Reproductive strategies of Amazonian stream fishes and their fine-scale use of habitat are ordered along a hydrological gradient

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SUMMARY

1. Macroecological comparisons have shown that habitat characteristics have a major influence on the evolution of life-history strategies of fishes in large river systems. Specifically, habitat variability and predictability seem to forge the reproductive strategies of fishes into a trilateral continuum with three main types: seasonal, opportunistic and equilibrium.

2. Despite the usefulness of such a broad categorisation, large-scale patterns of trait distribution should ultimately be influenced by selection at smaller (i.e. individual) spatial scales. However, the scarcity of studies developed at the organismal scale leaves gaps in our understanding of how habitat characteristics affect life history and behavioural tactics of stream fishes.

3. We examined the fine-scale spatial distribution of fishes in pristine Amazonian headwater streams over a full seasonal cycle to test whether segregation into main channel and associated temporary pond habitats was predictably related to hydrological variation and species' life-history traits.

4. Most species could not be unambiguously assigned to one of the three end points of the trilateral continuum, but seemingly occupied instead the intermediate multivariate space between the end points. However, species were clearly ordered along a gradient of relative habitat use, ranging from strict channel dwellers throughout the hydrological cycle to pond specialists, with some species occupying the ponds at high water levels. Hydrological effects on habitat use were significantly related to species' reproductive strategies, with the strongest responses found in species with large eggs and seasonally concentrated reproduction, which were most abundant in ponds at high water levels.

5. We suggest that trophic limitations and hydrological characteristics of Amazonian headwater forest streams may severely limit the seasonal strategy, one of the end points of the trilateral model. The species-specific habitat segregation reported here adds to the list of mechanisms, such as miniaturisation and isolation by distance, previously postulated to contribute to high diversity of stream fish faunas in the Amazon basin.

Keywords: freshwater fish, habitat segregation, life-history strategy, seasonal variation, temporary ponds

Introduction

Habitat variation influences the behaviour, ecology and evolution of organisms and plays a critical role in the maintenance of biodiversity (Morris, 2003). At shorter (ecological) time scales, habitat heterogeneity can facilitate spatial segregation and coexistence of species (Rosenzweig, 1987). At evolutionary time scales, habitat characteristics act as a templet, forging the life histories of organisms and leading to adaptations that improve individual survival and reproduction (Southwood, 1977). A review by Southwood (1988) evaluated the templet con-

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cept and noted that despite some differences in proximal variables and terminology used by various authors, the two main environmental axes selecting for biological traits were the frequency of disturbance and the harshness of the habitat. In this view, life-history traits result from adaptations to habitat-dependent selective pressures constrained by physiological limitations determined genetically (Stearns, 1992).

The habitat templet concept has provided background for studies examining the relationship between environmental characteristics and life-history strategies of fishes (Winemiller, 1989; Bruton & Merron, 1990; Gotelli & Pyron, 1991; Winemiller & Rose, 1992; Mérigoux, Dolédec & Statzner, 2001; Vila-Gispert & Moreno-Amich, 2002; Vila-Gispert, Moreno-Amich & García-Berthou, 2002; Tedesco *et al.*, 2008; Olden & Kennard, 2010; Mims & Olden, 2012). Winemiller (1989), later extended by Winemiller and Rose (1992), classified fish species as equilibrium, opportunistic or seasonal strategists on the basis of their reproductive traits and linked these strategies to specific environmental features. Equilibrium strategists, related to Pianka's (1970) 'K strategists', show strong investment in individual offspring; they have a long generation time and produce a small number of large oocytes continuously during a long spawning season. Equilibrium strategists tend to occur in habitats with low environmental variability. Opportunistic strategists are small-bodied and have a short generation time, early maturation, small oocytes and small clutches, which allow for high intrinsic rates of population growth. This strategy is associated with highly variable and unpredictable habitats. Seasonal strategists have medium-to-large body size, late maturation and long generation times and produce large clutches of small eggs periodically. Seasonal strategists are favoured in habitats with well-marked seasonal fluctuations (Winemiller, 1989; Winemiller & Rose, 1992). The three strategy types define the end points of a continuum that allows for intermediate strategies lying between the end points in a constrained multivariate space.

Studies focusing on general ecological strategies of groups of species help to explain macroecological biodiversity patterns and are attuned to the need for ecogeographical approaches in community ecology (Ricklefs, 2008). However, large-scale studies may not allow for a complete understanding of processes and relationships (including interspecific interactions, selective forces and environmental disturbances) at the scale of local ecological niches. At fine spatial and temporal scales, patterns of local habitat use are related to predation risk (e.g. Power, 1984; Gilliam & Fraser, 1987; Fraser & Gilliam, 1992), habitat profitability (e.g. Werner *et al.*, 1983) and physical habitat characteristics (e.g. Gorman & Karr,

1978). However, over evolutionary time scales, habitat use may interact with less labile biological traits to influence organism fitness and forge assemblage composition. The study of relationships between species biological traits and fine-scale habitat characteristics can yield insights into how trait selection at the organismal level becomes coupled to habitat use.

