

Energy allocation in relation to spawning and overwintering of a diadromous Puyen (*Galaxias maculatus*) population in the southernmost limit of the species distribution

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Abstract A population of *Galaxias maculatus*, a native species in southern South America, living under cold-temperate conditions (0°C water temperature with an ice layer covering the stream during the coldest days) and 7 h of light in winter undergoes high energetic demands in the population studied. We analyzed the energy density of gonads, liver, fat and muscle, through calorimetry for the first time in this species. Energy density of fat and liver were extremely high (34.77–56.52 and 29.54–40.77 kJ/g respectively). While perivisceral fat reserves were likely used for overwintering, liver and muscle reserves were used for reproduction. High energy densities were also found in gonads (27.76 kJ/g in ovaries and 25.84 kJ/g in testes). High investment in gonads of males suggests the presence of sperm competition. The temporal variation of the energy content of gonads, liver, fat and muscle indicates that the allocation of energy occurs by the internal transference of energy between organs and tissues.

Keywords Energy density · Calorimetry · Organs · Reproduction · Overwintering · *Galaxias maculatus*

Introduction

The understanding of the energy balance of an organism is based on knowledge of the temporal variation in energy

acquisition and its use in maintenance, growth and reproduction. Variation in these activities has been shown to be correlated with changes in the energy density of different organs (Jobling 1995; Lucas 1996). Energy density of an organism influences its survival and reproduction as well as growth; therefore, the knowledge of this variable enhances bioenergetics models of fish growth.

The broad seasonal fluctuations in temperature and productivity, characteristic of high latitude environments (Clarke 1983), influence the availability and quality of food for adults and young fish (Beamish and Bouillon 1995). Consequently, the accumulations of energy reserves in organisms is likely to show temporal variations. Then somatic growth, energy storage and reproduction must be synchronized with the more favorable environmental conditions, producing a mobilization of reserves between different somatic compartments (Calow 1985; Sibly and Calow 1986; Lucas 1996). The available energy intake is low during winter, and depletion of energy is regarded a major cause of mortality in freshwater fishes of cold and cold-temperate environments (Gardiner and Geddes 1980; Post and Evans 1989; Miranda and Hubbard 1994; Finstad et al. 2004).

The ‘inanga’ or ‘puyen’, *Galaxias maculatus* (Jenyns), is one of the world’s most widespread freshwater fish species in the southern hemisphere, with landlocked and diadromous populations, inhabiting lakes, rivers and sea coasts of Australia, New Zealand, Tasmania and southern South America (McDowall 1968; Waters and BurrIDGE 1999; Cussac et al. 2004). Few attempts have been made to estimate energy density in *G. maculatus*. Some earlier determinations of whole body energy density of this species reported values of 22 kJ/g dry mass in Lake Ellesmere, New Zealand (Ryan 1982), and 23 kJ/g dry mass in the Río Limay (39°S, 69°W), in South America, near the northern

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limit of species geographical distribution (Ciancio and Pascual 2006). The Río Ovando population is one of the most southerly known for this species (54°50'S, 68°34'W). It offers an opportunity to study the effects of strong seasonal variations in water temperature (between 0 and 15°C), photoperiod (7–17 h of light) and food availability (Boy et al. 2007). Studies on the dynamics of the energy allocation and, in particular, on the depletion of fat reserves in this species can contribute to the knowledge of its physiology.

The present study describes, for the first time in *G. maculatus*, energy density of gonads, liver, perivisceral fat and muscle; and analyzes the seasonal pattern of energy acquisition and allocation among the different organs. We hypothesize that the energetic requirements to overcome winter are supported by perivisceral fat and reproductive maturation (which occurs from spring to autumn) are supported by liver and muscle.

Materials and methods

Study site and sampling

The study was carried out in the Río Ovando estuary (54°50'S, 68°34'W) in the Parque Nacional Tierra del Fuego, Argentina. The Río Ovando estuary is a flat plain with tidal influence, where salinity fluctuates between 0 and 20‰ (Boy et al. 2007). Sea water temperature monthly means vary between a minimum of -2°C in winter and a maximum of 15°C in summer. Photoperiod varies between 7 h/day in winter to 17 h/day in summer (Iturraspe et al. 1989). In the coldest months the stream remains covered by a layer of ice (Boy et al. 2007).

