

# ECOGRAPHY

## Research

### Fish assemblage convergence along stream environmental gradients: an intercontinental analysis

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Species that pass through similar environmental filters, regardless of geographic proximity or evolutionary history, are expected to share many traits, resulting in similar assemblage trait distributions. Convergence of assemblage trait distributions among different biotic regions would indicate that consistent ecological processes produce repeated patterns of adaptive evolution. This study analyzes trait–environment relationships across multiple stream fish assemblages representing evolutionarily divergent faunas. We hypothesized that trait–environment patterns converge across regional faunas in response to a common set of environmental filters acting on functional traits. One hundred and ninety-seven species and forty streams were sampled from five regions: Belize, Benin, Brazil, Cambodia and USA. By examining trait–environment plots, multiple congruent trait–environment patterns were found across all regions, indicative of a consistent set of environmental filters acting on local community assembly. The consistency of these patterns strongly suggests that water velocity and habitat structural complexity function as universal environmental filters, producing similar assemblage trait distributions in streams across all regions. Bivariate relationships were not universal, and only one of the associations between a single functional trait and single environmental variable was statistically significant across all five regions. Strong phylogenetic signal was found in traits and habitat use, which implies that niche conservatism also influenced assemblage trait distributions. Overall, results support the idea that habitat templates structure trait distributions of stream fish assemblages and do so in a consistent manner.

Keywords: environmental filters, fishes, intercontinental, microhabitat, streams

#### Introduction

Although evolution plays a major role in establishing trait distributions in species assemblages (Webb et al. 2002, Cavender-Bares et al. 2009), contemporary ecological processes also have a significant influence, especially at local scales (Lebrija-Trejos et al. 2010, Kraft et al. 2015). For example, habitat template theory posits that spatial and temporal variation of habitat features selects for certain traits and, therefore, influences the structure of local communities (Southwood 1977, Poff 1997). In this manner, habitat features act as environmental filters that shape trait distributions of species assemblages by



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restricting establishment and persistence of organisms based on the suitability of their traits for living in a given environment (Weiher and Keddy 1995, Cornwell and Ackerly 2009, Lebrija-Trejos et al. 2010, Kraft et al. 2015). Therefore, it is predicted that species that pass through similar environmental filters, regardless of geographic proximity or evolutionary history, should share many convergent traits, and local assemblages should reflect nonrandom assembly. Assemblage trait convergence among evolutionarily independent communities would indicate common responses to selection producing repeated patterns of adaptive evolution as well as consistent processes of local community assembly. Here we examine the degree of convergent environmental–trait relationships in fish assemblages of lowland streams with similar environmental gradients in five different zoogeographic regions.

Stream fish are excellent model organisms for addressing questions about assemblage trait convergence in response to environmental conditions. First, stream fish often are isolated within a single drainage basin, which results in adaptation to local–regional conditions. Second, many functional traits of fish are well studied and therefore robustly quantified and interpreted (Keast and Webb 1966, Gatz 1979, Winemiller 1991). Third, local fish assemblages have been shown to be structured by environmental filters acting at multiple spatial and temporal scales (Poff and Allan 1995, Poff 1997, Hoinghaus et al. 2007). Certain selective pressures, such as bioenergetic costs associated with hydraulic drag as a function of body shape, are universal in fluvial habitats and should produce convergent assemblage structure. Several regional and local environmental filters have been found to be associated with fish assemblage structure. For example, hydraulic and geomorphic aspects of streams explain a large proportion of the trait variance between fish assemblages in North America and Europe at stream-reach and basin scales (Lamouroux et al. 2002). Finally, fluvial habitats, including lowland streams, have similar physicochemical characteristics worldwide, making them excellent model systems for comparing convergence across different zoogeographic regions.

Although there is great potential for finding general ecological patterns through the study of assemblage trait convergence in fishes (Winemiller 1991, Lamouroux et al. 2002), these studies face several challenges. First, assemblage-wide convergence studies require large data sets. Specifically, studies of different biogeographic regions are often problematic, with inconsistent site selection, scale of sampling and collection methods leading to incongruent data sets. Another potential problem arises from the data type and quality, with qualitative variables and coarse-scale data used for traits or habitat components (Lamouroux et al. 2002, Ernst et al. 2012). Finally, commonly used methods, such as community-weighted trait means or niche centroids, have been shown to be poor tests of trait–environment relationships and should be replaced with multivariate approaches such as RLQ and fourth-corner analyses (Peres-Neto et al. 2017).

Another factor that could contribute to lack of evidence of assemblage convergence is historical contingency. The unequal distribution of evolutionary lineages in different

biogeographic regions may result in local assemblages with disparate functional trait distributions and niches due to unique evolutionary histories and phylogenetic niche conservatism. Phylogenetic niche conservatism occurs when lineages retain ancestral niches over time (Harvey and Pagel 1991, Wiens and Graham 2005, Wiens et al. 2010) and has been shown to be common in various taxa, including mammals (Cooper et al. 2011, Peixoto et al. 2017), birds (Rangel et al. 2007), amphibians (Hof et al. 2010) and plants (Ackerly 2003, Crisp et al. 2009). Phylogenetic niche conservatism is often key to understanding niche–trait patterns observed in local assemblages (Vamosi et al. 2009, Wiens et al. 2010). For example, Ernst et al. (2012) inferred that trait–environment relationships were explained more by niche conservatism than convergent evolution. However, a review by Losos (2008) found that most studies of phylogenetic niche conservatism focus on only a few species at small geographic scales. He concluded that niche conservatism is not universal and should not be assumed.

Investigations testing for universal environmental filters based on congruent methods and data are rare, and most research on community structure and assembly has been site specific. Research that compares assemblages in similar habitats from different regions using functional traits could reveal assemblage-level convergence and general ecological patterns (Lamouroux et al. 2002, McGill et al. 2006). If local environmental conditions are an important driver of community structure (Southwood 1977), assemblages that pass through similar environmental filters should display similar patterns of trait distribution regardless of evolutionary history or geographic location. If these environmental filters are globally consistent, they should yield similar trait–environment relationships across evolutionarily distinct assemblages of different biogeographic regions.