In this study, we examined the influence of hydrological variability on the spatial segregation of Amazonian stream fishes between two habitats and the relationship between fine-scale habitat use and life history of individual fish species. We assessed fish abundance and environmental characteristics in the main channel and in associated temporary ponds at eight headwater stream sites along a full seasonal cycle and tested whether the distribution of species abundances between habitats (1) showed consistent spatial segregation, (2) was related to stream and pond hydrological characteristics and (3) was linked to species' life-history traits.

Methods

Study system

The study was conducted in first- and second-order streams (*sensu* Petts, 1994) and adjacent flooded areas in Reserva Ducke (02°53′S, 59°58′W), a protected area located in the central Brazilian Amazon near the confluence between the Negro and Solimões rivers. Vegetation cover in the stream basins is pristine 'terra-firme' rainforest. The seasonal flood pulse and prolonged lateral expansion of the mainstream channel typical of large floodplain rivers during the rainy season (Junk, Bayley & Sparks, 1989; Saint-Paul *et al.*, 2000) do not occur in the study area. Hydrological fluctuations in the study system are primarily controlled by local rainfall; stream discharge increases rapidly in response to local rainstorms and then recedes to pre-disturbance levels within a few hours. This response generates a long-term pattern of small, frequent pulses in discharge (Tomasella *et al.*, 2008). Pulses in stream discharge, coupled with the rise of the water table during the rainy season, generate numerous marginal temporary ponds, which are colonised by various species of fish (Pazin *et al.*, 2006). In the rainy season, marginal ponds can be linked to each other and to the stream channel through intermittent connections that appear during rainstorms and persist for a few hours. Seasonal persistence is highly variable among ponds, which can hold water for 1-11 months of the year. Over the year covered by this study, monthly rainfall was lowest near August and greatest near February; stream flow and pond development were at their

lowest in August and November (see Table S1 in Supporting Information).

Fish sampling and environmental measurements

We studied eight plots located in the western drainage basin of Reserva Ducke, which has broad valleys that favour the appearance of temporary ponds. After the dry season and following an initial stage of intermittent rainfall (when the newly formed ponds retain water for just a few days), the terrain alongside upland forest streams becomes gradually saturated with water as the wet season progresses, which results in pond hydroperiods lasting up to 11 months (Pazin *et al.*, 2006). Individual plots comprised an area that followed the stream meanders over a reach measuring 50 m in length; plot width (mean ± 1 SD: 49.4 ± 6.1 m) was determined by the width of the stream valley as delimited by the high-water mark. The distance between plots varied from 316 to 4300 m (mean ± 1 SD: 1999 ± 975 m). At each plot, we sampled fish and measured environmental characteristics of the mainstream channel and adjacent lateral ponds on five occasions (surveys) approximately evenly spaced in time. The surveys were conducted from April 2008 to March 2009, between two annual rainy seasons. On each survey date, fish were collected by three operators equipped with hand nets and seine nets (2-mm mesh). The stream reach was closed at its extremities with block nets (5-mm mesh) to prevent fish movements into or out of the reach; the operators then moved upstream along the reach attempting to cover all areas

systematically within a 2-h period (Mendonça, Magnusson & Zuanon, 2005). In ponds, fish were collected with hand nets until apparent depletion (no further specimens captured during a period of at least 5 min) (Pazin *et al.*, 2006). Captured fish were maintained in a plastic container holding stream water, with aeration by a battery-operated pump. All captured fish were identified and measured (standard length, mm) in the field and released at their capture location. For each plot and survey date, we measured stream width, depth and water velocity as described in Mendonça *et al.* (2005); we also counted the number of ponds and measured surface area, depth and distance to the stream channel for each pond (Pazin *et al.*, 2006). We included in our analyses 11 fish species that collectively accounted for 96% of all captures (see Table S2) and showed broad variation in distribution between pond and stream channel habitats (Fig. 1). Voucher specimens were deposited at the Instituto Nacional de Pesquisas da Amazônia (INPA) Fish Collection, Manaus.

