

Feeding, body condition and reproductive investment of *Astyanax intermedius* (Characiformes, Characidae) in relation to rainfall and temperature in a Brazilian Atlantic Forest stream

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Abstract – Life history theory seeks to explain how environmental variation selects for patterns of investment in growth and survival relative to production and survival of offspring. Seasonal variations in rainfall and temperature provide environmental cues for spawning by many tropical freshwater fishes. To investigate environment–life history associations, we conducted a one-year study of *Astyanax intermedius* in an Atlantic Forest stream of southeastern Brazil. Our analysis focused on temporal variation in feeding, body condition and reproduction in relation to rainfall and water temperature. For mature females, food intake was not significantly correlated with rainfall or temperature; however, body condition was negatively correlated with rainfall and water temperature. Female reproductive effort was positively correlated with water temperature, but did not vary with rainfall. For males and juveniles, there was no significant relationship between food intake or body condition and either environmental variable. Testis weight was negatively correlated with rainfall, but was not significantly correlated with water temperature. We detected a negative correlation between gonad mass with body condition and food intake for females but not for males. Our results differed from other studies in tropical and subtropical areas where rainfall has been shown to be positively correlated with fish reproductive effort. Our results indicate that reproductive effort of males is relatively constant throughout the year, whereas for females, it increases with increasing water temperature. This increase in reproductive investment in concert with an increasing temperature and metabolic rate may incur a trade-off with somatic growth and survival for this small stream fish.

Key words: fecundity; life history; oocyte size; physiological trade-offs; neotropical fishes

Introduction

Life history theories model relationships between growth, reproduction and survival as adaptive responses to variation in environmental factors, such as hydrology, water quality, food availability and

predation pressure (Roff 1984; Stearns 1992; Winemiller & Rose 1992). Life history strategies are defined by demographic, physiological and genetic constraints, or trade-offs, that influence patterns of energy and biomass allocation (Mathews 1998; Vila-Gispert & Moreno-Amich 2002; Blanck &

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Lamouroux 2007). Resources acquired by an organism can be divided into four broad categories: (i) maintenance (to sustain metabolic requirements and survival), (ii) production of somatic tissues (for current and future survival), (iii) reproduction (gonad development and related reproductive structures and activities) and (iv) energy reserves (for future survival, growth or reproduction). Energy budget models generally assume that the first priority is maintenance (proximate survivorship) and the second priority is growth, a portion of which enhances future survival and a portion that can be converted to gametes and other reproductive tissues at the appropriate time (Lester et al. 2004; Quince et al. 2008). Assimilated energy that exceeds these two needs is stored as reserves for either future growth or reproduction (Sibly et al. 2013).

Total energy is limited by the amount of food consumed, and both foraging and energy allocation depend on how an organism responds to environmental cues. More energy directed to one function results in less energy available for others. Hence, allocation of energy to reproduction trades-off with energy invested in somatic growth or storage that enhances survival and future reproduction (Stearns 1992; Link & Burnett 2001). In seasonal habitats, many fishes increase their feeding activity prior to spawning, which increases energy reserves or allows for recovery of energy depleted during previous reproductive bouts (Link & Burnett 2001). In these cases, feeding activity declines or may cease entirely during the reproductive period, when fat reserves and other storage tissues sustain metabolic requirements (Nikolsky 1963). This pattern has been observed for the stream-dwelling characids *Moenkhausia intermedia* (Braga & Gennari Filho 1990, 1991) and *Bryconamericus iheringi* (Lampert et al. 2004).

In lotic systems, rainfall regime and water temperature influence food availability and foraging success. In tropical and subtropical areas, these abiotic variables can provide reliable cues for the timing of reproduction by seasonal spawners. Temperature influences metabolic rate and gametogenesis (Kramer 1978), and precipitation affects habitat quality, quantity and connectivity, and also can trigger spawning and migration activities (Lowe-McConnell 1987; Chellappa et al. 2009). Even for species with extended reproductive seasons, higher rainfall and temperature sometimes are associated with higher reproductive effort (Kramer 1978; Mazzoni & Petitto 1999; Alkins-Koo 2000; Mazzoni et al. 2002; Paugy 2002; Andrade & Braga 2005; Gomiero & Braga 2007). However, in small headwater streams, high rainfall often is associated with habitat disturbance caused by high water velocity and sediment suspension (Gordon et al. 2004; Lewis 2008). High water velocity may cause mortality, directly through

displacement of larvae and juveniles by currents, or indirectly when high flows scour the streambed, reducing benthic algae and invertebrate prey (Oliveira et al. 1997). Prey capture rates of visually oriented predators also can be reduced by low water transparency (Sweka & Hartman 2001).

In the present study, we examined the patterns of food intake and resource allocation in a population of *Astyanax intermedius* Eigenmann, 1908, in relation to rainfall and water temperature in a headwater stream of the Atlantic Forest ecoregion of southeastern Brazil. *Astyanax intermedius* is part of a lineage known as the 'scabripinnis complex' (Characidae family) that apparently diversified within isolated hydrographic basins, with species restricted to small headwater drainages of the Paraíba do Sul River basin, adjacent coastal rivers of Rio de Janeiro state, and the Doce River basin, Minas Gerais state (Britski 1972; Moreira-Filho & Bertollo 1991; Kavalco & Moreira-Filho 2003; Lima et al. 2003; Bertaco & Lucena 2006; Lezama et al. 2011). Similar to other species within the genus, *A. intermedius* has a life history strategy that would be classified as opportunistic (*sensu* Winemiller 1989; Winemiller & Rose 1992), represented by small-bodied species with early maturation, continuous reproduction, low fecundity but high reproductive effort, rapid population turnover and capacity for rapid colonisation (Winemiller 1989; Lamouroux et al. 2002; Blanck et al. 2007). Size of maturation is about 4 cm standard length (SL), maximum size is 10 cm SL (Gomiero et al. 2012) and juveniles and mature adults are found throughout the year, indicating that the species has an extended reproductive period (U. P. Souza, unpublished data).