This study tests for convergence versus phylogenetic niche conservatism in trait–environment relationships across multiple stream fish assemblages from five zoogeographic regions on four continents. Specifically, we hypothesized that trait–environment relationships converge across distinct biogeographic regions as a result of a common set of environmental filters acting on functional traits. To address this hypothesis, three questions must first be answered: 1) are there correlations between functional traits and environmental variables? 2) If significant relationships among traits and environmental features exist within biogeographic regions, then are these trait–environment relationships consistent across biogeographic regions? 3) Are these relationships a result of common ancestry or convergence in response to a common set of environmental filters acting on fish assemblages?

## Methods

### Data acquisition and preparation

Streams and fish assemblages were surveyed at eight study locations spanning five zoogeographic regions – Benin

(Gulf of Guinea Coast, Afrotropical), Brazil (Amazon Basin, Neotropical), Cambodia (Mekong Basin, Indo-Malayan), Belize (Caribbean Coast, Mesoamerican), New Jersey, South Carolina and Texas (Atlantic and Gulf coasts, Nearctic). The inclusion of different zoogeographic regions allows for comparison of distantly related lineages and testing for convergent evolution and repeated patterns in community assembly. To minimize between-location differences in habitat features, survey sites were selected based on four criteria: 1) low stream order (1–3); 2) low level of disturbance (relatively little human impact to forested watersheds); 3) low gradient; and 4) similar geomorphology (meandering course with sandy substrate and presence of coarse woody structure). In each zoogeographic region, five to six wadeable streams were sampled to encompass a gradient of stream channel width: two small (<3 m average width), two medium (3–8 m) and two large (>8 m). Sampling took place during low-water periods (i.e. base-flow conditions) when streams were wadeable and fish capture was most efficient. Because this study is intrinsically scale dependent (Smith et al. 2013), a nested sample design was used (microhabitat within stream within zoogeographic region).

For each stream, a 200–500-m reach was sampled in an upstream direction to obtain a representative sample of fishes from all major types of microhabitats (modified from Barbour et al. 1999, Bower and Piller 2015, Troia and Gido 2015). Microhabitat types were identified as small patches with relatively consistent depth, water velocity, substrate composition and in-channel cover. A microhabitat was only sampled if its substrate composition could be categorized as sand (>90% cover), woody structure (>80% cover), aquatic macrophytes (>80% cover), leaf packs (>90% cover), root banks (banks with dense root structures, >90%), or gravel (6–25 cm diameter, >80% cover). In each microhabitat, we recorded water velocity, substrate composition and depth. We further classified each substrate category as having either low or high structural complexity. Given the challenge of sampling fish from diverse microhabitats, various methods were employed, including: seining, cast netting, dipnetting and backpack electrofishing. At each study site, habitat variables of water temperature (°C), dissolved oxygen (DO), pH, specific conductivity ( $\mu\text{S cm}^{-1}$ ) and salinity (ppt) were measured. Specimens were euthanized via anesthetic (MS222) overdose and then preserved in 10% formalin following Texas A&M University animal care protocols IACUC 2014-0173 and 2017-0233. Only data for common species were used for data analysis, and species having relative abundance <1% within the collective samples from a given region were excluded.

Twenty-one morphometric traits that reflect well-documented aspects of swimming performance and habitat use were selected (Gatz 1979, Winemiller 1991, Table 1). Traditional morphometric measurements for five individuals per species were made to the nearest 0.1 mm using calipers (for rare species  $n = 1-4$ ; sample sizes appear in Supplementary material Appendix 1 Table A1). To reduce potential ontogenetic biases, only adult size classes were used for analyses (size

at maturation information obtained from literature sources and FishBase, <www.fishbase.org>). Traits standardized by conversion to proportions using either standard length, body depth, body width, head length and head depth in the denominator (Winemiller 1991, Casatti et al. 2006).

Using a recently published time-calibrated tree, a majority-rule consensus tree was created (Rabosky et al. 2018). However, this tree did not include all species included in our study. Following Beaulieu et al. (2012), we replaced exemplar taxa (closely related taxa) to create a tree that included all of the species in our study.

## Statistical methods

Three-table ordination (RLQ) and fourth-corner analyses were applied to data sets for each zoogeographic region and one combined data set that included all zoogeographic regions ('global data set' hereafter) (Dray et al. 2014). For each zoogeographic region and the global data set, the two methods were used to test for specific regional and overall global species–trait–environment relationships, as well as to determine possible existence of a congruent trait–environment relationships across regions. Similar species–trait–environment relationships across regions as well as the global data set would suggest that a common environmental filter influences these assemblages. The RLQ method allows for the simultaneous analysis of three different datasets: species abundance and environmental data for sites (R), species traits (Q) and species abundance (L). This is accomplished by combining three different ordinations, 1) a Hill–Smith analysis for the R matrix because both continuous and categorical variables were used, 2) the Q matrix was created using multiple correspondence analysis and 3) a correspondence analysis to create the L matrix, into a final ordination (Ernst et al. 2012, Dray et al. 2014). The significance of the joint structure among these matrices was tested using a two-step permutation procedure (999 permutations). Model 2 tests the null hypothesis that no relationship exists between species presence–absence data with fixed traits and their environment permuting the row of dataset L; model 4 permutes the columns of dataset L, testing the null hypothesis that species composition is not influenced by species traits, given fixed environmental characteristics (Dray et al. 2014). If both null hypotheses are rejected, the R, Q and L matrices are effectively linked. To correct for multiple comparisons of environmental variables, all  $\alpha$ -values (0.05) were Bonferroni corrected so that  $\alpha_{\text{new}} = \alpha/N$ , where N is the number of environmental variables (Gallardo et al. 2009, Ernst et al. 2012). In addition, the  $\alpha_{\text{new}}$  values were then square-root adjusted to account for the combination of two models in the fourth-corner analysis (Dray and Legendre 2008, Ernst et al. 2012, Dray et al. 2014). Anguilliform species (eel-like body shape) were removed for both the RLQ and fourth-corner analyses due to their extreme morphology; their inclusion produced strongly skewed gradients and assemblage ordinations that separated anguilliform fish from all other species, and the latter tightly clustered within morphospace. The RLQ and

Table 1. Measured traits, trait codes and trait definitions.

