the experimental period were assessed using Mann-Whitney tests ($p < 0.05$), given that the low *n* prevented the use of ANOVA or the *t*-test.

In order to compare the distribution of muscle fiber diameter among treatments and time, non-parametric statistical techniques were used to fit smoothed probability density functions (PDF) with a kernel approach (Johnston et al., 1999), using software constructed with R language, kindly provided by I.A. Johnston. Values for the smoothing parameter *h*, which controls the variance of the kernel function, ranged between 0.1600 and 0.1926. Bootstrap techniques were used to distinguish the underlying structure of distributions from random variation. Probability density functions of each experimental group were compared using Kolmogorov-Smirnov tests ($p < 0.05$). The 5th, 10th, 50th, 95th and 99th percentiles of white muscle fiber diameter were calculated for the mean PDF of each experimental group, and compared with non-parametric Wilcoxon two-sample tests ($p < 0.05$).

2.2.6.3. Experiment 2

Differences between Summer/Winter treatments and times in TL, TM, K, GI, HI, FI, DI and ROS were studied with Kruskal-Wallis tests ($p < 0.05$), whereas differences in ED and TAC were studied with two-way ANOVA ($p < 0.05$).

2.2.6.4. Experiment 3

Differences between Summer/Winter treatments on TL, TM, BL, P, FS, Dur, Dur', FC, MS, ME, SDA and SDA Coeff were analyzed with *t*-tests ($p < 0.05$).

3. Results

3.1. Wild population

Mean daily water temperatures of AN fitted the polynomial function:

$$Y = -1E-08 * X^4 + 8E-06 * X^3 - 0.0016 * X^2 + 0.0400 * X + 11.8630 \quad (R^2 = 0.9583)$$

where Y = °C and X = Julian day (1 to 365/6).

All somatic indexes of *G. maculatus* from AN population differed significantly among seasons (Table 1, Kruskal-Wallis followed by nonparametric multiple comparisons, $p < 0.05$). Condition of individuals (K) was significantly lowest during winter. The GI was higher during spring-summer than during autumn-winter. The HI was highest during spring. The fat index (FI) was higher during spring and autumn than during summer and winter. The DI was higher during spring than during summer and autumn. The ED of individuals showed significant differences between seasons (one-way ANOVA, $p < 0.05$) (Table 1), being lower in summer than in spring and autumn (Tukey HSD multiple comparisons, $p < 0.05$).

Correlation coefficient values (r) were found to be significant between ED and FI during spring, autumn, and winter — where ED increases as accumulation of perivisceral fat increases ($r = 0.42–0.58$) — and also between ED and DI during summer — where ED decreases as stomach repletion increases ($r = -0.56$) — (Pearson correlations, $p < 0.05$). Among sexually mature and maturing individuals (IG > 5%) during summer, there was found a significant correlation between GI and DI ($r = -0.55$).

The minimum adequate model found was (parameter ± standard error):

ED = 23.6322 ± 0.2781 ∗ Int + 0.4628 ± 0.0655 ∗ FI − 0.2282 ± 0.0401 ∗ DI tumn + 0.4925 ± 0.1886 ∗ Spring − 0.4648 ± 0.2169 ∗ Summer.

($p < 0.05$, df = 157, AIC = 379.2088); and explained 48.22% of the variation of ED, which was related to FI, DI and season.

3.2. Experiment 1: Growth and food consumption

3.2.1. Total length, mass and condition

Neither during the experimental period nor between treatments TL showed significant differences (two-way ANOVA, $p > 0.05$; Table 2).

TM and K increased significantly during the experiment for the summer group, but no significant differences were found for 'Winter' conditions (two-way ANOVA, $p < 0.05$ and $p > 0.05$, respectively). 'Summer' group at 32 days and 59 days had higher TM and K than at 0 day (Post-hoc Tukey, $p < 0.05$). The TM in 'Summer' group increased by 43.18% at 32 days and by 7.14% from 32 days to 59 days, whereas K of the 'Summer' group increased by 25.71% at 32 days and by 3.60% from 32 days to 59 days (Table 2). After the experimental period, the 'Summer' group had significantly higher TM (about 26%) than the 'Winter' group (Post-hoc Tukey, $p < 0.05$; Table 2). Macroscopic aspect of the gonads of sacrificed individuals at 0, 32 and 59 days showed no signs of gonadal maturation in either 'Winter' or 'Summer' groups.