We tested the hypothesis that rainfall and water temperature influence feeding and biomass/energy allocation in *A. intermedius*. Feeding activity may decline during the spawning season, as has been observed for other characid fishes (Braga & Gennari Filho 1990; Costa & Braga 1993; Lampert et al. 2004). If rainfall or temperature provide cues for spawning, correlations would be expected between these variables and patterns of allocation for reproduction versus somatic growth. Temperature may trigger spawning directly by its effects on fish metabolism, whereas rainfall may affect fishes by changes in river discharge, water level or velocity (Baran 2006). In adults, feeding activity and body condition would be expected to decrease during the latter stages of gonad maturation and spawning when storage tissues are used to meet metabolic requirements (Nikolsky 1963; Link & Burnett 2001). Negative correlations between reproductive investment and body condition or food intake should be stronger in females because more biomass/energy is invested in ovaries compared

to testes. Juveniles would not be expected to reveal any relationships. In contrast, if high rainfall results in suboptimal conditions due to habitat disturbance, there should be negative correlations between precipitation and food intake or body condition, and reproductive investment, with juveniles, males and females revealing similar patterns.

Materials and methods

Study region and environmental data

Sampling was conducted in the Ribeirão Grande (23°26'S, 45°14'W), a headwater stream of the Paraíba do Sul River basin, one of the most impacted of southeast Brazil, threatened by urbanisation, industry development, agriculture and damming. Ribeirão Grande is a 4th-order stream at 757 m elevation with a drainage area of 4.59 km², located within 'Parque Estadual da Serra do Mar (PESM)', Santa Virginia Unit. Most of the park's landscape is covered by rainforest with patches of recovering native vegetation, abandoned eucalyptus plantation and small areas of agricultural plots. The region is mountainous with ridge tops between 960 and 1160 m within the core park area, and some reaching 1585 m at the headwaters of the Ribeirão Grande (São Paulo 1998). This region has a humid subtropical climate without a dry season, and the average rainfall is around 2180 mm per year, one of the highest rainfall levels of the São Paulo state. Historical data on rainfall (from 1936 to 2010) were obtained from the gauging station in São Luiz do Paraitinga (23°13'S, 45°19'W), 23 km downstream from our sampling site. Water temperature was measured once monthly during the afternoon of each fish survey with a thermometer.

Fish sampling and collection of life history data

Fishes were collected monthly between January and December 2004 with six gillnets (mesh sizes from 1.5 to 4.0 cm between adjacent knots), three funnel traps and a seine net (2.0 × 1.0 m, mesh = 0.25 cm between adjacent knots). Sampling effort was standardised to 12 h for gillnets and funnel traps, and seine net effort was standardised as 10 pulls taken during the morning along patches of the submerged macrophyte *Potamogeton polygonus* Cham. & Schldl. where fish were most abundant. The seine net yielded more specimens than gillnets and funnel traps. Two other sites positioned downstream from Ribeirão Grande (Paraibuna and Ipiranga rivers) were sampled monthly during the same period (Gomiero & Braga 2006), but yielded only three individuals of *Astyanax intermedius*, suggesting that the species does not have self-sustaining populations in those habitats.

Specimens were fixed in formalin 10% and preserved in alcohol 70%. Voucher specimens were deposited in the fish collection of Universidade Federal do Rio Grande do Sul (UFRGS 10821). In the laboratory, we measured standard length (*SL* - cm), total weight (*TW* - g), stomach weight (*SW* - g) and gonad weight (*GW* - g), and recorded sex, stage of gonad maturation, food quantity on stomach and degree of fat accumulation within the visceral cavity. Four different gonad maturation stages were identified (immature/juveniles, maturing, fully mature and spent) following standard macroscopically classifications based on size, shape and degrees of vascularisation and transparency (Vazzoler 1996; Alkins-Koo 2000).

To estimate fecundity, mature ovaries were weighed, immersed in Gilson's solution and periodically shaken to dissociate oocytes. Once separated, oocytes were preserved in 70% alcohol, and the number of oocytes was estimated according to the volumetric method (Vazzoler 1996) by the expression $N = S \times n/s$, where *S* is the volume of alcohol used to homogenise the mass of oocytes and *n* is the total number of oocytes counted in a sample of *s* volume obtained with a Stempel pipette (2 ml). A subsample of 100 oocytes was randomly selected from each ovary and measured using an ocular micrometre (at 10× magnification). *Astyanax intermedius* has fractional spawning, and the distribution of oocyte sizes has more than one mode (U. P. Souza, unpublished data). Therefore, the minimal diameter of mature oocytes was determined by their visual appearance (mature oocytes are opaque, West 1990). Also, mature oocytes were only considered to be those greater than or equal to the size of last mode of the distribution. Batch fecundity was then estimated as $Fec = p \times N$, where *p* is the proportion of mature oocytes estimated within each ovary. This procedure, although not as precise as histological determination (West 1990), has been useful to allow fecundity estimation in fractional spawners (Braga et al. 2007; Gomiero et al. 2007, 2008).

Data analysis

Analyses examined relationships between monthly rainfall (RF) and water temperature (TEMP) and indicators of food intake, body condition and reproductive investment. We used monthly rainfall data for the year of 2004 obtained from the historical series while water temperature was measured in the field during fish sampling. For all multiple regression models described below, the Pearson correlation coefficient was calculated to test for colinearity among the predictor variables.

Food intake

We evaluated the proportion of stomachs with food (ES) and the stomach weight (SW) as functions of rainfall and water temperature. The analyses were performed separately for immatures, males and females, and using fishes that came from all three sampling gears. ES was coded as 0 for empty stomachs and 1 for stomachs with any amount of food inside. ES was used as response variable in the following multiple logistic regression model:

$$\log\left(\frac{ES}{1-ES}\right) = \beta_0 + \beta_1 \times RF + \beta_2 \times TEMP \quad (1)$$

The statistical significance of each predictor was obtained by comparing the log-likelihood of the full (all predictors included) and reduced (excluding a single predictor variable) models using the likelihood ratio χ^2 statistic.