Trait	Transformation	Trait definition
Average standard length	SL	maximum standard length from the populations in this study
Head length	HEAD_L/SL	distance from the tip of the jaw to the posterior edge of the operculum
Head depth	HEAD_D/BOD_D	vertical distance from dorsum to ventrum passing through the pupil
Oral gape	GAPE/BOD_D	vertical distance measured inside of fully open mouth at tallest point
Mouth position	MOUTH_P	the angle between an imaginary line connecting the tips of the open jaws and an imaginary line running between the center of the pupil and the posterior-most vertebra (e.g. 90 representing a terminal mouth)
Eye position	EYE_POS/HEAD_D	vertical distance from the ventral pigmented region to the ventrum
Eye diameter	EYE_D/HEAD_D	vertical distance from eye margin to eye margin
Snout length	SNT_L/HEAD_L	distance from the posterior pigmented region of the eye to the tip of the upper jaw with mouth shut
Snout protrusion	SNT_PR/HEAD_L	additional distance from the posterior pigmented region to the tip of the upper jaw with mouth fully open and extended
Body depth	BOD_D/SL	maximum vertical distance from dorsum to ventrum
Body width	BOD_W/SL	maximum horizontal distance from side to side
Caudal peduncle length	PED_L/SL	distance from the posterior proximal margin of the anal fin to the caudal margin of the ultimate vertebra
Caudal peduncle depth	PED_D/BOD_D	minimum vertical distance from dorsum to ventrum of caudal peduncle
Caudal peduncle width	PED_W/BOD_W	horizontal width of the caudal peduncle at mid-length
Dorsal fin length	DORS_L/SL	distance from the anterior proximal margin to the posterior proximal margin of the dorsal fin
Dorsal fin height	DORS_HT/SL	maximum distance from the proximal to distal margin of the dorsal fin (excluding filaments)
Anal fin length	ANAL_L/SL	distance from the anterior proximal margin to the posterior proximal margin of the anal fin
Anal fin height	ANAL_HT/SL	maximum distance from proximal to distal margin of the anal fin
Caudal fin depth	CAUD_D/SL	maximum vertical distance across the fully spread caudal fin
Caudal fin length	CAUD_L/SL	maximum distance from proximal to distal margin of the caudal fin (excluding filaments)
Pectoral fin length	PEC_L/SL	maximum distance from proximal to distal margin of pectoral fin
Pelvic fin length	PELV_L/SL	maximum distance from the proximal to distal margin of the pelvic fin
Gut length	GUT_L/SL	length of gut from the beginning of the esophagus to the anus (extended without stretching)
Gill raker	RAKER	coded as 0 for absent, 1 for short, blunt or toothlike, 2 for intermediate or long and sparse and 3 for long and comb-like
Tooth shape	TOO_S	coded as 0 for absent, 1 for unicuspid (rasping), 2 for multicuspid (crushing), 3 for short conical (grasping), 4 for long conical (piercing) and 5 for triangular serrated (shearing)

fourth-corner analyses were conducted using the R package ‘ade4’ (Dray and Dufour 2007, <[www.r-project.org](http://www.r-project.org)>). A forest plot with averaged niche positions for each species was created using the ‘forestplot’ package in R to plot the average R site scores for each species (Gordon et al. 2017), where the average R site scores were considered as the niche position of each species. To determine if trait variance increased or decreased with water velocity, we used simple linear regression models to test for relationships body depth variance for every 0.05 units of water velocity and median water velocity for each water velocity sub-division.

Species traits and niche positions (average R site scores from the RLQ analysis of the global data set) were tested for phylogenetic signal using Abouhief’s test in the ‘adephylo’ package (Abouheif 1999, Jombart et al. 2010, Münkemüller et al. 2012), an autocorrelation index of phylogenetic proximity that does not rely on an evolutionary model (Pavoine et al. 2008). Original proximities of Abouhief’s test were used in this study. In addition, Mantel tests were performed to

test for a correlation between phylogenetic distance and co-occurrence distance for both the regional and global data sets using the vegan package in R (Oksanen et al. 2015).

## Data deposition

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.n53sh18>> (Bower and Winemiller 2019).

## Results

### Assemblage compositions

A total of 197 species was analyzed in this study, with the number of species collected in each region as follows: 57 USA, 52 Brazil, 41 Cambodia, 25 Benin and 21 Belize. The Brazil location had the most families represented (18), followed by Benin (17), Cambodia (13), USA (10) and Belize

(9) (Supplementary material Appendix 1 Table A1). No species occurred in more than one region.

### Global species–trait–environment relationships

Significant associations among the trait, microhabitat and species presence–absence data sets were revealed by the global RLQ permutation test ( $p < 0.001$  for model 2 and 4 in Dray et al. 2014), demonstrating strong, significant relationships between microhabitats and trait distributions across all regions and the potential existence of a global pattern for the species–trait–environment relationship at the assemblage level. Within all regions, model 2 was rejected, which suggests that species distributions were influenced by environmental conditions. Model 4 was rejected for all regions except Belize ( $p = 0.16$ ) and marginally for Cambodia ( $p = 0.069$ ), which suggests that the traits significantly influenced species composition in stream habitats for fish assemblages in Benin ( $p = 0.031$ ), Brazil ( $p = 0.028$ ) and the USA ( $p < 0.001$ ).

The first axis of the global RLQ indicated a gradient of water velocity and microhabitat structure, with unstructured, high-velocity habitats loading negatively, and low-velocity microhabitats with tree and shrub roots along the banks, aquatic vegetation, and, to a lesser extent, wood loading positively (Supplementary material Appendix 1 Fig. A1). For the most part, RLQ axis 2 discriminated deep from shallow microhabitats as well as those with substrates dominated by leaves or aquatic vegetation (Supplementary material Appendix 1 Fig. A1).

### Within and across-region, trait–environment relationships

Using the multivariate fourth-corner analysis, only one trait was found to have a consistent and statistically significant relationship with a microhabitat variable across all regions: body width with root bank (Fig. 1). Root bank and aquatic vegetation were the microhabitat variables that had the most significant relationships with fish traits, including body depth, body width, head length, dorsal fin length and anal fin height. In general, traits associated with high water velocity had a negative relationship, whereas positive relationships were observed between most functional traits and the variables aquatic vegetation and root bank (Fig. 1). Sandy habitats tended to have a negative association with morphological traits (Fig. 1). Two traits showed congruent habitat–traits relationships for four out of the five regions: dorsal fin length with root bank as well as body width with sand (Fig. 1). Analysis that included the rare species that had been omitted from the datasets produced similar results (Supplementary material Appendix 1 Fig. A2).