Fish were captured monthly with a seine net (10-m long, 1-m deep, 0.7-mm mesh) between February 2005 and February 2006. Specimens were anesthetized to death with MS-222, measured with a digital caliper (total length (TL), 0.01 mm), weighed (total wet mass (M), 0.01 g) and sexed. Adult individuals, larger than 57 mm, were selected for calorimetric determinations. The specimens were dissected and the wet mass of the gonads (M_G), the liver (M_L), perivisceral fat (M_F) and stomach and gut (M_D) obtained (0.0001 g). A portion of axial muscle (without bone and skin) was removed from the caudal end of the trunk with a scalpel.

The total eviscerated wet mass (EM) for each animal was calculated as $EM = M - (M_G + M_L + M_F + M_D)$. The Fulton condition index eviscerated ($Kevis = (EM \times 100 \times TL^{-3}) \times 10^3$) was calculated for each specimen. A factor of 10^3 in Kevis was added to reduce the decimal positions. The calculation of the Kev index was performed because it was previously found that the species exhibits a great

variation in total mass through the year mostly caused by the gonads that represent about 30% of the total mass of the fish during the reproductive season, from October to February (Boy et al. 2007). Differences on Kevis indexes between sexes could not be tested because the gonads were extremely reduced, to less than 1% of the total mass of fish (about 0.01–0.03 g wet mass of gonads), in winter and therefore the specimens could not be sexed.

The dry mass of gonads (DM_G), liver (DM_L) and perivisceral fat (DM_F) of each specimen was determined after drying at 70°C to a constant mass. In order to obtain the energy density of the whole fish, puyen of different sizes were measured, weighed, sexed and dried at 70°C to a constant mass. All dry samples were stored in sealed bags at -20°C until processing.

Given the low mass of gonads, liver and fat, calorimetric determinations of those organs and tissues were carried out exclusively during the reproductive season when they are at their maximum mass. We chose individuals with ripe gonads by macroscopic examination.

Calorimetric determinations

Dry samples for calorimetric determinations were ground and pellets were made with a press (Parr 2812). The caloric content of each sample was obtained by burning pellets of 20–200 mg in a micro-bomb calorimeter (Parr 1425). The values obtained were corrected for ash and acid content and expressed as kJ/g AFDW (energy density, ED). Benzoic acid calibrations were carried out periodically. The energy content of mature gonads, liver and perivisceral fat were calculated as: $EC = ED \times DM$. Whenever possible we used the same number of fish for each organ, but the difference in mass between organs sometimes made this impossible. Therefore, sample sizes are not consistent across organs and are limited in some cases.

Perivisceral fat was included in the study because preliminary data showed variation through the year suggesting it would play a role as energy reservoir in *G. maculatus* (Boy et al. 2007).

Statistical analysis

Monthly differences in Kev, and ED (kJ/g) and EC (kJ) of the gonads, liver and perivisceral fat were analyzed with a non-parametric analysis of variance (Kruskal–Wallis test). Differences between sexes were analyzed (when possible) through a Mann–Whitney test. Dunn's Multiple Comparisons tests were performed for each organ or tissue when significant differences between months were found (Zar 1984; Sokal and Rohlf 1995).

A regression analysis between TL and M was performed comparing months where Kev values were minimum (May,

June, July and August) and maximum (September, October and November). The regression slopes were compared with a Student's *t* test.

An analysis of energy density in relation to size was carried out as a preliminary study of the energy density of whole fish, although its variation over time was not studied. The relationship between ED (kJ/g) and size (TL, mm) was studied with a regression analysis.

Results

Fulton condition index

Key values ($n = 583$) varied highly significantly between months (Kruskal–Wallis; $H = 249.71$, $P = 0.00$, respectively), with lower values in winter than in summer. The progressive fall of the values during the cold season was reversed at the beginning of spring. Afterwards, Key index increased and maintained high values during the whole reproductive season (October–February). The increase of total mass in relation to size was significantly lower from May to August [M (g) = $-2.5183 + 0.0542TL$ (mm)] than from September to November [M (g) = $-4.0787 + 0.0804TL$ (mm)] (comparison of slopes: $t = -52.04$, $P < 0.05$).