SW was calculated only for stomachs containing some amount of food and used in the following multiple linear regression model:

$$\log(SW) = \beta_0 + \beta_1 \times \log(SL) + \beta_2 \times \log(RF) + \beta_3 \times \log(TEMP) \quad (2)$$

SL was included in this model to account for differences of body size on SW as larger fishes are also expected to have heavier stomachs. By doing so, the partial regression coefficients β_2 and β_3 measure the isolated effects of rainfall and temperature on stomach weights.

Body condition

We used the proportion of fishes with fat (FAT) in the visceral cavity and the eviscerated fish weight (EFW) to model the effect of rainfall and water temperature on the fish condition. The analyses were performed separately for immatures, males and females. FAT was coded as 0 for fishes without fat and 1 for fishes with fat, and used as response variable in a logistic regression model similar to Eq. 1.

We calculated the eviscerated fish weight (EFW) by subtracting the stomach (SW) and gonad (GW) weights from total weight (TW). EFW was considered a surrogate measure for fish condition, as it represents the weight of all somatic tissue plus the energy reserves that a fish can store in a given moment. For immature fishes, only the stomach weight was removed, as there was no developed gonad. Also, we did not consider the weight of the other organs given that these were only minor components of total weight when compared to gonads and stomachs. Multiple linear regression models were used to examine the influence of rainfall and water

temperature on log (EFW) following the same expression described in Eq. 2 when taking into account for differences in fish standard length.

Reproduction

The gonadosomatic index (GSI) was calculated by dividing gonad weight by total fish weight, and mean values for males and females were compared with a t test.

The same expression described in Eq. 1 was used to model the proportion of adults in reproduction as function of rainfall and water temperature. The response variable was coded as 0 for maturing adults and 1 for fully mature adults (those with gonads in the last stage of maturation). Immature fishes were not considered, and analyses were performed separately for males and females.

The combined influence of rainfall and water temperature with the logarithm of gonad weight (GW) of adults was tested using multiple linear regression using an expression similar to Eq. 2. We verified that log-transformation resulted in a linear relation between the response and predictor variables, but did not correct for the normality of residuals, implying that the standard t distribution could not be used to estimate the significance of the partial regression coefficients. Thus, probability values were calculated by comparing the observed partial regression coefficients with empirical distributions generated by a randomisation routine using 10,000 permutations (Manly 2007).

Overall, we ran 18 multiple regression models to test the effects of rainfall and temperature on the response variables for immatures, males and females. To control for the family-wise type I error rate, we applied the Bonferroni correction. For $\alpha = 0.05$, the significance level for each individual test was considered at $0.05/4 = 0.0125$ for immature, $0.05/6 = 0.008$ for males and $0.05/8 = 0.006$ for females. The Bonferroni method is considered a conservative correction, that is, it can result in higher type II error rates for individual tests (Quinn & Keough 2002). In the present paper, this implies that only individual traits strongly correlated with monthly rainfall or water temperature were considered statistically significant.

The relationship between gonad weight, food intake and body condition

A hypothesised reduction in food intake by *A. intermedius* during gonad maturation could result in two patterns. First, the incidence of stomachs with food in mature fishes could decrease if fish decrease foraging activity as gonads become larger. Second, there could be a negative correlation between stomach fullness (or weight) and gonad size if fish reduce foraging or

if enlarged gonads limit the space within the body cavity for expansion of the stomach.

The first pattern was tested with a logistic regression model, according to

$$\log\left(\frac{ES}{1-ES}\right) = \beta_0 + \beta_1 \times R_{GW \times SL} \quad (3)$$

ES is the same variable used in Eq. 1, and $R_{GW \times SL}$ is the residual obtained from the regression: $\log(GW) = \beta_0 + \beta_1 \times \log(SL)$. This was done to account for fish size differences in gonad weight.

The second pattern was tested by calculating the partial Pearson correlation (r_{Partial}) between $\log(GW)$ and $\log(SW)$ after accounting for the effect of fish size (SL). To visualise graphically the partial correlation patterns, we plotted the residuals from the regression between $\log(GW) \sim \log(SL)$ with the residuals from the regression between $\log(SW) \sim \log(SL)$.

If fish reduce their feeding during the spawning period, a trade-off between body condition and reproductive investment should appear. In this case, one would expect to find less fish with fat in the visceral cavity as gonad weight increases. This prediction was tested by a logistic regression using FAT as the response variable and $R_{GW \times SL}$ as predictor. This trade-off also could be evidenced by a negative correlation between EFW and GW , while accounting for fish size. This was tested by calculating the partial Pearson correlation coefficient between $\log(EFW)$ and $\log(GW)$ when controlling for SL . These analyses were performed separately for adult males and females.

Four tests for males and four tests for females were performed; therefore, Bonferroni correction was used to adjust the significance level to $P \leq 0.0125$.

Results

Environmental data

Historical rainfall averages 1246 mm per year, with a minimum of 729 mm in 1963 and a maximum of 1895 mm in 1983. There was no tendency for rainfall to increase or decrease from 1936 to 2010 ($r = 0.11$, $P = 0.34$). Intra-annual seasonality was apparent, with higher values from September to March and lower values from April to August (Fig. 1a). The wettest months (January and February, average rainfall of 207 mm) receive 6–7 times more rain than the driest (July and August, average rainfall of 30 mm).