Plots of habitat variables and traits reveal several consistent patterns (Fig. 2–4, Supplementary material Appendix 1 Fig. A3–A7). Fish with deep bodies, long dorsal fins and long anal fins were largely absent in microhabitats with relatively fast water (Fig. 2A, 5). Fish with intermediate anal fin height, head length, pectoral fin length, pectoral fin width

and pelvic fin length were found in high-velocity microhabitats (Fig. 2C–F). Low-velocity habitats had fish assemblages with greater interspecific variation for most traits (Fig. 2A–F, Supplementary material Appendix 1 Fig. A3–A7). This was also shown using simple linear regression models, wherein strong negative relationships between mean trait variance for every 0.05 units of water velocity and median water velocity of the water velocity sub-division were found for body depth ( $R^2 = 0.70$ ,  $p < 0.001$ ), head length ( $R^2 = 0.74$ ,  $p = 0.001$ ), anal fin height ( $R^2 = 0.75$ ,  $p = 0.002$ ), anal fin length ( $R^2 = 0.92$ ,  $p < 0.001$ ), caudal fin length ( $R^2 = 0.72$ ,  $p = 0.005$ ), pectoral fin length ( $R^2 = 0.51$ ,  $p = 0.013$ ) and pelvic fin length ( $R^2 = 0.61$ ,  $p = 0.004$ ) (Fig. 2E). A greater range of trait values was also found for species occupying structurally complex microhabitats compared to species residing in areas lacking structural complexity (Fig. 3). Similar trait–habitat patterns were found in each zoogeographic region for body depth, head length, anal fin height, pectoral fin length and pelvic fin length, where trait variance reduced with increasing water velocity (Fig. 4, Supplementary material Appendix 1 Fig. A3–A7). A strong negative relationships between body depth variance for every 0.05 units of water velocity and median water velocity for each water velocity sub-division was found for each region except Belize: A) Belize ( $R^2 = 0.17$ ,  $p = 0.150$ ); B) Benin ( $R^2 = 0.59$ ,  $p = 0.001$ ); C) Brazil ( $R^2 = 0.64$ ,  $p = 0.006$ ); D) Cambodia ( $R^2 = 0.57$ ,  $p = 0.005$ ); E) USA ( $R^2 = 0.63$ ,  $p = 0.002$ ); and F) global data set ( $R^2 = 0.93$ ,  $p < 0.001$ ) (Fig. 4).

### Habitat niche breath

High interspecific overlap in niche positions along microhabitat gradients as defined by RLQ analysis of the global data set was common within and among regions. Species of a given region were distributed more or less evenly along a gradient rather than clustered into regional groupings (Fig. 5). This suggests high similarity of microhabitat characteristics among streams of the five regions (i.e. environmental conditions of study systems were well matched). Several species from USA streams loaded along the negative end of the gradient, these being mostly from the same genus (*Lepomis*). Relatively few species from Cambodia were collected from structurally complex microhabitats with low water velocity. In contrast, Benin and Brazil fishes were infrequently collected from habitats with high water velocities and lacking structural complexity. In addition, relatively few Belize fishes were collected from microhabitats that loaded in the intermediate segment of the dominant habitat gradient (Fig. 5).

### Phylogenetic relationships

None of the regional assemblages had a statistically significant correlation between phylogenetic distance and co-occurrence distance (Mantel test,  $p > 0.05$ ), suggesting related species did not co-occur more than expected at random. However, phylogenetic distance and co-occurrence distance for the global data set were found to be smaller than expected at