Quantitative analyses

We examined the relationship between relative habitat use and seasonal environmental fluctuations in two steps. In the first step, we summarised habitat use at the assemblage level by means of an index that combined abundances in stream and ponds and used that index in an exploratory multivariate analysis that examined joint variation in habitat use by the 11 species in response to environmental fluctuations. For each species, survey and
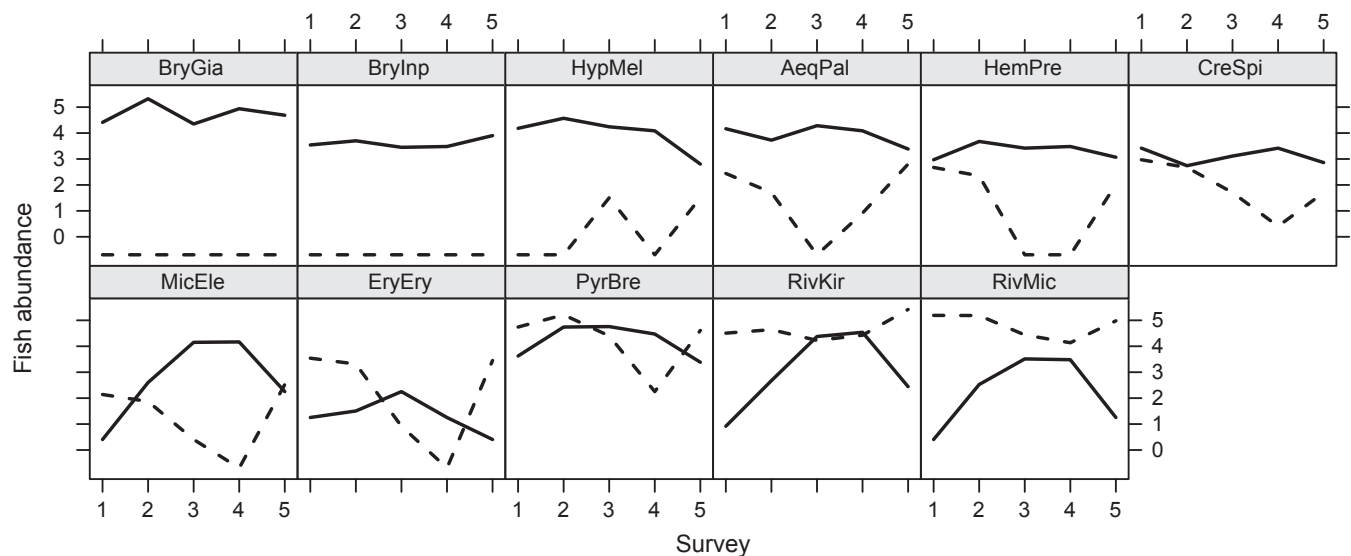
Fig. 1 Seasonal change in fish abundances and habitat characteristics for stream (continuous lines) and pond (dashed lines) habitats of Reserva Ducke. Abundance values are total counts from eight study sites, transformed as ln(X + 0.5). See Table 1 for species codes.

plot, the habitat use (HU) index was calculated as:

$$HU = \ln(P + 0.5) - \ln(S + 0.5) \qquad 1$$

where P represents abundance (total number of individuals) in marginal ponds, and S represents abundance in the stream channel. A small constant (0.5) was added to P and S to avoid indeterminate values when P or S was 0 (cf. empirical logit transformation in logistic regression). HU assumes positive values when abundance is greater in ponds than in the stream channel, negative values when abundance is greater in the stream channel than in ponds and zero when the two habitats are used equally. A total of 40 (eight plots and five surveys of each) HU values were obtained for each species. We then performed a redundancy analysis (RDA) that simultaneously related HU index values for the 11 study species (dependent variables) to seven environmental features (independent variables): stream width, stream depth, stream water velocity, number of ponds, pond surface area (sum across all ponds), mean pond depth and mean distance of ponds to stream channel. All 40 site–survey combinations were included as cases (objects) in the ordination procedure, which was based on the covariance matrix for the habitat use values. The vegan package (Oksanen *et al.*, 2012) was used to perform the ordinations in the R environment (v. 2.15.1; R Core Team, 2012).

Although RDA provides a useful overview of joint species' responses to environmental variation, it also relies on simple assumptions about the joint distribution of samples. In RDA and related canonical analyses, statistical inference is not straightforward under sampling designs that lead to complex error structures that may vary among species. Therefore, in the second step, we built more detailed generalised linear mixed models (GLMM) relating the relative use of pond and stream habitats by individual fish species (binomial responses characterised by variables P and S) to seasonal hydrological variation. To characterise hydrological variation, we used the first two components, PC1 and PC2, from a principal component analysis (PCA) based on the correlation matrix for the seven environmental features previously used in the RDA analysis. Only the first two components were retained because eigenvalues for the remaining five components were all <1 (Kaiser's criterion).

Random effects in the mixed models were used to account for potential intragroup correlations arising from the sampling design (measurements nested within sites). A temporal effect was also included in the models to account for trends in relative habitat use.