During 2004, total rainfall was 1323 mm, which is close to the historical average. From March to July, the monthly rainfall was around 100 mm, and the driest months were August (no registered rain) and September (25 mm). There were two peaks of rainfall in

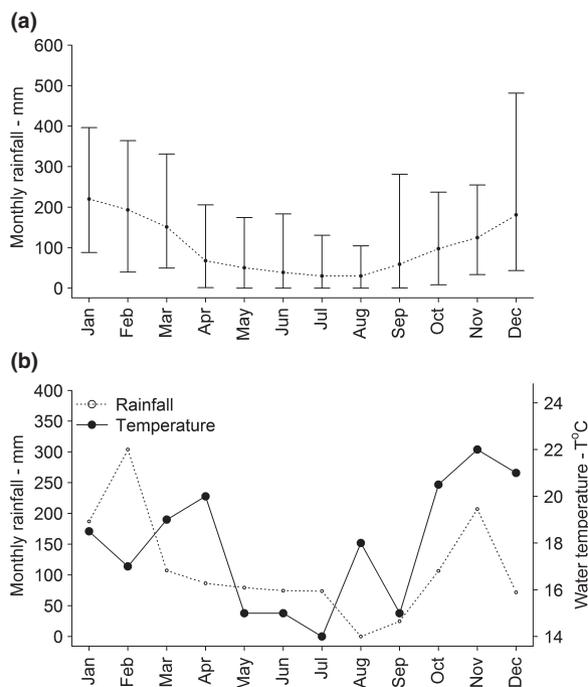


Fig. 1. Historical data of monthly rainfall between 1936 and 2010 with mean, maximum and minimum values measured at the gauging station of São Luiz do Paraitinga town (23°13'S, 45°19'W) (a) and data on monthly rainfall and water temperature in 2004 (b).

February (304 mm) and November (207 mm). In June (75 mm) and July (74 mm), rainfall was more intense than the historical average and in December (72 mm), rainfall was less than half the long-term average.

Mean water temperature during 2004 was 17.9 °C, with highest values in November (22 °C) and lowest in July (14 °C) (Fig. 1b). There was no significant correlation between monthly rainfall and water temperature in 2004 ($r = 0.26$, $P = 0.41$), which allowed us to estimate their independent effects on response variables using multiple regression models.

Effects of rainfall and water temperature

Based on our sample of 761 fishes, the number of stomachs with food and stomach weight was not correlated with rainfall or water temperature, and the only significant result was a strong positive relationship between stomach weight and fish size, which is expected given that larger fish have larger stomachs. The proportions of males and immatures with visceral fat were not correlated with rainfall and temperature, whereas the proportion of females with fat decreased with rainfall ($N = 302$, $\beta_{RF} = -0.008$, $P < 0.001$) and eviscerated female weight decreased with both rainfall ($N = 203$, $\beta_{RF} = -0.02$, $P < 0.001$) and temperature ($N = 203$, $\beta_{TEMP} = -0.23$, $P < 0.001$). For

males, eviscerated body weight decreased with rainfall ($N = 170$, $\beta_{RF} = -0.01$, $P = 0.005$). As expected, the correlation between eviscerated body weight and SL was positive and highly significant for all groups (Table 1).

Fish with mature gonads were collected throughout the year, and the proportional weight of testes was approximately half that of ovaries ($GSI_{testes} = 0.05$, $GSI_{ovaries} = 0.11$, $t = 14.93$, d.f. = 343, $P < 0.001$). Standard lengths for mature females ranged from 5.3 to 9.0 cm and for mature males, from 4.7 to 7.8 cm. We analysed a total 212 mature paired ovaries, and batch fecundity ranged from 538 to 6727 mature oocytes (mean = 2688.7, SD = 1278.8).

The proportion of mature males did not vary with rainfall or water temperature, whereas the proportion of mature females increased with water temperature ($N = 302$, $\beta_{RF} = 0.215$, $P < 0.001$). Testis weight was negatively correlated with rainfall ($N = 133$, $\beta_{TEMP} = -0.05$, $P = 0.001$); temperature was posi-

tively correlated with ovarian weight ($N = 209$, $\beta_{TEMP} = 1.15$, $P < 0.001$) and oocyte size ($N = 203$, $\beta_{TEMP} = 0.29$, $P = 0.001$), but not with fecundity ($N = 203$, $\beta_{TEMP} = 0.54$, $P = 0.026$). As expected, body length was positively correlated with ovarian weight and fecundity, but not with oocyte size (Table 1). Appendix S1 provides full descriptions of the regression models.

The relationship between gonad weight, food intake and body condition

The proportion of stomachs with food had a significant and negative correlation with ovarian weight ($N = 275$, $\beta_{GW} = -0.589$, $P = 0.002$), but this pattern was not observed for testis weight ($N = 170$, $\beta_{GW} = -0.046$, $P = 0.880$). The partial correlation coefficient (i.e. corrected for differences of fish size) between stomach weight and gonad weight was non-significant for males ($N = 169$, $r_{Partial} = -0.09$, $P = 0.268$, Fig. 2a) but significant and negative for females ($N = 275$, $r_{Partial} = -0.16$, $P = 0.007$, Fig. 2c).

The proportion of females with fat decreased with gonad weight ($N = 275$, $\beta_{GW} = -0.669$, $P < 0.001$), but this relationship was nonsignificant for males ($N = 170$, $\beta_{GW} = -0.312$, $P = 0.302$). The partial correlation coefficient was positive between eviscerated body weight and testis weight ($N = 170$, $r_{Partial} = 0.37$, $P < 0.001$, Fig. 2b), and negative between eviscerated body weight and ovarian weight ($N = 275$, $r_{Partial} = -0.22$, $P < 0.001$, Fig. 2d).

Discussion

For fish that have opportunistic life history strategies involving multiple spawnings over long reproductive periods, relationships between environmental factors and indices of growth, condition and reproduction can elucidate the influence of environmental variation on allocation strategies. In Ribeirão Grande, *Astyanax intermedium* reproduced throughout the year, as evidenced by the presence of both small juveniles and mature adults from January to December 2004. The hypothesis that rainfall and temperature are correlated with patterns of biomass acquisition and allocation was partially supported for females. The only significant findings for males were negative correlations between rainfall, and eviscerated fish weight and testis weight.

The hypothesis that rainfall would result in sub-optimal conditions for *A. intermedium* due to habitat disturbance was not supported by our results, because stomach weight and proportion of stomachs with food were not correlated with rainfall. It is

Table 1. Summary of regression models for immatures, males and females of *Astyanax intermedium* showing samples sizes (N) and partial regression coefficients for fish standard length (β_{SL}), monthly rainfall (β_{RF}) and water temperature (β_{TEMP}).