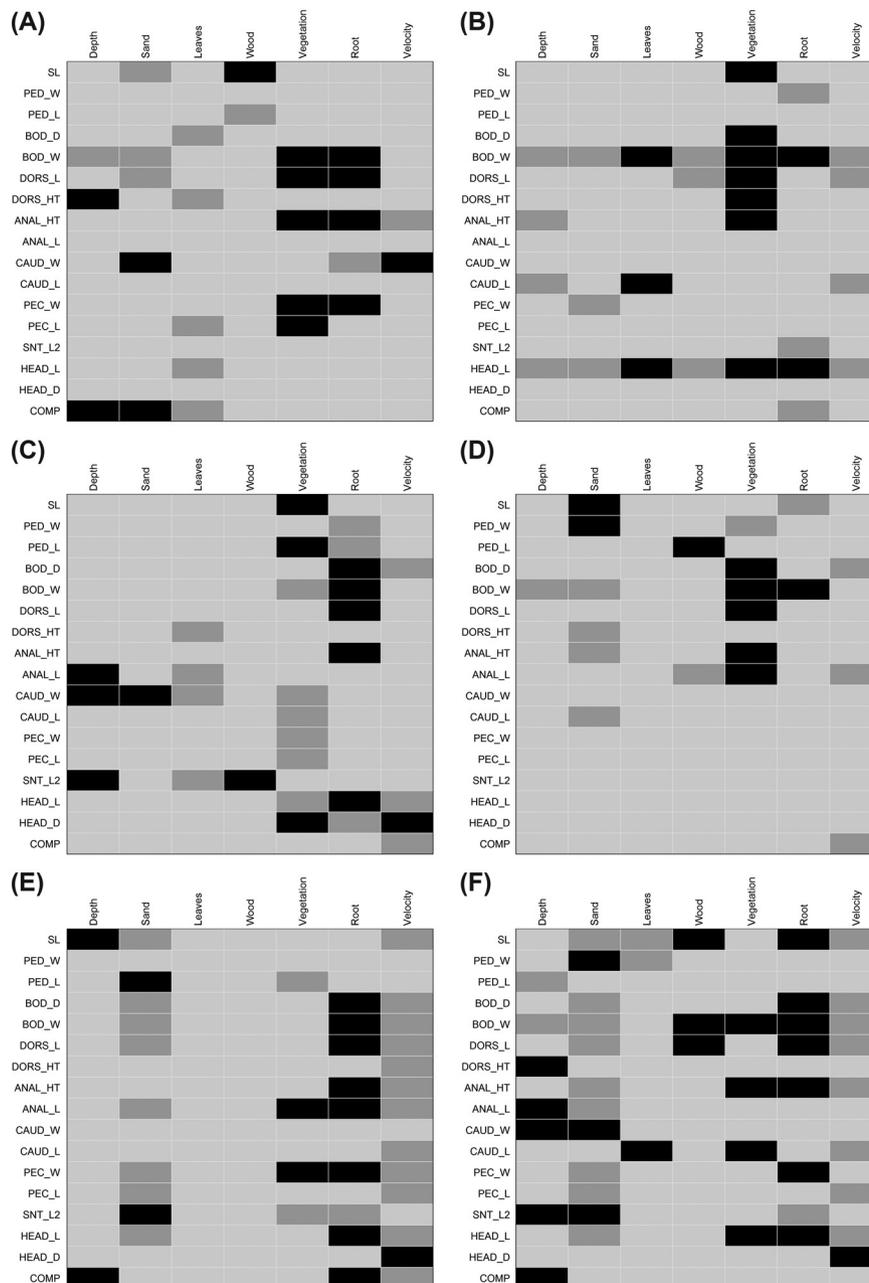


Figure 1. Results of the fourth-corner analyses for each region and the global dataset: (A) Belize, (B) Benin, (C) Brazil, (D) Cambodia, (E) USA and (F). Black denotes a positive relationship (Pearson's correlation) between microhabitat variable and trait, dark grey denotes a negative relationship, and light grey denotes non-significant relationships.

random ( $p < 0.001$ ), confirming that each region was phylogenetically distinct.

A high prevalence of strong phylogenetic signal, indicating that closely related species have more similar traits, was found for all traits across all regions except for Benin. Phylogenetic signal varied among regions, ranging from all traits exhibiting phylogenetic signal in North American species, to only seven traits in West African species (Supplementary material Appendix 1 Table A2). Strong phylogenetic signal was also found for species niche positions (R site scores for each

species from the RLQ analysis) (Abouhief's number = 0.94,  $p = 0.001$ ).

## Discussion

This study revealed patterns of trait–environment relationships that were congruent across distinct zoogeographic regions that apparently derive from consistent sets of environmental filters acting on functional traits. This provides

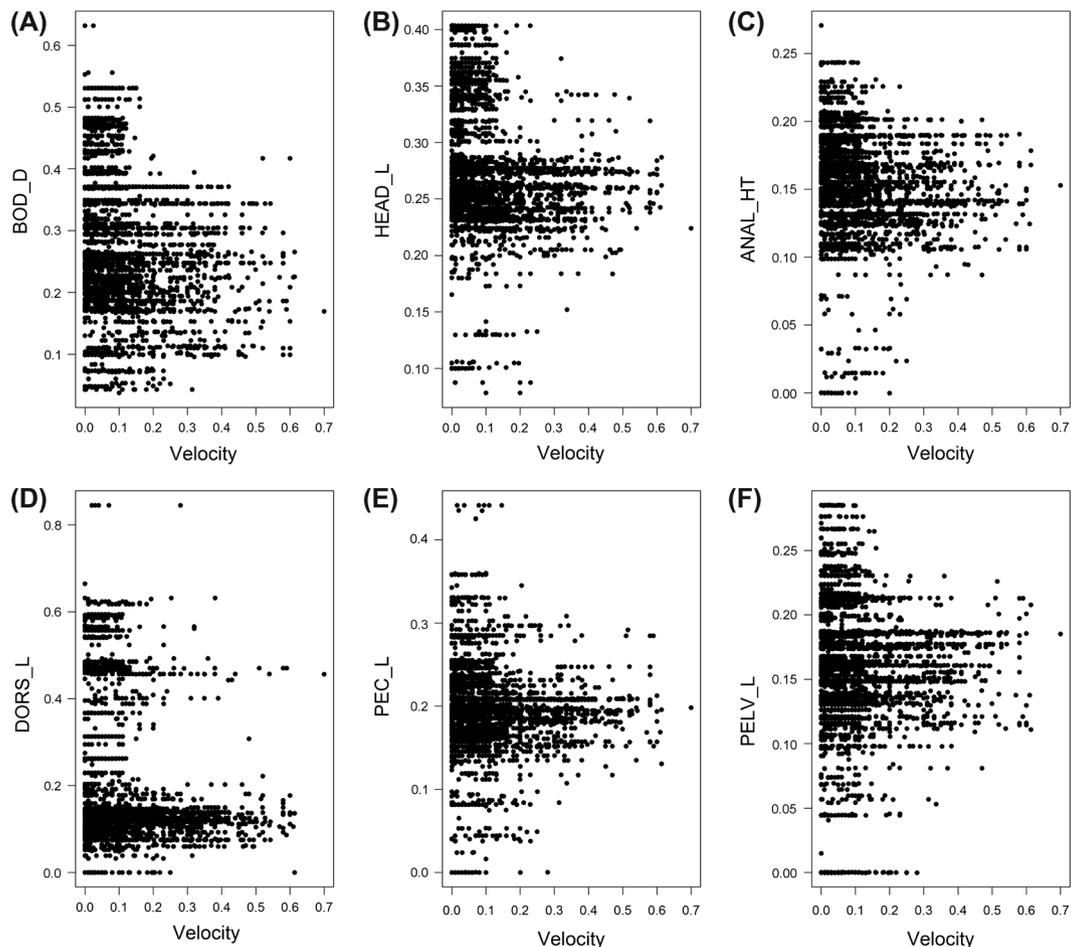


Figure 2. Graphs showing mean trait values for each fish species plotted against water velocity for each specimen ( $\text{m s}^{-1}$ ): (A) body depth, (B) head length, (C) anal fin height, (D) dorsal length, (E) pectoral fin length and (F) pelvic fin length.

support for the idea that habitat templates structure trait distributions within stream fish assemblages. Even though fourth-corner analysis only revealed one trait–environment relationship that was congruent across every region, trait–environment plots indicated the existence of congruent environmental filters across all zoogeographic regions for several traits (Fig. 4, Supplementary material Appendix 1 Fig. A3–A7). In some cases, a strong phylogenetic signal implied that historical contingency played a significant role in determining trait–environment relationships; thus, trait–environment relationships revealed evidence of both convergent evolution and phylogenetic niche conservatism.