Table 1
Mean (± standard deviation) seasonal values of Fulton condition index (K), gonadosomatic index (GI, %), hepatosomatic index (HI, %), perivisceral fat index (FI, %), digestive index (DI, %) and energy density (ED, kJ/g AFDW) of *Galaxias maculatus* from the Arroyo Negro diadromous population, Parque Nacional Tierra del Fuego. N in brackets. Equal lower-case letters as superscripts indicate significant differences ($p < 0.05$) between seasons; [A] One-way ANOVA; [K] Kruskal-Wallis test.

Season	K[K]	GI (%)[K]	HI (%)[K]	FI (%)[K]	DI (K)[K]	ED (kJ/g)[A]
Summer (28)	0.44 ± 0.09	11.22 ± 8.67^{cd}	1.16 ± 1.07^{a}	0.19 ± 0.36^{ac}	3.64 ± 2.17^{a}	20.47 ± 1.77^{ac}
Autumn (60)	0.43 ± 0.06^{b}	0.56 ± 0.52^{ac}	1.21 ± 0.38^{b}	1.16 ± 1.20^{cd}	4.84 ± 1.37^{b}	21.28 ± 1.23^{c}
Winter (30)	0.39 ± 0.05^{ab}	0.87 ± 1.90^{bd}	1.49 ± 0.32^{c}	0.46 ± 0.73^{bd}	5.81 ± 1.76	20.72 ± 1.39
Spring (39)	0.43 ± 0.05^{a}	3.71 ± 2.37^{ab}	2.27 ± 0.87^{abc}	1.14 ± 0.96^{ab}	6.25 ± 1.12^{ab}	21.54 ± 1.20^{a}

Table 2
Experiment 1. Variables measured on *G. maculatus* (mean ± standard deviation) under 'Summer' and 'Winter' experimental conditions of temperature and day length at 0, 32 and 59 days of the growth and food consumption experiment (0 day, 32 days and 59 days respectively). Total length (TL, mm), total mass (TM, g), Fulton condition index (K), energy density of individuals (ED, kJ/g), ingested food or food consumed as percentage of aquarium biomass (MIFD, %), ingested food on a daily basis on mass units ($MIFD_m$, g/g day) and expressed on energy units ($MIFD_e$, kJ/g day), gross conversion efficiency (GCE), fiber number (F), indicating the number of white fibers per cross sectional area; percentage of new fibers (NF, %) indicating the percentage of fibers < 20 μm; Cross sectional area (CA, mm^2). N in brackets. * Significant differences ($p < 0.05$) between 'Summer' and 'Winter' treatments. Equal lower-case letters indicate significant differences ($p < 0.05$) between 0 day, 32 days and 59 days times. [A] Two-way ANOVA; [M] Mann-Whitney test; [T] *t*-test.