Gonads

Median ED was significantly higher in females than in males (Mann–Whitney; $U = 239.00$, $P = 0.00$; $n = 27$ females and 52 males). Median values were 27.76 kJ/g in ovaries and 25.84 kJ/g in testes (Fig. 1a). Monthly differences were significant in males (Kruskal–Wallis; $H = 9.10$, $P = 0.03$) but not in females (Kruskal–Wallis; $H = 8.18$, $P = 0.47$). Dunn's multiple comparisons of ED of different months performed in males were not significant in any contrast, due to the low statistical power of the test, but February showed the highest energy density value.

Median EC was significantly higher in females than in males (Mann–Whitney; $U = 490.00$, $P = 0.01$), median EC was 2.46 kJ for ovaries and 1.73 for testes (Fig. 2a). Median EC of gonads between months did not show significant differences for females (Kruskal–Wallis; $H = 7.59$, $P = 0.11$) but was significantly different for males (Kruskal–Wallis; $H = 16.21$, $P = 0.00$). In February, the gonads of males had lower EC values than those for November and January (Dunn's Multiple Comparisons, $P < 0.05$).

Liver

The ED showed significant monthly differences for females (Kruskal–Wallis; $H = 11.91$, $P = 0.02$; $n = 42$) with values

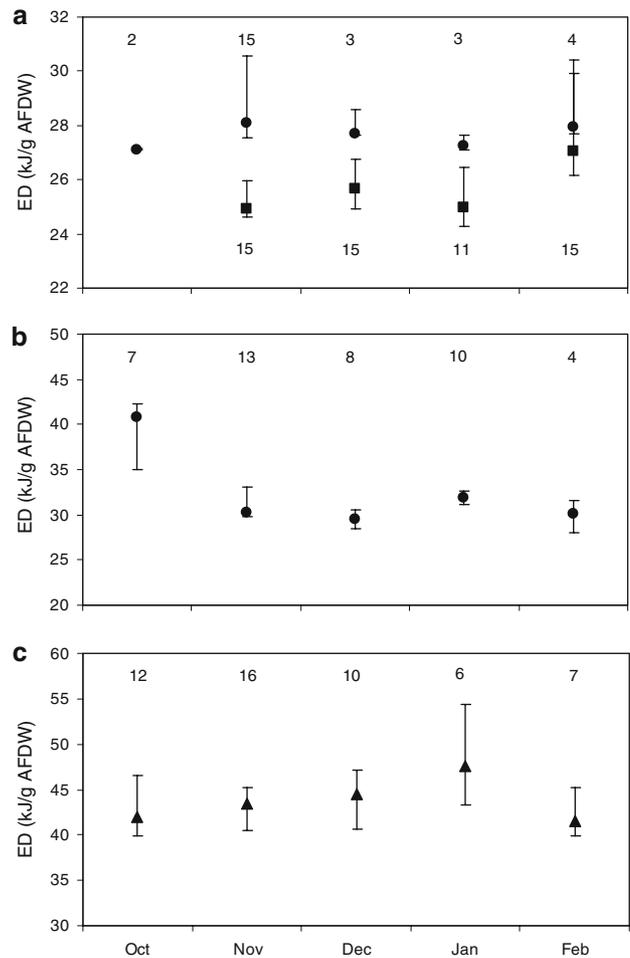


Fig. 1 Monthly variation of energy density (ED, kJ/g AFDW) of *G. maculatus* during the spawning period. Median, second and third quartiles, n given above and below bars. **a** Ripe ovaries (filled circle), Ripe testes (filled square). **b** Liver. **c** Perivisceral fat

varying between 29.54 and 40.77 kJ/g (Fig. 1b). ED values for October were significantly higher than those for December (Dunn's Multiple Comparisons, $P < 0.05$). Only females were analyzed because the male liver dry masses were under the minimum needed for calorimetric determinations. There were no significant differences in EC monthly median values of the livers between months (Kruskal–Wallis; $H = 7.66$, $P = 0.10$), the median EC of liver was 0.62 kJ (Fig. 2b).