For each species, the GLMM had the following form:

$$\text{logit}(\pi_{it}) = \beta_0 + \beta_1 PC1_{it} + \beta_2 PC2_{it} + \beta_3 T_t + \upsilon_i + \varepsilon_{it} \qquad 2$$

where β are regression coefficients, T represents time, υ are random effects associated with sites, and ε are normally distributed errors with zero mean. The indices i ($i = 1\ldots 8$) and t ($t = 1\ldots 5$) correspond to site and time. The random effects account for lack of independence among samples from a given site; they are assumed to

Table 1 Life-history data for the eleven most abundant fish species in the study streams of Reserva Ducke. Standard lengths are maximum values from the Fishbase Website (http://www.fishbase.org) or from our field measurements. When information for a given species was not available, we used data from a closely related species (provided under 'Source')

Species	Code	Standard length (mm)	Fecundity (eggs per batch)	Oocyte diameter (mm)	Spawning strategy			Source
					Diffuse	Polymodal	Unimodal	
Aequidens pallidus	AeqPal	150	871	1.75	0	1	0	*A. pulcher* (Winemiller, 1989)
Bryconops giacopinii	BryGia	125	2858	0.7	1	0	0	*Bryconops* sp. (Mérigoux *et al.*, 2001)
Bryconops inpai	BryInp	110	2858	0.7	1	0	0	*Bryconops* sp. (Mérigoux *et al.*, 2001)
Crenuchus spilurus	CreSpi	50	180*	0.8**	0	1	0	*Zuanon, J., pers. obs.; **Axelrod & Schultz (1990)
Erythrinus erythrinus	EryEry	200	43748	1.2	0	0	1	Mérigoux *et al.* (2001)
Hemigrammus cf. *pretoensis*	HemPre	67	1930	0.5	0	0	1	*H. ocellifer* (Mérigoux *et al.*, 2001)
Hyphessobrycon aff. *melazonatus*	HypMel	41	882	0.6	1	0	0	*H.* aff. s*ovichthys* (Mérigoux *et al.*, 2001)
Microcharacidium eleotrioides	MicEle	23	209	0.5	1	0	0	Mérigoux *et al.* (2001)
Pyrrhulina brevis	PyrBre	76	2207	0.85	0	0	1	*Pyrrhulina filamentosa* (Mérigoux *et al.*, 2001)
Rivulus micropus	RivMic	58	92	2.1	0	0	1	Dias, M.S., unpubl. data
Rivulus kirovskyi	RivKir	50	18	2.0	0	0	1	Dias, M.S., unpubl. data

be normally distributed and have zero mean. Parameter estimation was done within a Bayesian framework, with prior distributions specified as follows:

$$\beta \sim N(0, 10); \sigma_v \sim IG(1, 1); \sigma_\varepsilon \sim IG(1, 1) \qquad 3$$

where IG is the inverse Gamma distribution, and the σ represents the standard deviations of the random effects and the residual errors, in both cases parameterised to yield diffuse priors.

The GLMM were fit using the MCMCglmm package in R (Hadfield, 2010). For each species, the GLMM coefficients β_1 and β_2 quantify the effects of hydrology on relative habitat use by that species. To visualise these effects and compare the responses of individual species, we created an ordination of the 11 species in the parameter space defined by coefficients β_1 and β_2. To determine whether hydrological effects on habitat use were related to reproductive strategies, we first selected a set of biological traits to characterise the reproductive strategies of the study species: maximum standard length, fecundity (number of mature oocytes), egg size (mean diameter of mature oocytes) and spawning schedule. Values for these variables were obtained from various literature sources (Table 1). When information was not available for a given species, we used data from a closely related species. Spawning schedule was inferred from changes in frequency distributions of fish length across the five surveys (Nunn, Harvey & Cowx, 2007). For each species, we followed the transitions in frequency distributions across the surveys and interpreted the appearance of a cohort of young-of-the-year in a given survey as evidence of spawning prior to that survey. We then established three spawning schedules: polymodal, for species that spawn throughout the year and have more than one reproductive peak; diffuse, for species that spawn throughout the year but show no apparent peaks; and unimodal, for species that concentrate spawning over a short period during the rainy season. These schedules are similar to the multiple (or fractional), protracted and total spawning strategies described in Nunn *et al.* (2007). Associations between variables characterising species' reproductive strategies were examined by means of a PCA based on the correlation matrix for reproductive traits. The first three components from this PCA (the only components with eigenvalues >1) were used as surrogate measures of reproductive strategies. We tested whether hydrological effects on habitat use were related to reproductive strategies by examining the Pearson correlations between the β_1 coefficients from the GLMM models and the first three components of the PCA of reproductive traits. In

Table 2 Loadings of environmental variables on the first two components of a PCA based on the matrix of correlations between environmental variables. The two components account for 54.7% (PC 1) and 22.9% (PC 2) of the variance in the environmental data

Environmental variable	PC 1	PC 2
Stream width*	0.253	0.608
Stream velocity[†]	0.284	0.043
Stream depth[‡]	0.141	0.684
Mean pond depth[‡]	0.462	0.092
Total pond area[‡]	0.463	−0.215
Total number of ponds[‡]	0.468	−0.198
Mean pond distance to stream[‡]	0.436	−0.259