	Summer			Winter		
	0 day	32 days	59 days	0 day	32 days	59 days
TL (mm)[A]	62.63 ± 2.43 (8)	65.29 ± 2.80 (8)	66.04 ± 3.15 (8)	63.42 ± 3.28 (8)	64.51 ± 1.90 (8)	64,08 ± 3.11 (8)
TM (g)[A]	0.88 ± 0.16^{ab} (8)	1.26 ± 0.25$^{*\,a}$ (8)	1.35 ± 0.33$^{*\,b}$ (8)	0.90 ± 0.21 (8)	1.08 ± 0.13* (8)	1.07 ± 0.20* (8)
K[A]	0.35 ± 0.00^{ab} (8)	0.44 ± 0.00$^{*\,a}$ (8)	0.46 ± 0.01$^{*\,b}$ (8)	0.34 ± 0.00 (8)	0.41 ± 0.01 (8)	0.41 ± 0.00 (8)
ED (kJ/g)[A]	19.93 ± 0.81 (8)	–	20.09 ± 1.04 (8)	20.62 ± 0.96 (8)	–	20.29 ± 1.83 (8)
MIFD (%)[M]	–	–	0.10 ± 0.02 (8)	–	–	0.11 ± 0.06 (8)
$MIFD_m$ (g/g day)[T]			0.03 ± 0.01 (8)			0.03 ± 0.02 (8)
$MIFD_e$ (kJ/ day)[T]			0.64 ± 0.05 (8)			0.69 ± 0.29 (8)
GCE[T]	–	–	20.97 ± 4.16 (8)	–	–	9.26 ± 2.55 (8)
F (fibers)[M]	619 ± 73.87 (5)	585 ± 50.92 (5)	628 ± 45.39 (7)	608 ± 43.92 (5)	649 ± 72.53 (6)	659 ± 123.83 (4)
NF (%)[M]	1.49 ± 1.42 (5)	0.67 ± 0.54 (5)	0.19 ± 0.25 (7)	1.21 ± 0.46 (5)	1.26 ± 1.18 (6)	1.05 ± 0.61 (4)
CA (mm^2)[M]	1.56 ± 0.18^{b} (5)	2.35 ± 0.37^{b} (5)	2.53 ± 0.39* (7)	1.52 ± 0.32^{a} (5)	2.00 ± 0.38^{a} (6)	1.97 ± 0.21* (4)

3.2.2. Energy density (ED)

The ED (kJ/g) did not differ significantly among experimental groups or during the experiment (two-way ANOVA, $p > 0.05$). Mean ED was 20.23 kJ/g (Table 2).

3.2.3. Food consumption/intake

The MIFD (%) did not differ between treatments (Mann-Whitney, $p < 0.05$; Table 2), being about 11% of biomass. Neither $MIFD_m$ (g/g day) nor $MIFD_e$ (kJ/g day) differed between the Summer and Winter treatments (*t*-test, $p < 0.05$; Table 2).

3.2.4. Energy assimilation

Gross conversion efficiency was significantly lower in 'Winter' than in Summer treatment (*t*-test, $p < 0.05$; Table 2), being winter efficiency 44% of the 'Summer' efficiency.

3.2.5. Muscle fibers

The F and NF did not differ statistically between treatments or during the experimental period (Mann-Whitney, $p > 0.05$). Mean F value per cross sectional area was 625 fibers. Mean NF values accounted for up to 1% of the total number of white fibers (Table 2).

Significant differences were found for cross sectional area (CA) between experimental groups at 59 days (Mann-Whitney, $p < 0.05$), which was about 28% higher in the 'Summer' than in the 'Winter' group. In addition, the CA of both groups increased significantly between 0 day and 32 days (about 31% of initial CA values in the winter group and about 51% in the summer group) (Table 2).

The PDF of muscle fiber diameter differed significantly between treatments at 32 days and at 59 days (Kolmogorov-Smirnov, $p < 0.05$; Fig. 1). The peak of the distribution remained at about 40–45 μm during the whole experimental period for the 'Winter' group indicating the absence of muscle growth, whereas the peak for the 'Summer' group shifted from 40 μm (at 0 day) to 50 μm (at 32 days), and finally to 60 μm (at 59 days), summarizing a rate of 10 μm/month. Furthermore, the 'Winter' group had a higher proportion of smaller fibers, while the 'Summer' group had a higher proportion of fibers on the right-hand tail (highest fiber diameters) compared to the 'Winter' group; this difference between treatments became more evident at 59 days (Fig. 1c). All calculated percentiles (5, 10, 50, 95 and 99) of mean PDF for the summer groups were higher than those of the winter groups, both at 32 days and at 59 days. Nevertheless, significant differences were found between treatments only for the tenth percentile at 59 days (Wilcoxon, $p < 0.05$, Table 2).