Perivisceral fat

The ED values for each individual varied between 34.77 and 56.52 kJ/g (Fig. 1c). No significant differences were found in the ED of perivisceral fat between sexes (Kruskal–Wallis; $H = 0.77$, $P = 0.38$; $n = 32$ females and 19 males) or months (Kruskal–Wallis; $H = 4.17$, $P = 0.38$). Ninety (90) percent of individual values were higher than 38 kJ/g. Median was 43.37 kJ/g. The EC median monthly values of perivisceral

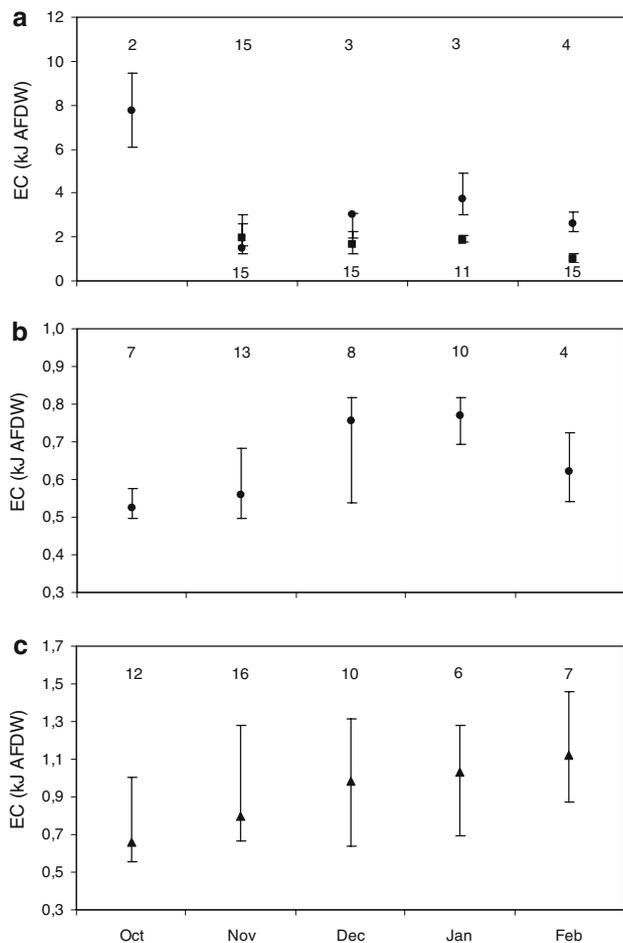


Fig. 2 Monthly variation of energy content (EC, kJ AFDW) of *G. maculatus* during the spawning period. Median, second and third quartiles, *n* given above and below bars. **a** Ripe ovaries (filled circle), Ripe testes (filled square). **b** Liver. **c** Perivisceral fat

fat did not vary between months (Kruskal–Wallis; $H = 2.96$, $P = 0.5651$). The median EC was 0.90 kJ (Fig. 2c).

Muscle

No significant differences attributable to sex were found in the ED (Kruskal–Wallis; $H = 0.31$, $P = 0.58$; $n = 121$ females and 84 males) (Fig. 3). ED values differed significantly between months (Kruskal–Wallis, $H = 32.52$, $P = 0.00$), with median values between 23.77 and 25.76 kJ/g. ED values for February were significantly lower than those for May and June, and values for May, June and August were significantly higher than those for September, November and December (Dunn's Multiple Comparisons; $P < 0.05$).

Whole fish

Energy density varied between 22.86 and 26.87 kJ/g (mean = 24.90, SD = 0.96, $n = 43$). The energy density

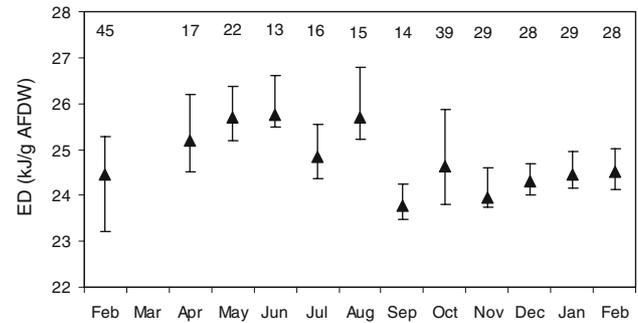


Fig. 3 Monthly variation of muscle energy density (ED, kJ/g AFDW) of *G. maculatus*. Median, second and third quartiles, *n* given above bars

decreases with size (ED (kJ/g) = $-0.0358 \times TL$ (mm) + 27.2760, $R^2 = 0.1107$, $P = 0.03$).

