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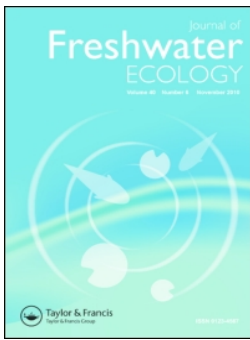
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Evidence of morphological and functional variation among Bluegill *Lepomis macrochirus* populations across Grand Lake St Mary's watershed area

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ABSTRACT

A myriad of factors have been shown to influence the morphology of freshwater fish. However, studies that parse out where variation is coming from (e.g. body size, sex, and habitat) as well as what potential these changes have to influence function (e.g. swimming performance) are understudied. Therefore, the objectives of this study were to describe morphological variation of Bluegill *Lepomis macrochirus* across the Grand Lake St Mary's watershed area (northwest OH, USA) and test for covariation of morphology with size, sex, and habitat as well as to assess swimming performance to discern whether any differences in habitat (and morphology) correspond with functional aspects related to critical swimming velocity. Geometric morphometric methods were used to assess shape variation among individuals and general linear models were used to test for covariation of morphology with size, sex, and habitat. Analyses indicated that body size was the strongest driver of morphological variation followed by sex, habitat, and interactions – indicating the presence of allometry, sexual dimorphism, and the potential for habitat induced plasticity. In general, more robust morphologies tended to correspond with larger individuals, males, and/or individuals from lentic habitats. Swimming performance trials supported functional differences as individuals from lotic habitats demonstrated significantly higher U_{crit} swimming performance values ($\sim+20\%$) than lentic individuals. Broader applications of these findings can link to evolutionary ecology, management, and conservation.

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Introduction

The relationship between organismal morphology and environment is a long standing topic of interest among ecologists and has been steadily gaining interest since the first ecomorphology studies began to relate form, function, and environment (Delacour & Mayr 1945; Verheyen 1953; Delacour 1959). Ecomorphology has helped facilitate understanding of what can potentially drive body shape and niche and has established templates for the study of morphological relationships with resource use, life history, and evolution. Specifically, the application of ecomorphology can provide valuable insight into ontogeny, phylogeny, and evolution. However, despite the obvious utility of ecomorphology and its growing literature base – there is still much room for improving our understanding of the various taxonomic groups and clades of North American fishes.

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Morphological variation is driven by the influence of evolutionary (Felsenstein 1976; Fransen 2011) and environmental factors (Green & Côté 2014; Santos & Araújo 2014) which contribute to both intraspecific and interspecific variation. Such selective pressures are not mutually exclusive, however, as both couple together in driving morphological variation on an individual and population level (Dhuyvetter et al. 2007). While the comparison of morphological characters and molecular markers provide valuable insights into evolutionary and environmental processes that contribute to morphological variation, the relative contributions made by either factor is understudied. Furthermore, while morphological variation is expected in freshwater fishes (Alexander et al. 2006), how much or what proportion of variation is attributable to physiological changes in body size or sex or environmental changes in habitat is largely unknown for many taxa.

The development of morphological features as a function of body size is a major source of shape variation within fish species. Across a given species complete ontogeny the morphological variation exhibited is often the greatest contributor to shape change (Wainwright 1996). Ontogenic variation in growth-morphology trajectories are shaped by selective pressures relating to a variety of functional factors, including diet, maturity, predation pressures, and habitat type (Hjelm et al. 2001; Neves et al. 2015) that coincide with change during a lifetime of development. Morphological changes during ontogeny strongly influence performance and can provide implications for success of particular size classes dependent on diet, sex, and/or habitat. The effects of available food resources, sexual maturity or reproductive life history, as well as habitat differences on morphological variation in growth trajectories coinciding with body size between fishes have been identified and tied back to increased advantages for some individuals (Wainwright 1996). For example, the complex set of interrelationships that accompany ontogenic changes has been well documented in populations of Eurasian Perch (*Perca fluviatilis*) collected from different habitats where ontogenic trends in morphology have been shown to differ between littoral and pelagic microhabitats in the same ecosystem (Svanbäck & Ecklöv 2002).

Sexual dimorphism between male and female individuals provides an additional source of morphological variation. Although not necessarily independent of ontogenic changes, as many species do not diverge in morphology until reaching sexual maturity (Jacquemin & Pyron 2013), sexual dimorphism is widespread among many organisms and suggests that variation between sexes is a result of adaptation (Shine 1989). Describing shape variation between male and female individuals can provide valuable information on the mechanisms present in reproductive processes. Although not universal across species, numerous species which demonstrate sexual dimorphism in morphology (as well as size) have been linked back to a balance between fecundity constraints and success (Svensson et al. 1986). To further complicate this interpretation, recent studies have identified variation in size, age, and morphology of those individuals that become sexually mature that can be contingent on levels of intraspecific competition (Ehlinger 1997). Although differences in morphology between sexes can be nuanced as a result of habitat, diet, competitive interactions, etc. at a very fundamental physiological level, these differences are typically the result of females carrying ovaries (typically a larger organ) filled with eggs (a larger gamete) compared with males carrying testes filled with sperm (Parenti & Grier 2004).