3.3. Experiment 2: Energy allocation and oxidative metabolism parameters

3.3.1. Total length, mass and condition

The TL did not show significant differences during the experimental period or between treatments (Kruskal-Wallis, $p > 0.05$; Table 3). After the experimental period the 'Summer' group had significantly higher TM and K (34.33% and 28.57%, respectively) than the 'Winter' group (Kruskal-Wallis, $p < 0.05$; Table 3).

3.3.2. Somatic indexes and energy density (ED)

The DI and GI indexes did not differed significantly between treatments nor times (Kruskal-Wallis, $p < 0.05$, Table 3), mean values were 3.76% and 3.12%, respectively. The HI increased significantly during the experiment (Kruskal-Wallis, $p < 0.05$, Table 3), about 29% and 15% during 'Summer' and 'Winter' treatments, and the FI decreased significantly during Winter treatment (Kruskal-Wallis, $p < 0.05$, Table 3), about 66%. The ED (kJ/g) did not differ significantly among experimental groups or during the experiment (two-way ANOVA, $p > 0.05$). Mean ED was 21.01 kJ/g (Table 3).

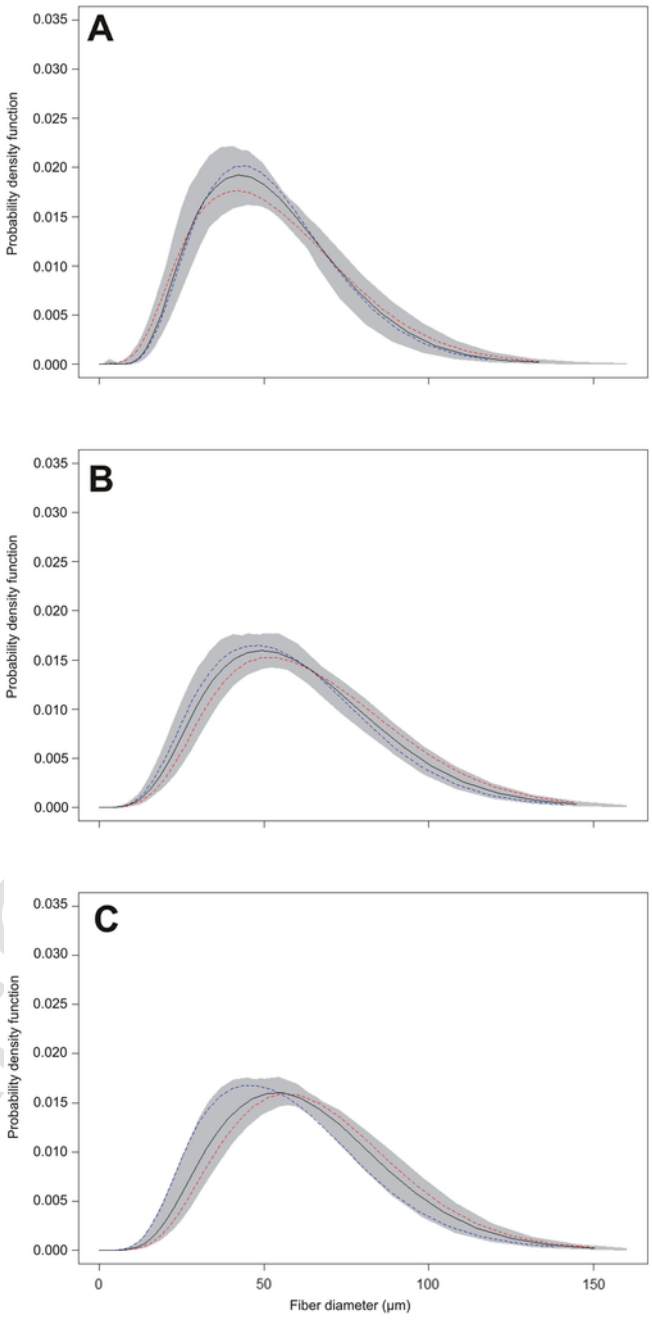

Fig. 1. Mean value (lines) and variability band (shaded area) of the probability density function (Prob. density function) of muscle fiber diameter (μm) of experimental groups of *Galaxias maculatus* at 'Summer' (blue) and 'Winter' (red) treatments at 0 (A), 32 (B) and 59 (C) days of experiment (0 day, 32 days and 59 days respectively). The shaded area represents 1000 bootstrap estimates of the combined groups of 'Winter' and 'Summer' treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3.3. Oxidative metabolism parameters