Response variable	Predictors	Immature	Males	Females
Feeding				
Proportion of stomachs with food	N	244	215	302
	β_{RF}	ns	ns	ns
	β_{TEMP}	ns	ns	ns
Stomach weight	N	57	200	295
	β_{SL}	1.40*	2.32*	2.83*
	β_{RF}	ns	ns	ns
	β_{TEMP}	ns	ns	ns
Body condition				
Proportion of fishes with fat	N	244	215	302
	β_{RF}	ns	ns	-0.008*
	β_{TEMP}	ns	ns	ns
Eviscerated weight	N	57	170	203
	β_{SL}	3.01*	3.03*	2.86*
	β_{RF}	ns	-0.01*	-0.02*
	β_{TEMP}	ns	ns	-0.23*
Reproduction				
Proportion of mature adults	N		215	302
	β_{RF}		ns	ns
	β_{TEMP}		ns	0.215*
Gonad weight	N		133	209
	β_{SL}		3.50*	2.89*
	β_{RF}		-0.05*	ns
	β_{TEMP}		ns	1.15*
Fecundity	N			203
	β_{SL}			2.93*
	β_{RF}			ns
	β_{TEMP}			ns
Oocyte size	N			203
	β_{SL}			ns
	β_{RF}			ns
	β_{TEMP}			0.29*

ns, nonsignificant results.

*Indicates $P \leq 0.0125$ for immatures, $P \leq 0.008$ for males and $P \leq 0.006$ for females.

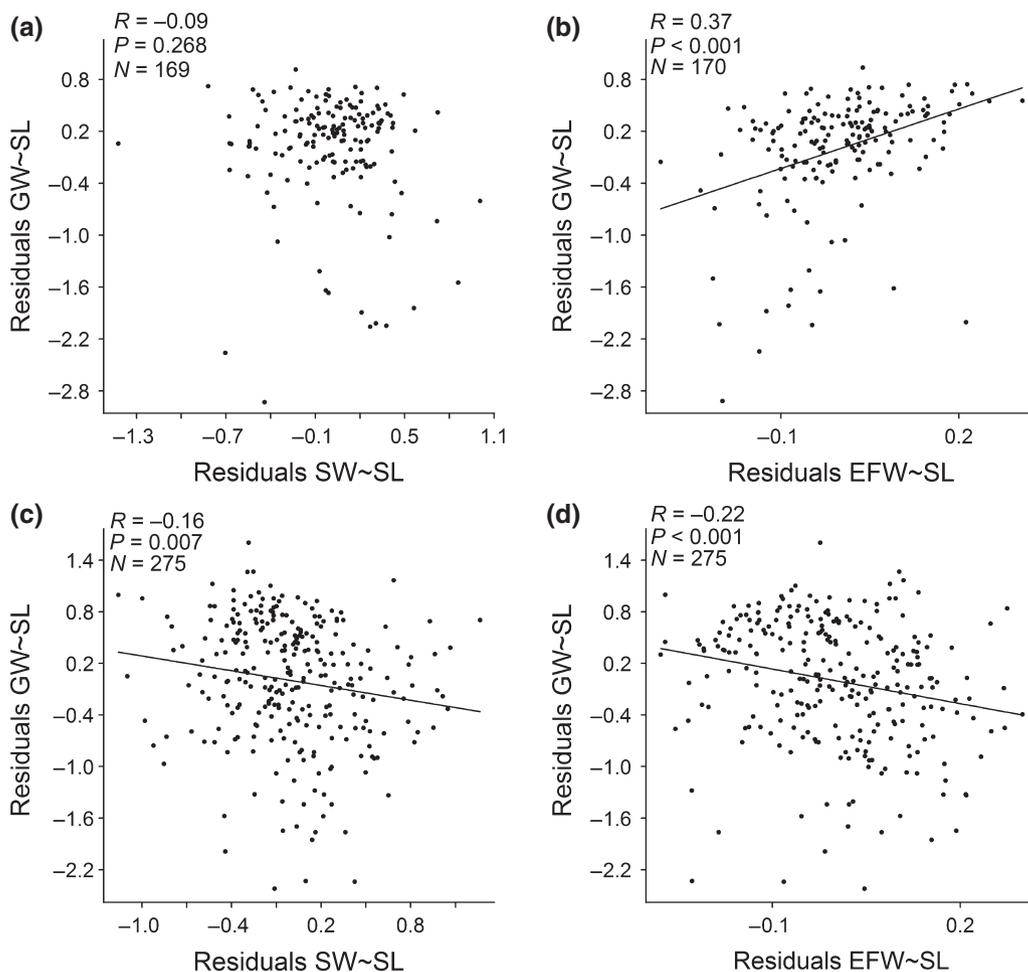


Fig. 2. Relationships between gonad weight (GW) versus stomach weight (SW) and gonad weight versus eviscerated body weight (EFW) for males (a and b) and females (c and d) of *Astyanax intermedius* controlling for the effect of fish standard length (SL). R : partial Pearson correlation coefficient, N : sample size.

possible that stomach weight was not a reliable measure of food intake because we analysed together fishes captured by seine nets with those captured gill nets that remained in water for a 12-h period. If there was a bias associated with differential digestibility of ingested items between these groups, it would be difficult to observe a significant pattern. We attempted to reduce this effect by complementing the analyses of stomach weight with the analyses of proportion of stomach with or without food. Thus, even stomachs with small amounts of food, including those in advanced stages of digestibility, were categorised in the former group. However, if increased rainfall actually reduces food intake, then body condition should be negatively correlated with rainfall. This relationship was significant only for females, was weak for males and was not significant for immature fish.