Discussion

The maximum values of ED in ovaries of puyen (29.7 kJ/g; Fig. 1a) were higher than those registered for most teleost species, such as 16 sub-Antarctic and Antarctic notothenioids (Vanella et al. 2005; Fernández et al. 2008), *Merluccius hubbsi* (Montecchia et al. 1990), *Clupea pallasii* (Paul and Paul 1999), *Engraulis mordax* (Hunter and Leong 1981) and *Pomoxis annularis* (Bunnell et al. 2007).

The fall of the liver ED at the beginning of the reproductive season (Fig. 1b) suggests a transference of energy from the liver to the gonads, as was found in several teleost species (Eliassen and Vahl 1982; Smith et al. 1990; Montecchia et al. 1990; Kamler et al. 2001; Huntingford et al. 2001; Vanella et al. 2005). Accumulated perivisceral fat is used by puyen (Boy et al. 2007) and other fishes (Newsome and Leduc 1975; Foltz and Norden 1977; Adams et al. 1982; Booth and Keast 1986) to overwinter instead of being used for gonadal growth (Giussani et al. 1989, quoted by Luzzana et al. 1996). The very high energy density values found in puyen fat (up to 56 kJ/g; Fig. 1c) are greater than those reported in the literature. However, there is very limited information about energy density of fat in fishes determined directly by calorimetry. Energetic budgets are usually calculated indirectly from the proximal biochemical composition, which assume about 39 kJ/g of dry mass for the complete oxidation of fatty acids. High energetic values similar to the ones determined in this study (more than 50 kJ/g) were also found in calorimetric analyses of whole body of small size specimens of sub-Antarctic mesopelagic fish, such as *Gymnopolus braueri*, *Protomyctophum andriashevi* (Tierney et al. 2002) and *Theragra chalcogramma* (Paul et al. 1998). There are no clear explanations for these unexpected high values. van de Putte et al. (2006) assume

that the highest values obtained by Paul et al. (1998) could be caused by the existence of “undetermined random factors”, and the high values obtained by Tierney et al. (2002) could be caused by “possible outliers which could have led to an overestimate of the reported mean value in small fish”. We believe those high values will become more common when most studies performed direct measurements. The ED values of whole fish found in the present study are higher than the mean of 23 kJ/g reported by Ciancio and Pascual (2006) in puyen from northern continental Patagonia (39°S). This difference could be due to geographical differences probably related to variation in the diet (Lumb et al. 2007).

In puyen, energy content of testes is approximately 8% of the somatic energy (Fig. 2a). This value is similar to about 10% found in parr of anadromous brown trout (Jonsson and Jonsson 2005). Salmon parr often compete for fertilization of eggs producing much more milt than species that do not exhibit sperm competition (Jonsson and Jonsson 2005). Vladoic et al. (2002) found a positive association between spermatocrit and the energy content of the milt in salmon, so males with large gonads produce more sperm than those with smaller ones thus increasing their success by partitioning relatively more energy for sperm production. The high energy density and energy content found in the testes (Figs. 1a, 2a) provides evidence for the sperm competition suggested in a previous work for the same puyen population (Boy et al. 2007).

The added energy content of gonads, liver and perivisceral fat (Fig. 2) is 12–24% of the energy content of fish during the reproductive season. Likewise, Boy et al. (2007) found that in the non-reproductive season, gonad and liver represent less than 1 and 1.5% of total mass respectively, and there is no fat accumulation. On the contrary, during the reproductive season, gonad and liver reach 30 and 2.5% of total mass, respectively. Moreover, depletion of energy density of muscle during the reproductive season (Fig. 3) compensates for the increased contribution of gonads, liver and fat in the energy budget. These data support that muscle represents a main energy reservoir during the non-reproductive season in puyen and that the allocation of energy is mostly represented by internal transference of energy from muscle to gonads, liver and perivisceral fat.

Further studies should be conducted in order to determine the lipidic composition of perivisceral fat of puyen and to understand the extremely high values obtained for this tissue in this study, and the influence of latitude in explaining the high energy density values. More studies on energy density of fishes through a direct method such as calorimetry are needed for a better understanding of internal transference of energy in fishes.

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