The formation of reactive oxygen species (ROS) in muscle increased significantly during the experimental period, from about 147 to about 293 (Relative area/WW) in both treatments (Kruskal-Wallis, $p < 0.05$, Table 3). Whereas the total antioxidant capacity (TAC) in

Table 3
Experiment 2. Variables measured on *G. maculatus* (mean ± standard deviation) under 'Summer' and 'Winter' experimental conditions of temperature and day length at 0 and 32 days of the energy allocation and oxidative metabolism parameters experiment (0 day and 32 days respectively). Total length (TL, mm), total mass (TM, g), Fulton condition index (K), energy density of individuals (ED, kJ/g), gonadosomatic index (GI, %), hepatosomatic index (HI, %), perivisceral fat index (FI, %), digestive index (DI, %), formation of reactive oxygen species (ROS) and TAC (Relative area/WW). N in brackets. * Significant differences ($p < 0.05$) between Summer and Winter treatments. Equal lower-case letters indicate significant differences ($p < 0.05$) between 0 day and 32 days times. [K] Kruskal-Wallis test; [A] Two-way ANOVA; [M] Mann-Whitney test; [T] *t*-test.

	Summer		Winter	
	0 day	32 days	0 day	32 days
TL (mm)[K]	62.40 ± 1.55 (16)	62.39 ± 2.45 (16)	61.60 ± 1.57 (16)	61.77 ± 2.41 (16)
TM (g)[K]	0.82 ± 0.26 (16)	0.90 ± 0.24* (16)	0.73 ± 0.25 (16)	0.67 ± 0.10* (16)
K[K]	0.33 ± 0.08 (16)	0.36 ± 0.07* (16)	0.31 ± 0.07 (16)	0.28 ± 0.02* (16)
ED (kJ/g)[A]	21.06 ± 0.97 (8)	20.50 ± 1.39 (8)	20.93 ± 1.54 (8)	21.54 ± 0.87 (8)
GI (%)[K]	6.12 ± 9.19 (16)	2.01 ± 2.99 (15)	3.28 ± 8.30 (16)	1.06 ± 1.59 (16)
HI (%)[K]	1.05 ± 0.17[d] (16)	1.35 ± 0.37[d] (17)	1.00 ± 0.10[c] (12)	1.15 ± 0.17[c] (24)
FI (%)[K]	0.59 ± 0.50 (14)	0.53 ± 0.47 (15)	0.67 ± 0.45[e] (14)	0.23 ± 0.26[e] (18)
DI (%)[K]	3.40 ± 0.40 (16)	4.64 ± 1.72 (16)	3.44 ± 1.03 (16)	3.58 ± 0.47 (16)
ROS (Relative area/WW)[K]	145.72 ± 63.59[g] (8)	287.54 ± 114.23[g] (9)	148.02 ± 41.89[f] (8)	298.64 ± 115.71[f] (7)
TAC (Relative area/WW)[A]	0.56 ± 0.12[i] (8)	0.38 ± 0.13[i] (9)	0.56 ± 0.09[h] (8)	0.32 ± 0.07[h] (7)

muscle decreased significantly from 0.057 to 0.036 (Relative area/WW) during the same period (two-way ANOVA, $p < 0.05$; Table 3).