Overall, our findings suggest that rainfall did not strongly influence resource acquisition and biomass allocation of *A. intermedius* in Ribeirão Grande. This

contrasts with studies conducted in other tropical and subtropical regions where reproductive effort of stream fishes was strongly associated with rainfall and river discharge (Kramer 1978; Winemiller 1989; Mazzoni & Petito 1999; Alkins-Koo 2000; Mazzoni et al. 2002; Paugy 2002; Andrade & Braga 2005; Gomiero & Braga 2007). We attribute the lack of a correlation between rainfall and reproduction to the absence of a well-defined dry season in Ribeirão Grande, and to the unpredictable nature of local rainfall and flow variation in this small headwater stream. It is possible that monthly rainfall might have not captured information on the occurrence of discrete events of intense precipitation that would affect resource acquisition. In this case, data related to the number of daily precipitation events above a predetermined level would be more informative, provided that this level is well chosen.

Unlike rainfall, temperature was correlated with several of our response variables. Temperature has a strong influence on the metabolism of ectotherms

(Rankin & Jensen 1993; Lewis 2008). For example, Patterson et al. (2013) found a positive effect of temperature on mass-specific metabolic rate of paddlefish (*Polyodon spathula*), and Pauly and Pullin (1988) provided evidence that time to hatching for eggs of 84 teleost fishes is reduced at higher temperatures. The influence of temperature on physiology should be particularly strong for temperate-zone fishes, but could temperature also affect life history strategies of subtropical and tropical fishes? We did not find evidence of an association between temperature and food intake, nor between temperature and body condition of males and immature fish. Male reproductive investment also did not correlate with temperature. In contrast, temperature was positively correlated with reproductive investment in females in terms of the proportion of mature females, gonad weight and oocyte diameter.

Temperature can influence fish reproduction by means other than its direct effects on physiology. If seasonal temperature variation is a reliable predictor or indicator of conditions in stream ecosystems, it could influence fitness and life history as a cue for spawning (Beschta et al. 1989). In this case, strategies favouring biomass allocation to reproductive tissues would be correlated with temperature variation as a consequence of adaptive evolution. During our study period, water temperature ranged from 14 °C (July) to 22 °C (December), and monthly temperature variation in this headwater stream may be more predictable than flow variation that is strongly controlled by unpredictable local rainfall. Thus, the influence of temperature on reproductive effort in females might have resulted from a combination of proximate factors related to higher fish metabolism (promoting gametogenesis) and ultimate factors related to adaptive processes that direct a higher proportion of assimilated mass/energy to reproductive tissues at the appropriate time. In addition, given that the increase in gonad weight as function of temperature was more strongly associated with larger oocytes than with higher fecundity, it is possible that larger larvae emerge during warmer periods. Pauly and Pullin (1988) showed that interspecific variation in egg diameter is positively correlated with water temperature. Our results suggest that this positive relationship may also be an intraspecific tendency. If so, it would be interesting to test the hypothesis that higher eggs and larvae result in higher larval survivorship. For example, Nunn et al. (2003) found that years with warmer temperatures resulted in stronger early life stage recruitment, although the authors could not separate effects of higher temperatures from lower discharges.

We observed a negative correlation between reproductive investment and body condition of females,

which is consistent with the hypothesis that females with developing ovaries cannot assimilate energy sufficient to produce significant energy reserves. Ovarian weight was negatively correlated with both stomach weight and the proportion of stomachs with food. Thus, it appears that food intake and body condition are lower during the spawning period when energy and biomass are allocated to reproduction. Similar patterns were observed for other characid fishes that have lower food intake during the spawning season (Braga & Gennari Filho 1990; Lampert et al. 2004) when energy reserves are mobilised for maintenance and reproduction (Nikolsky 1963; Costa & Braga 1993; Link & Burnett 2001). Reduced food intake is most conspicuous when reproduction involves parental care or migration, but in the case of a small fish, this pattern could arise from limited space within the visceral cavity. For example, Braga (2004) examined the gonad structure of small stream fishes in another basin of the same region as our study site (species of the genera *Characidium*, *Trichomycterus* and *Imparfinis*). These fishes possess mature ovaries that form a single structure along the superior wall of visceral cavity in an arrangement that appears to maximise egg storage within a small space. The morphology of the paired ovaries in *A. intermedius* is not as specialised, but mature ovaries of *A. intermedius* often fill most of the visceral cavity, compressing most of the other organs (F.M.S. Braga, personal observation). Male reproductive investment was uncorrelated with temperature and rainfall, and seemed to be relatively constant throughout the year. Production of spermatozoa requires less energy and material than egg production; therefore, it might be expected that reproductive investment by males revealed no significant relationship with food intake and body condition.

Our findings differed from other studies in tropical and subtropical regions; patterns of food acquisition and biomass allocation were not correlated with rainfall. In addition, monthly variation in water temperature influenced reproductive effort only in females, and this higher reproductive investment in concert with higher temperatures may incur a trade-off with somatic growth and survival. Given that several species within the 'scabripinnis complex' are found in small headwater streams over a broad region of southeastern Brazil (Moreira-Filho & Bertollo 1991; Bertaco & Lucena 2006), future comparative studies could reveal whether patterns observed in the present study are consistent for different populations or species from different locations. Also, we should mention that a sampling date effect may be present in our results. In this case, the fish traits would be more similar within than among months due to other factors not related to water temperature or monthly rainfall, so that the results presented herein should be

confirmed with longer time series or with controlled experiments.