### Within-region, trait–environment relationships

We first tested for trait–environment relationships within biogeographic regions, finding strong trait–environment relationships for each biogeographic region using RLQ and fourth corner methods. Local environmental conditions clearly played a role in shaping the traits of stream fish assemblages within each biogeographic region, which supports the habitat template theory (Southwood 1977, Townsend and

Hildrew 1994). This implies that neutral assembly, by itself, does not apply to these fish assemblages, even though dispersal and other processes may often have stochastic aspects (Hubbell 2001). Instead, the interaction between microhabitat variables and species traits were involved in structuring local assemblages. In general, structurally complex microhabitats had positive relationships with certain traits, whereby species with larger trait values tended to occupy structurally complex microhabitats. Deep-bodied fish, such as centrarchids and cichlids, tended to occupy structurally complex microhabitats, which likely caused the positive relationship between microhabitat complexity and several traits, such as body depth and dorsal fin length. A gibbon (deep-body) body and broad medial fins facilitate maneuverability in horizontal and vertical dimensions, which is advantageous near structurally complex microhabitats, such as submerged logs and sticks, aquatic vegetation or root tangles along stream banks (Keast and Webb 1966, Webb 1984, Helfman et al. 2009). Body depth, body width, head length and the length and height of medial fins (Fig. 2) generally had negative relationships with water velocity. A possible explanation for this negative relationship is discussed below.

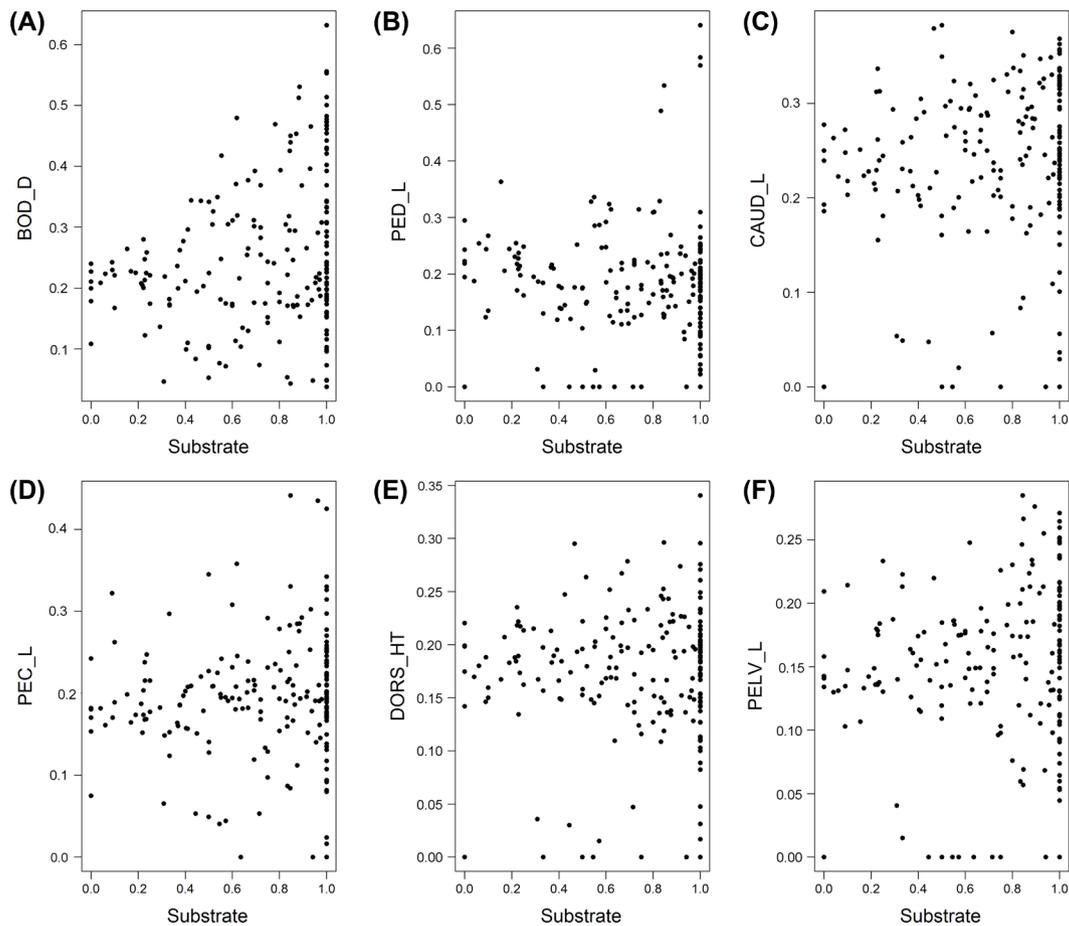


Figure 3. Graphs showing species mean trait values plotted against the proportion of individuals collected in structurally complex microhabitats weighted by species abundance on the x-axis (substrate complexity): (A) body depth, (B) caudal peduncle length, (C) caudal fin length, (D) pectoral fin length, (E) dorsal fin length and (F) pelvic fin length.

### Across-regions, trait–environment relationships

The second question addresses the consistency of trait–environment relationships across all zoogeographic regions. Using fourth corner analyses, only one of the documented trait–environment relationships was perfectly consistent across all regions. We expected more inter-regional consistency for relationships between individual traits and individual environment variables. For instance, a strong negative relationship should exist between body depth and water velocity due to the high energy costs associated with maintaining position in fast water given the hydraulic drag exerted on a large body surface area (Webb 1984, 1988). However, fourth-corner analysis did not reveal a significant body depth–flow relationship for Belize or Benin, and this may be due to low statistical power, owing to the relatively small numbers of species collected from streams in these regions. This result also could have been influenced by the small sample sizes for fishes collected from microhabitats with high water velocities in these regions, and the non-linear relationship observed between water velocity and many morphological traits (Fig. 2).

Some consistent trait–environment patterns were identified across all regions, suggesting similar filters acting on species traits (Fig. 2, 3, Supplementary material Appendix 1 Fig. A3–A7). For example, fishes with greater body depth were absent from habitats with high water velocities, and only fishes with slender or fusiform body shapes occupied these habitats (Fig. 2). A similar pattern has been described by other studies (Willis et al. 2005, Oliveira et al. 2010, Bower and Piller 2015). In small, low-gradient streams, many deep-bodied fishes are restricted from occupying areas with high water velocities due to hydraulic drag and the high energetic costs of maintaining position (Webb 1984, 1988). In microhabitats with low water velocities, this source of selection on body form is relaxed, and species with diverse body shapes often coexist. This pattern of reduced trait diversity in microhabitats with fast-flowing water was found in every region (Fig. 4, Supplementary material Appendix 1 Fig. A3–A7). This widespread pattern suggests that water velocity acts as a universal filter that selects for traits in the same manner across zoogeographic regions. Instream structures that enhance physical habitat complexity also seems to be