*transformed as ln(X).
†transformed as $X^{0.5}$.
‡transformed as ln(X + 1).
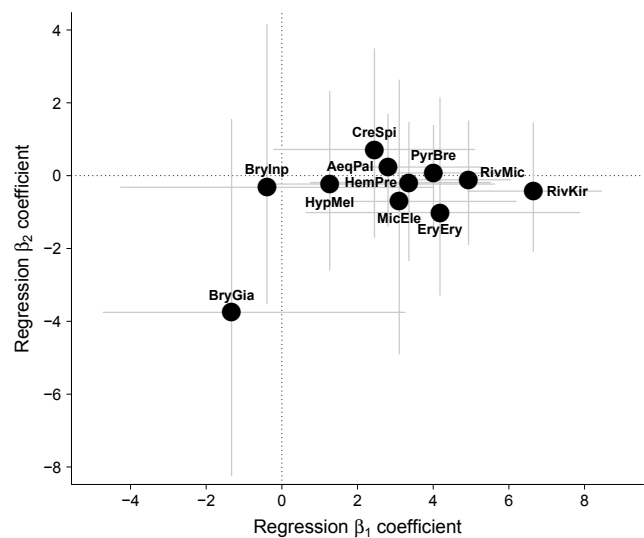
Fig. 2 Species ordination in the parametric space defined by the β_1 and β_2 coefficients of GLMM models of habitat use. Horizontal and vertical lines represent 95% credible intervals from the posterior distributions of the coefficients. See Table 1 for species codes.

all statistical analyses, environmental and life-history variables were transformed as required to linearise the relationships and reduce the influence of outliers.

Results

Seasonal patterns of habitat use differed markedly among species (Fig. 1). The two *Bryconops* species did not occur in ponds and showed little variation in abundance in the stream channel. Despite temporal fluctuations in abundance, *Aequidens pallidus*, *Crenuchus spilurus*, *Hemigrammus* cf. *pretoensis* and *Hyphessobrycon* aff. *melazonatus* were always most abundant in streams, whereas *Erythrinus erythrinus*, *Microcharacidium eleotrioides* and
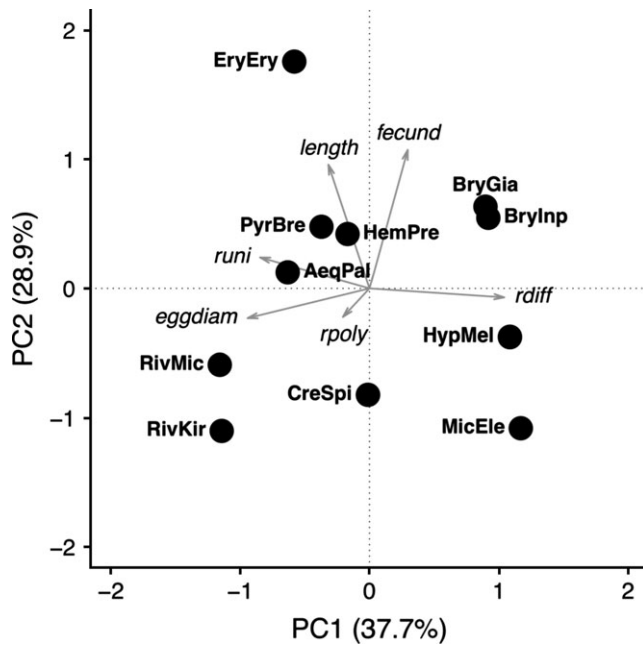
Fig. 3 Biplot for principal component analysis based on species reproductive traits. Fecund, total number of mature oocytes; length, maximal standard length for the species; eggdiam, mean diameter of mature oocytes; rdiff, rpoly and runi refer to spawning timing (see Methods). The variables fecund and length were ln-transformed. See Table 1 for species codes.
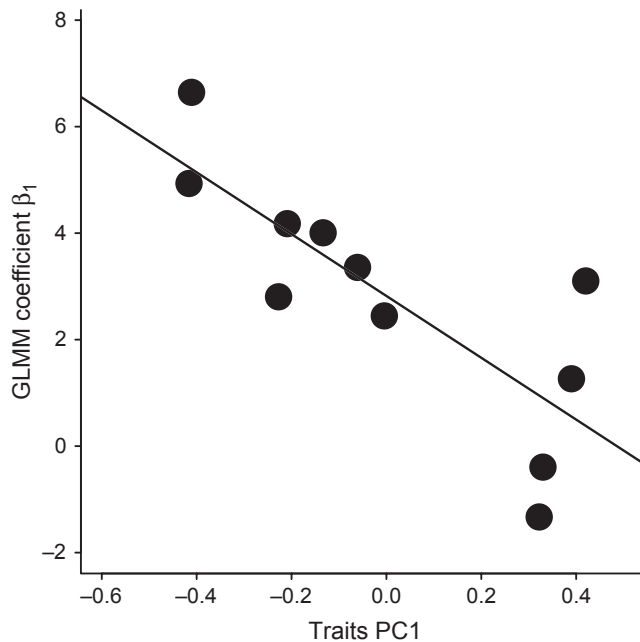

Fig. 4 Scatter plot showing the relationship between coefficient β_1 of the GLMM and the first component of the traits PCA for the 11 fish species. A linear regression line is shown ($y = 2.820 - 5.805x$; $r = -0.799$, $P = 0.003$).