3.4. Experiment 3: Oxygen consumption

The O_2 consumption of *G. maculatus* at Winter and Summer treatments is shown in Fig. 2. Baseline (BL), peak (P), factorial scope (FS), and duration (Dur) did not show significant differences between treatments (*t*-test, Table 4). Duration based on meal size (Dur') resulted in 'Winter' about 1.5 fold than 'Summer' treatment, being this a significant difference (*t*-test, $p < 0.05$). Food consumed (FC), meal size (%M) and meal energy (ME) were significantly higher for 'Summer' treatment compared to 'Winter' one (*t*-test, $p < 0.05$), being the difference about 1.5 fold too. Q_{10}' values were near 2 for FC, MS, and ME, and near 0.5 for Dur' (Table 4).

3.5. Power budget

The partial power budget for one-year old *G. maculatus* under experimental 'Summer'/'Winter' conditions of day length and temperature is shown in Table 5. The energy intake retained as growth varied from about 8 to 4 J/g h in 'Summer' and 'Winter', respectively. The energy allocated to basal metabolism (R_BL) and to process food (R_SDA) did not vary between treatments, being about 1.6 and 0.3 J/g h, respectively. The remainder of energy intake (17 and 23 J g/h under 'Summer' and 'Winter', respectively) would be allocated to unmeasured excretion, lost in faeces and also to locomotor activity.

4. Discussion

Present results suggest that both winter and summer are 'energetically high-cost seasons' for individuals of the diadromous *G. macula-*

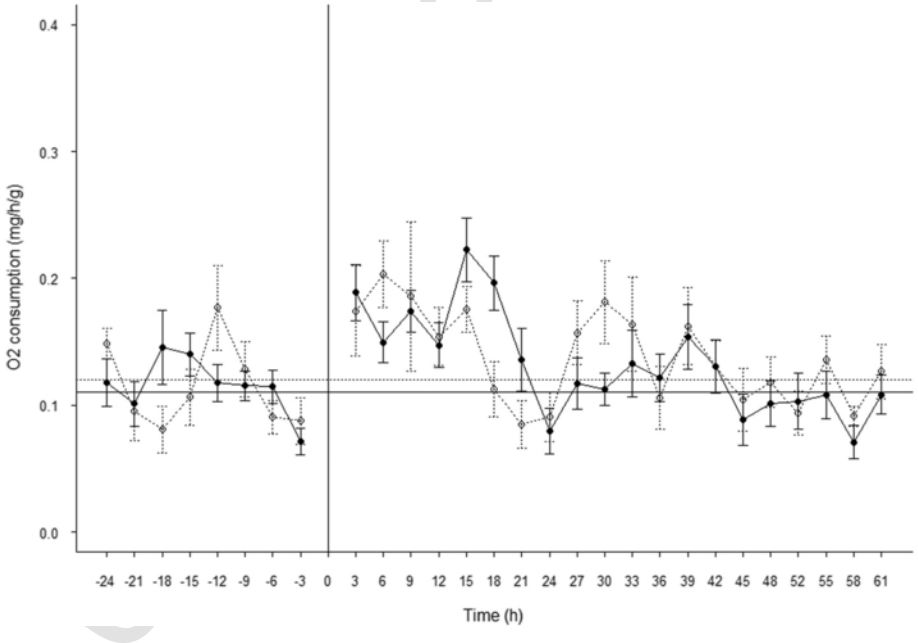

Fig. 2. Pre- and post-prandial oxygen consumption (mg/h/g wet mass, mean ± standard deviation) of experimental groups of *G. maculatus* at 'Summer' (solid circles) and 'Winter' (open circles) treatments. Fish were fed at 0 h (vertical line). In order to avoid the influence of handling stress, baseline was calculated using data from last 24 h before feeding at Summer (solid line) and Winter (dotted line) treatments.