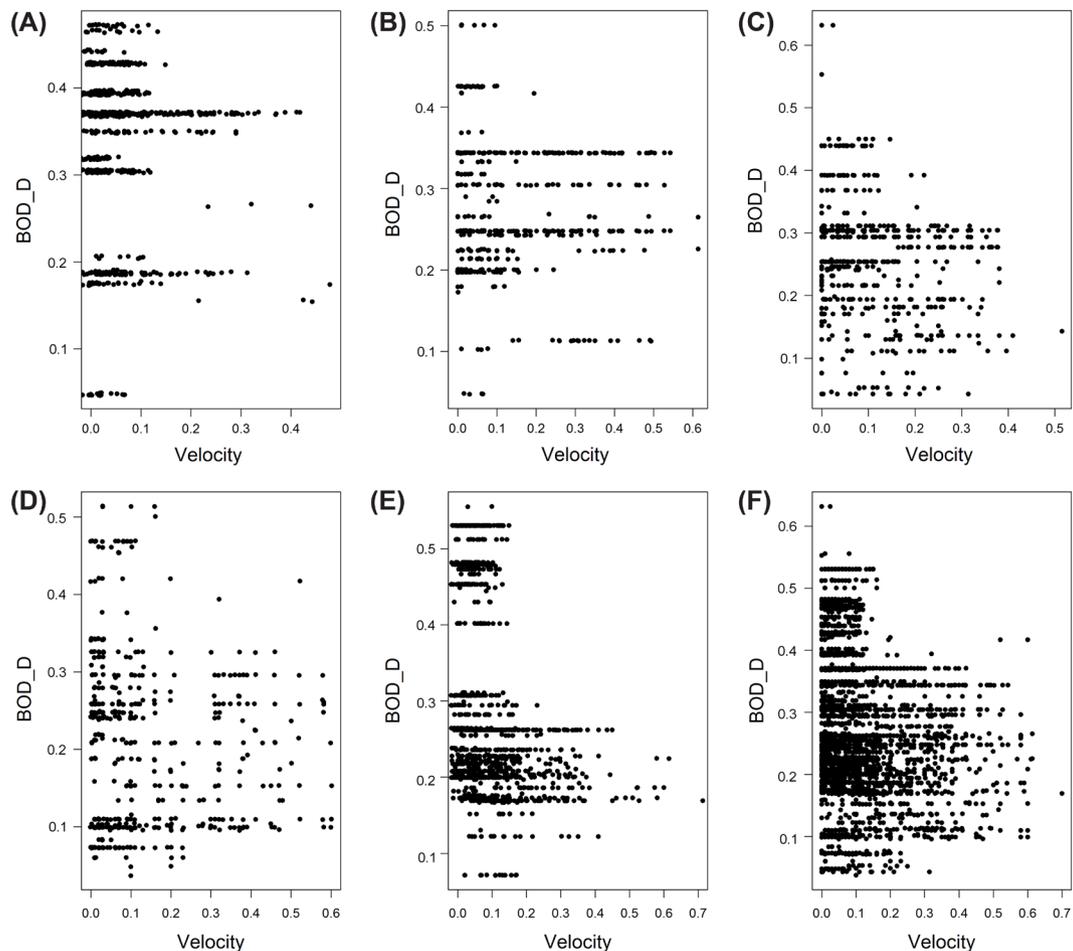


Figure 4. Scatter plots of mean body depth of each species against water velocity ( $\text{m s}^{-1}$ ) for all fish specimens and each region: (A) Belize, (B) Benin, (C) Brazil, (D) Cambodia, (E) USA and (F) Global dataset.

a universal environmental filter that structures fish assemblages. Trait diversity was greater for fishes inhabiting microhabitats with more structural complexity; body width, anal fin, caudal fin, dorsal fin and pectoral fin all had greater variation among fishes occupying structurally complex microhabitats. Structurally complex microhabitats may contain more abundant or higher quality food resources and also provide shelter for avoidance of predators and harsh environmental conditions, such as high water velocity (Kovalenko et al. 2012). Thus, the loss of instream structures, such as large woody debris, generally reduces fish functional diversity (Kovalenko et al. 2012, Mouillot et al. 2013, Emslie et al. 2014, Ceneviva-Bastos et al. 2017). Many human impacts tend to reduce the structural complexity of stream habitats, resulting in the decline or elimination of ecological specialists (Brejão et al. 2018) and lower functional trait diversity (Leitão et al. 2017). No apparent patterns were evident between traits and water depth, suggesting that depth was not a strong filter for traits examined in this study. However, it should be noted that streams selected for this study were small and wadeable, with limited variation in depth.

Several other factors could explain the relatively limited inter-regional similarity obtained for trait–environment relationships. First, the lack of congruent trait–environment relationships between regions suggests that idiosyncratic selective pressures may have prevented convergent trait–environment relationships. Although sites were selected to minimize environmental differences, the observed trait patterns may reflect unique environmental conditions in certain streams or regions. In that case, assemblage trait distributions might be influenced more strongly by unique environmental filters than by one or a few universal filters. A similar pattern was obtained from an inter-continental study of tropical anurans (Ernst et al. 2012). Regionally unique trait–environment relationships would imply that associations between traits and environmental features are either unpredictable or else influenced by other factors that vary in space and/or time. If this were the case, assemblage function structure could only be studied and predicted based on regional or perhaps even local species assemblages, and the link between traits and environmental variables should not be assumed to be universal. Another explanation for the lack of globally consistent