Pyrrhulina cf. *brevis* were more abundant in ponds than in the stream channel during the wettest months (surveys 1 and 5). The two rivulids were generally most

abundant in ponds, especially during the wettest months. Species showing strong seasonal variation tended to increase their use of ponds, decrease their use of the stream channel or both, during the driest months (surveys 3 and 4).

Habitat use at the assemblage level was structured by hydrological variation, as shown by the configuration of species arrows along axis 1 of the RDA ordination (see Fig. S1 in Supporting Information). The environmental features contributing most strongly to axis 1 were those reflecting the availability of ponds, as indicated by the length and alignment of the arrows for pond number, area, depth and distance to the stream channel (Fig. S1). The three species with lowest values along RDA axis 1, *Bryconops giacopinii*, *B. inpai* and *Hyphessobrycon* aff. *melazonatus*, primarily used the stream channel throughout the year and thus showed limited (*H.* aff. *melazonatus*) or no (*B. giacopinii* and *B. inpai*) seasonal variation in habitat use (Fig. 1). At the other extreme of the axis are the two *Rivulus* species that were abundant in ponds in all surveys but had seasonally varying abundance in the stream channel (Fig. 1).

Similar to results from the RDA, pond features had the greatest loadings on the first axis of the PCA of environmental features, whereas stream features contributed most strongly to the second axis (Table 2). The GLMM models showed that habitat use of seven species responded to variation along the first PCA axis (i.e. confidence intervals for their β_1 coefficient did not overlap zero; Fig. 2). In contrast, none of the species showed responses to variation in the second PCA axis (confidence intervals for all of the β_2 coefficients overlap zero; Fig. 2).

In the PCA of reproductive traits, the first component was negatively related to the size of mature oocytes and to unimodal spawning and positively related to diffuse spawning (Fig. 3). The second component was positively related to the number of eggs and to maximum standard length (Fig. 3). The first PCA axis ordered species along a gradient reflecting spawning schedules and mean egg size. Species with small (≤0.7 mm) eggs and diffuse reproduction had large positive values on the first axis, whereas species with large (≥1.2 mm) eggs and unimodal reproduction had large negative values on this axis (cf. Fig. 3 and Table 1). The extent to which species modified their habitat use in response to hydrological variation was linked to their reproductive strategies, as shown by the relationship between the β_1 coefficients from the GLMM and the first component from the PCA of reproductive traits (Fig. 4). This relationship indicates

that hydrological effects on habitat use were strongest for species with large eggs and seasonally concentrated reproduction (unimodal spawners; negative values for PC1 in Fig. 3), which were most abundant in ponds at high water levels. In contrast with this pattern, species with smaller eggs and a seasonal reproduction (diffuse spawners; positive values for PC1 in Fig. 3) were always dominant in the stream channel and showed no change in habitat use in response to hydrological variation. The second and third components of the PCA of reproductive traits showed no apparent relationship with the β_1 coefficients from the GLMM.

Discussion

Habitat use patterns

Although it is well known that the seasonal expansion of large Amazonian rivers into flooded forest is followed by lateral migration of fish (Lowe-McConnell, 1987; Goulding, 1989), the size of Amazonian floodplains and the logistical difficulties inherent to sampling fish in this system have hindered quantification of habitat shifts at this spatial scale (but see Cox-Fernandes, 1997; Castello, 2008; Osorio *et al.*, 2011). In contrast, the comparatively smaller scale of headwater streams allows for effective sampling of fish abundances within well-delimited habitat boundaries. Indirect evidence of fish movements between headwater stream channels and adjacent temporary habitats in Reserva Ducke includes the nested pattern of fish assemblages in ponds (Pazin *et al.*, 2006) and a marked decline in the abundance of fish in the main channel of headwater streams during the rainy season (Espírito-Santo *et al.*, 2009). By sampling streams and temporary ponds simultaneously along a complete seasonal cycle, the present study provides more direct evidence of seasonal habitat shifts and shows that the extent of such shifts varies markedly among species.