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References

- Alkins-Koo, M. 2000. Reproductive timing of fishes in a tropical intermittent stream. *Environmental Biology of Fishes* 57: 49–66.
- Andrade, P.M. & Braga, F.M.S. 2005. Reproductive seasonality of fishes from a lotic stretch of the Grande River, High Paraná River Basin, Brazil. *Brazilian Journal of Biology* 65: 387–394.
- Baran, E. 2006. Fish migration triggers in the Lower Mekong Basin and other tropical freshwater systems. MRC Technical Paper No. 14. Vientiane: Mekong River Commission, 56 pp.
- Bertaco, V.A. & Lucena, C.A.S. 2006. Two new species of *Astyanax* (Ostariophysi: Characiformes: Characidae) from eastern Brazil, with synopsis of the *Astyanax scabripinnis* species complex. *Neotropical Ichthyology* 4: 53–60.
- Beschta, R.L., Bilby, R.E., Brown, G.W., Holtby, L.B. & Hofstra, T.D. 1989. Stream temperature and aquatic habitat: fisheries and forestry interactions. In: Salo, E.O. & Cundy, T.W., eds. *Streamside management: forestry and fishery interactions*. Seattle: Institute of Forest Resources, University of Washington, pp. 191–232.
- Blanck, A. & Lamouroux, N. 2007. Large-scale intraspecific variation in life-history traits of European freshwater fish. *Journal of Biogeography* 34: 862–875.
- Blanck, A., Tedesco, P.A. & Lamouroux, N. 2007. Relationships between life-history strategies of European freshwater fish species and their habitat preferences. *Freshwater Biology* 52: 843–859.
- Braga, F.M.S. 2004. Habitat, distribuição e aspectos adaptativos de peixes da microbacia do Ribeirão Grande, Estado de São Paulo, Brasil. *Acta Scientiarum Biological Sciences* 26: 31–36.
- Braga, F.M.S. & Gennari Filho, O. 1990. Contribuição para o conhecimento da reprodução de *Moenkhausia intermedia* (Characidae, Tetragonopterinae) na represa de Barra Bonita, rio Piracicaba, SP. *Naturalia* 15: 171–188.
- Braga, F.M.S. & Gennari Filho, O. 1991. Estudos sobre a fecundidade, desova e mortalidade natural de *Moenkhausia intermedia* (Characidae, Tetragonopterinae), na represa de Barra Bonita, rio Piracicaba, SP. *Naturalia* 16: 55–68.
- Braga, F.M.S., Souza, U.P. & Carmassi, A.L. 2007. Dinâmica populacional de *Characidium lauroi* e *C. alipioi* (Teleostei, Chrenuchidae) na microbacia do Ribeirão Grande, serra da Mantiqueira Oriental, Estado de São Paulo. *Acta Scientiarum Biological Sciences* 29: 281–287.
- Britski, H.A. 1972. Peixes de água doce do Estado de São Paulo. Sistemática. Comissão internacional da bacia do Paraná-Uruguai: Poluição e Piscicultura. São Paulo: Faculdade de Saúde Pública da USP/Instituto de Pesca. 79–108 pp.
- Chellappa, S., Bueno, R.M.X., Chellappa, T., Chellappa, N.T. & Val, V.M.F.A. 2009. Reproductive seasonality of the fish fauna and limnoecology of semi-arid Brazilian reservoirs. *Limnologia* 39: 325–329.
- Costa, F.E.S. & Braga, F.M.S. 1993. Estudo da alimentação natural de *Astyanax bimaculatus*, *Astyanax schubarti* e *Moenkhausia intermedia* (Characidae, Tetragonopterinae) na represa de Barra Bonita, rio Piracicaba (SP). *Revista Unimar* 15: 117–134.
- Gomiero, L.M. & Braga, F.M.S. 2006. Diversity of the ichthyofauna in the Serra do Mar State Park-Núcleo Santa Virgínia, São Paulo State, Brazil. *Acta Scientiarum Biological Sciences* 28: 213–218.
- Gomiero, L.M. & Braga, F.M.S. 2007. Reproduction of a fish assemblage in the state of São Paulo, southeastern Brazil. *Brazilian Journal of Biology* 67: 283–292.
- Gomiero, L.M., Souza, U.P. & Braga, F.M.S. 2007. Reproduction and feeding of *Rhamdia quelen* (Quoy & Gaimard, 1824) in rivers of the Santa Virginia Unit, State Park of the Serra do Mar, São Paulo, SP. *Biota Neotropica* 7: 127–133.
- Gomiero, L.M., Garuana, L. & Braga, F.M.S. 2008. Reproduction of *Oligosarcus hepsetus* (Cuvier, 1829) (Characiformes) in the Serra do Mar State Park, São Paulo, Brazil. *Brazilian Journal of Biology* 68: 187–192.
- Gomiero, L.M., Souza, U.P. & Braga, F.M.S. 2012. Condition factor of *Astyanax intermedius* Eigenmann, 1908 (Osteichthyes, Characidae) parasitised by *Paracymothoa astyanaxi* Lemos de Castro, 1955 (Crustacea, Cymothoidae) in the Grande River, Serra do Mar State Park - Santa Virgínia Unit, São Paulo, Brazil. *Brazilian Journal of Biology* 72: 379–388.
- Gordon, N.D., McMahon, T.A., Finlayson, B.L., Gippel, C.J. & Nathan, P.J. 2004. *Stream hydrology: an introduction for ecologists*, 2nd edn. Chichester: John Wiley & Sons Ltd. 429 pp.
- Kavalco, K.F. & Moreira-Filho, O. 2003. Cytogenetical analyses in four species of the genus *Astyanax* (Pisces, Characidae) from Paraíba do Sul River Basin. *Caryologia* 56: 453–461.
- Kramer, D.L. 1978. Reproductive seasonality in the fishes of a tropical stream. *Ecology* 59: 976–985.
- Lamouroux, N., Poff, N.L. & Angermeier, P.L. 2002. Intercontinental convergence of stream fish community traits along geomorphic and hydraulic gradients. *Ecology* 83: 1792–1807.
- Lampert, V.R., Azevedo, M.A. & Fialho, C.B. 2004. Reproductive biology of *Bryconamericus iheringii* (Ostariophysi: Characidae) from rio Vacacaí, RS, Brazil. *Neotropical Ichthyology* 2: 209–215.