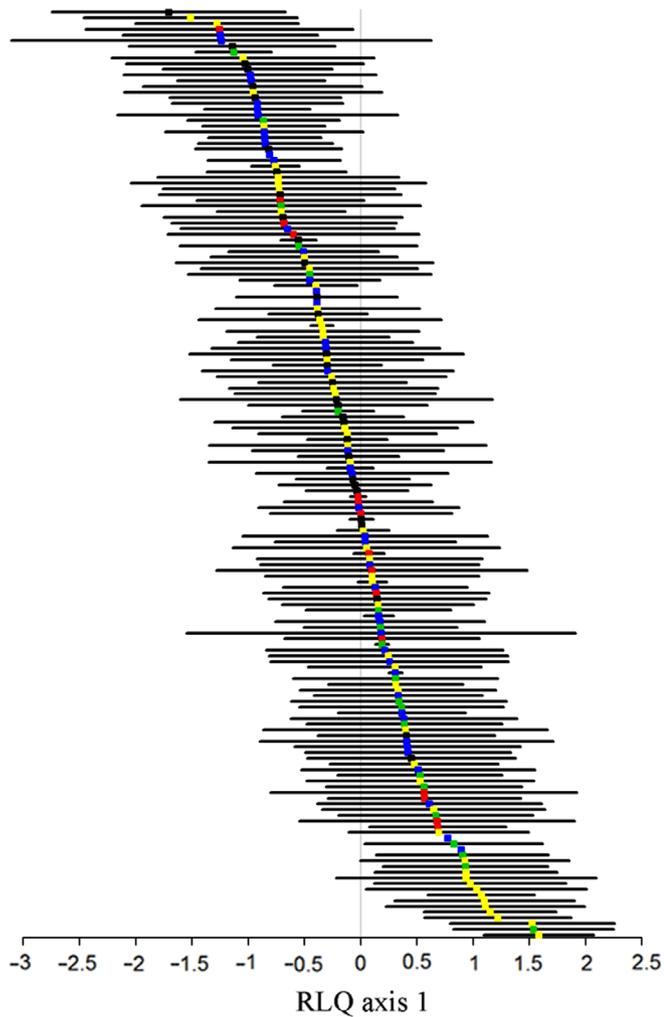


Figure 5. First axis of the global RLQ representing the habitat niche position (mean) and breadth (SD). Each bar represents one species, and color indicates country: Benin (red), Belize (green), Brazil (blue), Cambodia (black) and USA (yellow). Positive scores are associated with low water velocity microhabitats with root bank, plants and wood substrate and negative scores are associated with high flow microhabitats with sand or gravel substrates.

trait–environment relationships is the potential influence of species-specific behavior and ontogeny; for example, when an organism selects particular microhabitats during certain stages of its life cycle (Ross and Brenneman 2001, Bower and Piller 2015). Some fish, such as the North American minnow *Cyprinella lutrensis*, move into habitats with faster water velocities during breeding (Ross and Brenneman 2001). In some cases, human environmental impacts that degrade streams and natural assemblage structure might have reduced our ability to detect convergent trait–environment relationships. Some of our Cambodian streams were subject to fishing pressure and watershed impacts that probably affected certain fish stocks. Relatively large fishes known to inhabit small streams of the region (e.g. snakeheads (Channidae), catfishes (Clariidae), climbing perch (Anabantidae) and leaffish

(Pristolepidae)) were uncommon in our samples. Snakeheads and clariid catfishes have elongate bodies, climbing perch and leaffish have gibbose body shapes, and these species likely would have strengthened relationships had they been more prevalent in the dataset. Furthermore, our assemblage and habitat surveys represent snapshots in time. All stream surveys were conducted under base-flow conditions, and temporal variation in habitat conditions and local assemblage composition was not evaluated. Moreover, regional influences on assemblage structure were not accounted for in this study, and these can affect the manner in which local process influence assemblage patterns (Ricklefs 1987).

In our study, regional faunas were well separated phylogenetically, and only one congruent trait–environment relationship was detected by fourth-corner analysis. These results were not entirely unexpected. Studies comparing traits of regional assemblages with species from widely divergent lineages separated by deep evolutionary time often find a strong influence of phylogenetic constraint (Schoener 2009). The strong phylogenetic signals in niche positions and assemblage functional trait distributions in our study imply that phylogenetic constraints and niche conservatism could have influenced assemblage trait distributions (Wiens et al. 2010, Ernst et al. 2012), whereby closely related species have more similar traits and niches than distantly related taxa, and to a degree greater than expected by chance in the case of phylogenetic niche conservatism. Species functional traits and habitat niche positions were to some degree constrained by phylogeny. However, Peixoto et al. (2017) found that the degree of phylogenetic signal in bats varied depending on the extent of phylogenetic inclusiveness. In our study, strong phylogenetic signal in niche position and traits was detected when all species were included in the analysis, but tests for phylogenetic niche conservatism within a family or order may produce different results (Peixoto et al. 2017). Despite the strong influence of evolutionary history on assemblage trait patterns, a high proportion of fishes were found to be convergent with at least one other species (unpubl.). For example, *Melanocharacidium dispilomma* (order Characiformes) and *Etheostoma thalassinum* (Perciformes) had similar traits and niches. Other examples of species converging on similar traits and niches were *Laubuka caeruleostigmat* (Cypriniformes) and *Carnegiella strigata* (Characiformes) as well as *Umbra pygmaea* (Esociformes) and *Erythrinus erythrinus* (Characiformes). Clearly, both convergent evolution and phylogenetic constraint influenced assemblage trait distributions in our dataset.

### Moving forward

Our study focused on traits associated with fish locomotion and microhabitat use, but additional niche dimensions, such as diet, life history, defense and metabolism, would further our understanding of community assembly (Laughlin and Messier 2015, Winemiller et al. 2015). Presently, natural history data are lacking for many freshwater fishes, especially in the tropics and including some of the species included in this study.

Such data are essential, not only for achieving a more complete understanding of community ecology (Winemiller et al. 2015, Able 2016), but also for natural resource management (Able 2016). In addition, new methods to integrate phylogenetic influences on trait–environmental relationships are needed to improve inferences about niche convergence and phylogenetic niche conservatism. As new methods are combined with larger and more comprehensive data sets for traits and environmental variables, more phylogenetically inclusive and detailed analyses will elucidate mechanisms that create assemblage structure at various scales of time and space.

Our findings support the idea that environmental conditions of local habitats influence trait patterns of stream fish assemblages in a consistent manner worldwide. Water velocity and structural complexity appear to function as universal environmental filters that produce similar assemblage trait distributions in low-gradient streams across multiple zoogeographic regions. Our findings imply that trait–environmental relationships derive both from common ancestry (promoting the strength of within-region relationships, but inhibiting inter-regional congruence) as well as evolutionary convergence in response to a common set of environmental filters (promoting inter-regional congruence). If certain environmental filters can be shown to have consistent effects on local community assembly, this information would significantly enhance our ability to predict outcomes from human interventions, including environmental degradation and actions aimed at ecological restoration.

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Supplementary material (available online as Appendix ecog-04690 at <[www.ecography.org/appendix/ecog-04690](http://www.ecography.org/appendix/ecog-04690)>). Appendix 1.