Despite their complex life-history schedules, which usually involve movements between different habitats to complete their life cycles (Schlosser & Angermeier, 1995), fish can show highly predictable habitat associations. Hydrological characteristics of streams, such as depth or discharge, have been effectively used to explain the distribution and habitat use of fish (Gorman & Karr, 1978). Our results show that fish species can be ordered along a gradient of relative habitat use and that habitat segregation between ponds and stream channel is more strongly influenced by the availability of ponds than by stream hydrology. The gradient in habitat use ranged

from species that occurred mainly in the stream channel under all hydrological conditions, such as the characids *Bryconops* and *Hyphessobrycon*, to species that occupied ponds in response to increased pond availability at high water levels, such as the rivulids and the erythrinid *Erythrinus erythrinus*. Species with a more equitable distribution of abundance between the two habitats were positioned in the middle of this gradient.

Life-history strategies

As reported by Winemiller and Rose (1992), the species ordination based on life-history traits identified two general gradients of variation. Most of the fish species studied could not be unambiguously assigned to one of the three end points (equilibrium, opportunistic or seasonal) of the trilateral continuum proposed by Winemiller (1989) and Winemiller & Rose (1992), but may occupy instead the intermediate multivariate space between the end points. For example, the two *Rivulus* species show some characteristics of the equilibrium strategy but have a long spawning period and do not show parental care. *Hyphessobrycon* aff. *melazonatus*, *Microcharacidium eleotrioides* and *Crenuchus spilurus* present traits (small body and egg size, several reproductive events) that match the opportunistic strategy, but at least *C. spilurus* shows uniparental care of eggs and larvae (J. Zuanon, pers. obs.). The larger-sized species (*Bryconops* spp. and *E. erythrinus*) have some characteristics of seasonal spawners, but in contrast with expectations for the seasonal strategy, both *Bryconops* species have a long reproductive period, and *E. erythrinus* have large eggs that are protected by one or both parents. The cichlid *Aequidens pallidus* show reproductive characteristics typical of equilibrium species (multiple spawning and well developed parental care of eggs, larvae and young), but it is among the largest species in the study streams. Small (<10 cm SL) species, which comprise a major portion of the fish faunas in low-order forest streams of the Amazon basin, have been underrepresented in previous studies of life-history strategies in Neotropical fishes (but see Mérigoux *et al.*, 2001). Our study expands the scope of previous assessments of the model of Winemiller and Rose (1992) taxonomically and functionally (by examining a set of species of small body size) as well as geographically (by focusing on first- and second-order forest streams that are both numerous and widespread in the Amazon basin).

The lack of species representative of the seasonal strategy may result from environmental constraints specific to Amazonian upland forest streams. The seasonal strategy

requires a large body size to allow for the development and accommodation of a large number of oocytes (Winemiller, 1989). However, the small dimensions of headwater forest streams (both stream width and depth) presumably limit the maximum body size of fishes that complete their life cycles in this environment. Furthermore, the low productivity of Amazonian headwater streams (Walker, 1995) may reduce their suitability for seasonal strategists by limiting somatic growth and the accumulation of fat reserves necessary for seasonal migration. Finally, the absence of some potential environmental triggers to fish migration, such as marked seasonal change in photoperiod or temperature, may also contribute to the apparent paucity of seasonal strategists in Amazonian forest streams. In the highly dendritic networks of the Brazilian Amazon, most of the headwater streams are distant from the main river stems so that very different fish assemblages are found in small streams and larger rivers (hydrological order >5) (Crampton, 2011). Accordingly, we would expect the seasonal strategy to become progressively more common as river size, autochthonous productivity and floodplain development increase along the river continuum (Vannote *et al.*, 1980).

Habitat templet and reproductive strategies

Temporal variation in water level of rivers and streams is one of the main driving forces of fish movements among habitats. Regularity and predictability in hydrological variation provide favourable environments for completion of the life cycle of many species (Schlosser, 1991). In tropical floodplain river systems, many fish species have developed life-history strategies that allow them to use highly productive temporary habitats for reproduction and early development of larval and juvenile phases during the flood season (Lowe-McConnell, 1987; Winemiller, 1989). Rapid growth under favourable conditions in these habitats allows young-of-the year to attain a larger body size by the end of the flood season, leading to greater chances of survival and dispersal under the harsh environmental conditions of the subsequent dry season (Junk *et al.*, 1989).

Our results show that habitat segregation between ponds and the stream channel is strongly related to both availability of ponds and life-history strategies. Upland forest streams are not directly affected by the seasonal flood pulse, but strong hydrological variation is nonetheless induced there by local rainfall (Tomasella *et al.*, 2008). In these streams, life-history strategies could therefore be expected to reflect adaptations to highly

variable and unpredictable environments. However, despite frequent small-scale fluctuations in stream level in response to rainfall, the availability of marginal ponds is to some extent predictable. On an intra-annual time scale, Pazin *et al.* (2006) showed that some ponds could keep water for as long as 11 months and that fish assemblage composition was linked to pond hydroperiod. Furthermore, most of the ponds in that study were still present during the present study, 6 years later, suggesting that individual ponds show persistence across years. The channels of Amazonian upland forest streams are temporally very stable and can show only minor apparent changes over a period of decades (J. Zuanon, pers. obs. of streams in Reserva Ducke since 1986). Stability of the stream channel and banks in Amazonian upland forests is enhanced by the naturally stable soil structure, densely interwoven roots of the riparian vegetation and protection from direct impact of rainfall by the forest canopy. Interannual persistence in pond availability also contributes to long-term predictability and may thus favour the development of couplings between fish reproductive traits and relative use of channel and pond habitats in upland forest streams.