Table 4
Experiment 3. Respirometry variables (mean ± standard deviation) of *G. maculatus* from 'Summer' and 'Winter' treatments. Total length (TL, mm), total mass (TM, g), baseline (BL, mg O_2/h/g wet mass), peak (P, mg O_2/h/g wet mass), factorial scope (FS, P/BL), duration (Dur, h), duration based on meal size (Dur', h/kJ food), food consumed (FC, g), meal size or food consumed as percentage of body mass (MS, %), meal energy (ME, kJ/g), specific dynamic action (SDA, kJ/g), SDA coefficient (SDA Coeff, %) and Q_{10}' (calculated for variables with significant differences), (N) = sample size. * Significant differences (*t*-test, $p < 0.05$) between 'Summer' and 'Winter' treatments.

	Summer	Winter	Q_{10}'
TL (mm)	62.27 ± 1.49	63.15 ± 1.91	–
TM (g)	0.86 ± 0.12	0.78 ± 0.13	–
BL (mg O_2/h/g wet mass)	0.11 ± 0.04	0.12 ± 0.06	–
P (mg O_2/h/g wet mass)	0.25 ± 0.08	0.31 ± 0.13	–
FS (P/BL)	2.26 ± 0.49	2.83 ± 0.98	–
Dur (h)	29.16 ± 9.76	28.10 ± 11.71	–
Dur' (h/kJ food)	63.96 ± 27.99*	92.80 ± 33.11*	0.54
FC (g)	0.10 ± 0.03*	0.06 ± 0.01*	2.26
MS (%)	11.15 ± 2.83*	7.89 ± 1.91*	1.78
ME (kJ/g)	0.57 ± 0.13*	0.40 ± 0.09*	1.81
SDA (kJ/g)	0.30 ± 0.25	0.37 ± 0.22	–
SDA Coeff (%)	4.73 ± 3.29	5.64 ± 3.33	–
N	12	11	

Table 5
Partial power budget of *Galaxias maculatus* under 'Summer' and 'Winter' experimental conditions of temperature and day length in the 59 days growth experiment; sensu Brett and Groves (1979; C = G + R + F + U). Energy intake (C, J/g h), energy stored in body (growth, G, J/g h), energy consumed in metabolism (R, J/g h), energy consumed in basal metabolism (R_BL, J/g h), energy consumed to process food (R_SDA, J/g h). The energy lost in faeces and excretion (F + U, J/g h) and consumed in locomotion (R_LOC, J/g h) were calculated by difference F + U + R_LOC = C − G − R, where R = R_BL + R_SDA. Values (mean ± standard deviation).

	Summer	Winter
C (J/g h)	26.46 ± 2.25	28.95 ± 12.01
G (J/g h)	7.74 ± 0.77	3.90 ± 0.56
R (J/g h)	1.88 ± 0.68	1.97 ± 0.85
R_BL (J/g h)	1.58 ± 0.52	1.71 ± 0.83
R_SDA (J/g h)	0.30 ± 0.26	0.26 ± 0.18
F + U + R_LOC (J/g h)	14.96 ± 0.68	21.11 ± 0.85

tus population, given that they show their minimum values of ED and accumulation of perivisceral fat during those seasons. Experimental 'Winter' and 'Summer' groups both showed increased ROS production and decreasing TAC. The combination of studies on a wild population under experimental conditions allowed us to explore the causes of this variation, despite differences between wild and experimental diets. Seasonal variation of ED (about 48% of its variation) is explained by food consumption and perivisceral fat accumulation. Although accumulated perivisceral fat is used to maximize winter survival in this population (Boy et al., 2007), this energy reservoir could not attain the required level to maintain constant autumn ED values of individuals during winter (Table 1), nor to sustain growth (Table 2). Moreover, the high energy requirements for reproduction during summer (Boy et al., 2007, 2009), added to the cost of growing, could lead to the surprising a priori lowest energy density of the individuals. The inverse relationship found between DI and the degree of gonadal maturation could be another reason for the low-energy density values during summer. Mature individuals reduce their ingestion, probably given that gonads fill the body cavity almost completely, as it occurs in other species (e.g. *Ammodytes hexapterus*, Robards et al., 1999).