- Lester, N.P., Shuter, B.J. & Abrams, P.A. 2004. Interpreting the von Bertalanffy model of somatic growth in fishes: the cost of reproduction. *Proceedings of the Royal Society B: Biological Sciences* 271: 1625–1631.
- Lewis, W.M. Jr 2008. Physical and Chemical Features of Tropical Flowing Waters. In: Dudgeon, D., ed. *Tropical streams ecology*. New York: Elsevier, pp. 1–21.
- Lezama, A.Q., Triques, M.L. & Queiroz, F.M. 2011. *Astyanax intermedius* Eigenmann, 1908 (Actinopterygii: Characiformes): distribution extension in eastern Brazil. *Check List* 7: 583–584.
- Lima, F.C.T., Malabarba, L.R., Buckup, P.A., da Silva, J.F.P., Vari, R.P., Harold, A., Benine, R., Oyakawa, O.T., Pavanelli, C.S., Menezes, N.A., Lucena, C.A.S., Malabarba, M.C.S.L., Lucena, Z.M.S., Reis, R.E., Langeani, F., Casatti, L., Bertaco, V.A., Moreira, C. & Lucinda, P.H.F. 2003. Genera Incertae Sedis in Characidae. In: Reis, R.E., Kullander, S.O. & Ferraris, C.J. Jr, eds. *Check list of the freshwater fishes of south and central America*. Edipucrs: Porto Alegre, pp. 106–169.
- Link, J.S. & Burnett, J. 2001. The relationship between stomach contents and maturity state for major northwest Atlantic fishes: new paradigms? *Journal of Fish Biology* 59: 783–794.
- Lowe-McConnell, R.H. 1987. *Ecological studies in tropical fish communities*. Cambridge: Cambridge University Press. 382 pp.
- Manly, B.J.F. 2007. *Randomization, bootstrap and monte carlo methods in biology*. Boca Raton: Chapman & Hall/CRC. 455 pp.
- Mathews, W.J. 1998. *Patterns in freshwater fish ecology*. New York: Chapman & Hall. 756 pp.
- Mazzoni, R. & Petito, J.T. 1999. Reproductive biology of a Tetragonopterinae (Osteichthyes, Characidae) of the Ubatiba fluvial system, Maricá – RJ. Brazil. *Brazilian Archives of Biology and Technology* 42: 455–461.
- Mazzoni, R., Caramaschi, E.P. & Fenerich-Verani, N. 2002. Reproductive biology of Characidiinae (Osteichthyes, Characidae) from the Ubatiba river, Maricá-RJ. *Brazilian Journal of Biology* 62: 487–494.
- Moreira-Filho, O. & Bertollo, L.A.C. 1991. *Astyanax scabripinnis* (Pisces, Characidae): a species complex. *Brazilian Journal of Genetics* 14: 331–357.
- Nikolsky, G.V. 1963. *The ecology of fishes*. Translated from the Russian by L. Birkett. New York: Academic Press. 351 pp.
- Nunn, A.D., Cowx, I.G., Frear, P.A. & Harvey, J.P. 2003. Is water temperature an adequate predictor of recruitment success in cyprinid fish populations in lowland rivers? *Freshwater Biology* 48: 579–588.
- Oliveira, L.G., Bispo, P.C. & Sá, N.C. 1997. Ecologia de Comunidades de Insetos Bentônicos (Ephemeroptera, Plecoptera e Trichoptera), em córregos do Parque Ecológico de Goiânia, Goiás, Brasil. *Revista Brasileira de Zoologia* 14: 867–876.
- Patterson, J.T., Mims, S.D. & Wright, R.A. 2013. Effects of body mass and water temperature on routine metabolism of American paddlefish *Polyodon spathula*. *Journal of Fish Biology* 82: 1269–1280.
- Paugy, D. 2002. Reproductive strategies of fishes in a tropical temporary stream of the Upper Senegal basin: Baoulé River in Mali. *Aquatic Living Resources* 15: 25–35.
- Pauly, D. & Pullin, R.S.V. 1988. Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. *Environmental Biology of Fishes* 22: 261–271.
- Quince, C., Abrams, P.A., Shuter, B.J. & Lester, N.P. 2008. Biphasic growth in fish I: theoretical foundations. *Journal of Theoretical Biology* 254: 197–206.
- Quinn, G.P. & Keough, M.J. 2002. *Experimental design and data analysis for biologists*. Cambridge: Cambridge University Press. 537 pp.
- Rankin, J.C. & Jensen, F.B. 1993. *Fish ecophysiology*. New York: Chapman & Hall. Fish and Fisheries Series 9. 421 pp.
- Roff, D.A. 1984. The evolution of life history parameters in teleosts. *Canadian Journal of Fisheries and Aquatic Sciences* 41: 989–1000.
- São Paulo. 1998. *Planos de Manejo das Unidades de Conservação: Parque Estadual da Serra do Mar – Núcleo Santa Virgínia*. São Paulo: Secretaria do Meio Ambiente. Governo do Estado de São Paulo. 128 pp.
- Sibly, R.M., Grimm, V., Martin, B.T., Johnston, A.S.A., Kulakowska, K., Topping, C.J., Calow, P., Nabe-Nielsen, J., Thorbek, P. & DeAngelis, D.L. 2013. Representing the acquisition and use of energy by individuals in agent-based models of animal populations. *Methods in Ecology and Evolution* 4: 151–161.
- Stearns, S.C. 1992. *The evolution of life histories*. New York: Oxford University Press Inc. 249 pp.
- Sweka, J.A. & Hartman, K.J. 2001. Influence of turbidity on brook trout reactive distance and foraging success. *Transactions of the American Fisheries Society* 130: 138–146.
- Vazzoler, A.E.A.M. 1996. *Biologia da reprodução de peixes teleósteos: Teoria e Prática*. Maringá, São Paulo: EDUEM, SBI. 169 pp.
- Vila-Gispert, A. & Moreno-Amich, R. 2002. Life-history patterns of 25 species from European freshwater fish communities. *Environmental Biology of Fishes* 65: 387–400.
- West, G. 1990. Methods of assessing the ovarian development fishes: a review. *Australian Journal of Marine and Freshwater Research* 41: 199–222.
- Winemiller, K.O. 1989. Patterns of variation in life history among South American fishes in seasonal environments. *Oecologia* 81: 225–241.
- Winemiller, K.O. & Rose, K.A. 1992. Patterns of life-history diversification in North American fishes: implications for population regulation. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 2196–2218.

Supporting Information

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

Appendix S1. Multiple regression models for the effects of rainfall, water temperature, fish size and gonad weight on feeding, body condition and reproductive parameters of *Astyanax intermedius*.