Mechanistic processes

The strong association of *Rivulus* species with pond habitats could be explained by the ability of *Rivulus* to occupy the ponds as soon as these become seasonally available (Pazin *et al.*, 2006). *Rivulus* appear to be poor swimmers but are capable of reaching pond habitats independently of permanent or temporary aquatic connections, by jumping repeatedly over dry land (as reported for other cyprinodontiformes; Gibb *et al.*, 2011 and references therein). This behaviour could be related to avoidance of predators in the stream channel (e.g. Fraser & Gilliam, 1992), greater availability of food in ponds or use of ponds as spawning and nursery areas. Young-of-the-year of both *Rivulus* species were mostly captured in ponds throughout the study and were only captured in the stream channel in the dry season surveys, when pond availability was reduced. *Rivulus* species are usually the sole occupants of the uppermost stream reaches, in which aquatic habitat comprises predominantly very shallow water underlain by a thick bed of decaying leaf litter. The increases in size and discharge of streams along the river continuum are accompanied by changes in the aquatic environment (i.e. deeper channels, more rapid flow and reduced availability of leaf-packed habitats), which may result in poorer habitat for *Rivulus*. Preference for marginal ponds by

rivulids may thus be a strategy that allows for colonisation of a variety of suitable lateral habitats and leads to increased dispersal potential along the river continuum (Fraser, Gilliam & Yip-Hoi, 1995).

In contrast, *Bryconops* are primarily nektonic, rheophilic stream dwellers that tend to shoal and show generalised body morphology and habits (Géry, 1977). The restriction of *Bryconops* to stream channel habitat may arise from low tolerance to hypoxia or from ecological constraints related to a reproductive strategy requiring medium-to-long-distance dispersal of offspring (Winemiller, 1989). Alternatively, this restriction may be related to the feeding mode of *Bryconops*, which are highly dependent on large inputs of allochthonous food transported by the current, such as terrestrial insects (Silva, Ferreira & de Deus, 2008). Moreover, pond occupancy by *Bryconops* may be impaired by structural habitat features (e.g. depth) that constrain the use of the water column by these shoaling active swimmers.

Species that showed more equitable use of pond and channel habitats share features of equilibrium strategists. *Aequidens pallidus* and *C. spilurus* are small-to-medium-sized fish that have low fecundity and variable forms of parental care; moreover, they seem to prefer highly structured habitats and display territorial or philopatric behaviour (*A. pallidus*: Bührnheim & Cox-Fernandes, 2004; *C. spilurus*: J. Zuanon, pers. obs.).

The Amazon basin is one of the few regions on the planet that still allow for examination of relationships between biological traits and environmental characteristics under pristine conditions, at spatial scales ranging from microhabitat to biome. Although habitat stability has been shown to have a major influence on the evolution of reproductive strategies in riverine fishes (Tedesco *et al.*, 2008; Olden & Kennard, 2010), most studies to date have focused on macroecological comparisons of large river systems. However, small-scale habitat characteristics affect life history and behavioural tactics (Gross, 1991), and therefore, large-scale patterns of trait distribution may be influenced by selection at smaller spatial scales than those commonly studied. Our results support this notion by showing that species reproductive strategies are ordered predictably along a gradient of hydrological variation at a fine spatial scale. The species-specific habitat segregation reported here can be added to the list of mechanisms, such as miniaturisation (Weitzman & Vari, 1988) and isolation by distance (Castro, 1999), postulated to contribute to the high diversity of the stream fish fauna in the Amazon basin. The observed coupling of fish reproductive strategies, habitat use and temporal dynamics may enhance species richness by allowing for increased specialisation in the exploitation of spatially and temporally varying resources in these small forest streams.

Acknowledgments

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Supporting Information

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

Figure S1. RDA ordination of relative habitat use by 11 fish species in relation to hydrological characteristics of stream and pond habitats. The first RDA component accounts for 37.0% of the total variation in habitat use and 62.7% of the variation in habitat use explained by the hydrological variables. The second RDA component accounts for 11.0% of the total variation in habitat use and 18.6% of the variation in habitat use explained by the hydrological variables. See Table 1 for species codes.

Table S1. Environmental characteristics of streams and ponds on five surveys between April 2008 and February 2009 for the eight study plots in Reserva Ducke. Medians (25th – 75th percentiles) are reported.

Table S2. Number of fish of each species captured in stream channels and temporary ponds between April 2008 and February 2009 at eight study sites in Reserva Ducke.

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