One-year-old adult individuals of *G. maculatus* exhibit a three times lower basal metabolic rate than the juveniles studied by Urbina and Glover (2015). Differences found could be attributed not only to experimental conditions (said study was performed under 14 °C and a photoperiod of 12L:12D), but mainly to the effect of body size. It is already known that small fish will consume more oxygen per gram of tissue than larger conspecifics, and also that this relationship is allometric (Jobling, 1994). Individuals ingest similar volumes of food under experimental 'Winter'/'Summer' conditions of day length and temperature (Tables 2, 4); and a similar situation could also occur in the wild, as it is suggested from DI values (Table 1). They also spend the same amount of energy to maintain basal metabolism (about 6% of C) and to process ingested food (about 1% of C) under 'Winter' and 'Summer' conditions (Table 5). Nevertheless, individuals allocate twice as much energy to growing in 'Summer' (28% of C) than in 'Winter' (14% of C), when the energy allocated is just enough to maintain body mass (Tables 2, 4) but not for growth, resulting that experimental 'Summer' groups gained more energy in weight (127%; gross conversion efficiency, GCE) than 'Winter' ones. Condition (K) and energy reserves (FI, HI) of *G. maculatus*' individuals diminish under 'Winter' experimental conditions (Tables 2, 3), suggesting a mobilization of energy from reserves to face the winter costs which could not be fueled with assimilated food. A similar response was observed by Urbina and Glover (2015) in *G. maculatus* exposed to different salinities. These authors observed that oxygen consumption was not affected by external salinity, but nitrogen excretion was. The authors explained this as a switch in substrate use, similar to the proposal made in this work. The findings of Urbina and Glover (2015) and present study suggest a capacity of the species to sustain the metabolic rate under a broad range of external conditions at the expense of obtaining energy from somatic reserves. Future measurements of nitrogen excretion under experimental 'Summer/Winter' treatments would help to elucidate this question.

We propose a hypertrophic muscle growth rate for *G. maculatus* of about 10 μm/month under 'Summer' experimental conditions. The absence of growth during winter conditions was observed not only in TL and TM, but also on the cross-sectional area of the caudal peduncle and white fiber diameter. Differences found between summer/winter growth may result from differences in 'terms' of the power budget not measured here (faeces, excretion and locomotion), that shape the scope for growth, i.e. the surplus of energy available for growth beyond that required for maintenance. It has long been recognized that the swimming performance of fish is influenced by water temperature. Likewise, similar level of observed locomotor activity not necessarily would mean similar cost of this activity (e.g. Webb, 2002; Alexander, 2005; Claireaux et al., 2006). There is no consensus about the thermal effect on the net costs of transport (the metabolic rate during swimming/standard metabolic rate) at the whole-animal level. There are some examples in the literature where swimming cost was shown to be influenced by water temperature (reviewed by Johnston and Temple, 2002; Fernández et al., 2002; Vanella et al., 2012), though in some species there seems to be no thermal effect (e.g. Claireaux et al., 2006; Ohlberger et al., 2007). Summarizing, more research is needed, e.g. on costs of activity of *G. maculatus*.

Present results provide evidence of the potential ability of this diadromous population of *G. maculatus* to maintain basal metabolism, locomotion and feeding activity during both winter and summer, and that both are highly energy demanding seasons. A partial bioenergetics equation for adult *G. maculatus* is also proposed, complementing the findings of Urbina and Glover (2015) for juveniles, in order to provide a tool for estimating the costs of the different activities of this species. This work highlights the need to integrate the study of wild populations with experimental work in order to enhance the comprehension of energy utilization strategies in relation to environmental factors. The information provided herein from growth and respirometry experiments, and previous information about the puyen wild population (Boy et al., 2007, 2009, 2013) suggest that the energy alloca-

tion strategy differs between winter and summer, with a higher proportion of energy that could be allocated to growth and reproduction in summer, while in winter a high proportion of energy could be allocated to maintain activity. Further research is needed to elucidate if there is a trade-off in the allocation of energy for physiological functions and how it could be affected by winter and summer conditions and its impact on the *fitness* of the individuals in the natural population.

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