Morphological variation can also be induced by environmental factors. This can occur as a result of phenotypic plasticity, selection, or a combination (Hollander et al 2006). The ability of a single genotype to produce more than one phenotype as a result of environmental heterogeneity (at multiple scales) is a response that is well documented in freshwater fishes (Franssen 2011). However, it is important to note, that environmentally induced differences are indirect measures, rather than direct, where other factors associated with the change in habitat play a larger role. Similar to other factors that relate to morphological variation, environmentally induced shape divergence in fish has been associated with a variety of parameters, including risk of predation (Reznick 1982; Brönmark & Miner 1992; Godin & Briggs 1996), diet availability (Showalter et al. 2016), differences in flow regime (Hendry et al. 2006), and more. For example, studies on populations of Poeciliidae indicate

that those experiencing increased levels of predation tend to exhibit larger caudal and smaller head regions than those without high levels of predation risks (Langerhans & Makowicz 2009) and that this relationship can be sex specific (Hendry et al. 2006). Moreover, these variations in shape as a result of habitat have been linked to swimming performance, a major driver of escape behavior and dispersal ability in moving water, where as a result of developing a deeper body one could be expected to reduce swimming efficiency due to drag (Law and Blake 1996; Zimmerman 2007). Variation in flow regimes contribute to many habitat differences and have been shown to have a profound effect on morphological characters in freshwater fishes (Langerhans et al. 2003; Langerhans et al. 2007). Populations which inhabit regions with increased flow (lotic) tend to develop streamlined bodies which correspond with reduced drag and increased abilities to maintain steady swimming speeds in flow. By contrast, individuals from habitats with no flow (lentic) tend to develop deeper bodies which elevate its ability to traverse structurally complex habitats (Langerhans 2008; Santos & Araújo 2014). Water flow is thought to drive predictable morphological variation, but comparative evidences have shown that fish can develop different environmental responses based on energetics and cost reduction strategies leaving many unanswered questions regarding phenotypic variation across flow regimes (Pakkasmaa & Piironen 2000; Langerhans 2008).

Overall, evidence coupling together size, sex, and environment indicates that a suite of factors – albeit individually or interacting – can potentially drive morphological variation among populations (Hendry et al. 2006; Langerhans & Markowicz 2009). These variations in morphology are important to quantify as they can facilitate a greater understanding of ecology by providing clear functional links that can then be used to better understand a host of issues associated with niche, adaptation, persistence, performance, and more. Despite the breadth of the aforementioned literature and clear applications, morphological responses are not fully understood for all taxonomic groups and clades, where many species are understudied or could benefit from additional inquiries pulling from other populations, methodologies, or functional approaches. The study organism associated with this research, the Bluegill (*Lepomis macrochirus*), represents a case where a growing literature base already describes a variety of aspects of biology but could benefit from additional morphological study. Bluegill is endemic to freshwater ecosystems in North America and is considered abundant across a wide variety of habitats. From a management perspective, Bluegill is a highly sought after sportfish and are heavily stocked in many lakes, reservoirs, and ponds (Jackson 2002; Yokogawa 2013; Taguchi et al. 2014). From a biological perspective, past works have described their general niche (Spotte 2007), diet (Werner & Hall 1988; Masagounder et al. 2014), behavior (Werner & Hall 1977; Partridge et al. 2015), and aspects of morphology ranging from variation between sizes, sexes, particular habitats (micro and macro), reproductive state, and life histories, to the role that museum preservation may play on shape (Layzer and Clady 1987; Ehlinger 1990; Ehlinger 1991; Ehlinger et al. 1997; Yokogawa 2009; Colborne et al. 2011; Gaston et al. 2013; Yokogawa 2013; Gaston and Lauer 2015). However, despite this growing literature base there is a lack of watershed level information on the relative influence of body size, sex, and habitat (particularly with regard to morphological responses to flow) that controls for covariates, accounts for interactions, and uses laboratory testing to directly verify and test any resulting hypothesized links with any functional application to the observed morphological variation in Bluegill. Therefore, the primary objectives of this study were to (1) describe variation in Bluegill body morphology across the Grand Lake St Mary's (GLSM's) watershed area by testing for morphological covariation with body size, sex, and local habitat using novel morphometric methodologies as well as (2) to review and establish potential functional mechanisms related to morphological variation that could be tied to diet, fecundity, and/or swimming performance using a combination of literature reviews and laboratory testing. We predicted that morphological variation would be exhibited in populations across the watershed consistent with body size, sex, and habitat type as a result of selection for ontogeny, sexual dimorphism, and environmental plasticity.

Methods

Study area

The GLSM watershed covers about 239 km² in northwest Ohio and eventually empties into the Wabash River and ultimately contributes to the area of the Ohio River drainage basin (Figure 1). However, one important peculiarity to note is that as a result of anthropogenic influences on the basin during widespread shipping canal use in the mid-1800s, Grand Lake is also connected to the St Mary's River, a major tributary of the Maumee River and ultimately Lake Erie drainage basin (Figure 1). Past studies (Hoorman et al. 2008; Filbrun et al. 2013; Dumouchelle & Stelzer 2014) have documented highly variable water quality parameters within the GLSM watershed area consistent with seasonal variation in water temperature, dissolved oxygen, turbidity, nutrient loading (e.g. nitrates and phosphates), and specific conductance.

The aquatic habitat of the GLSM watershed area is composed mostly of low gradient streams of small to medium size with primarily sand and silt substrate resultant of glacial till depositions. These tributaries primarily drain in to the main body of water – GLSM, which is one of the largest man-made lakes in North America, covering about 54.4 km² in Mercer and Auglaize counties (Ohio, USA). Hand dug in 1837, GLSM functioned as a supply reservoir for the Miami and Erie Canal (Clark 1960) but currently serves as a public water supply for the city of Celina and recreational outlet (Davenport & Drake 2011). The GLSM terrestrial and riparian area is primarily composed of row crop and animal based agriculture (82%), residential (5%), forest (4%), and animal feeding operations (1%) (GLSM, 2005). Specific to biota, Ohio EPA fisheries surveys have identified

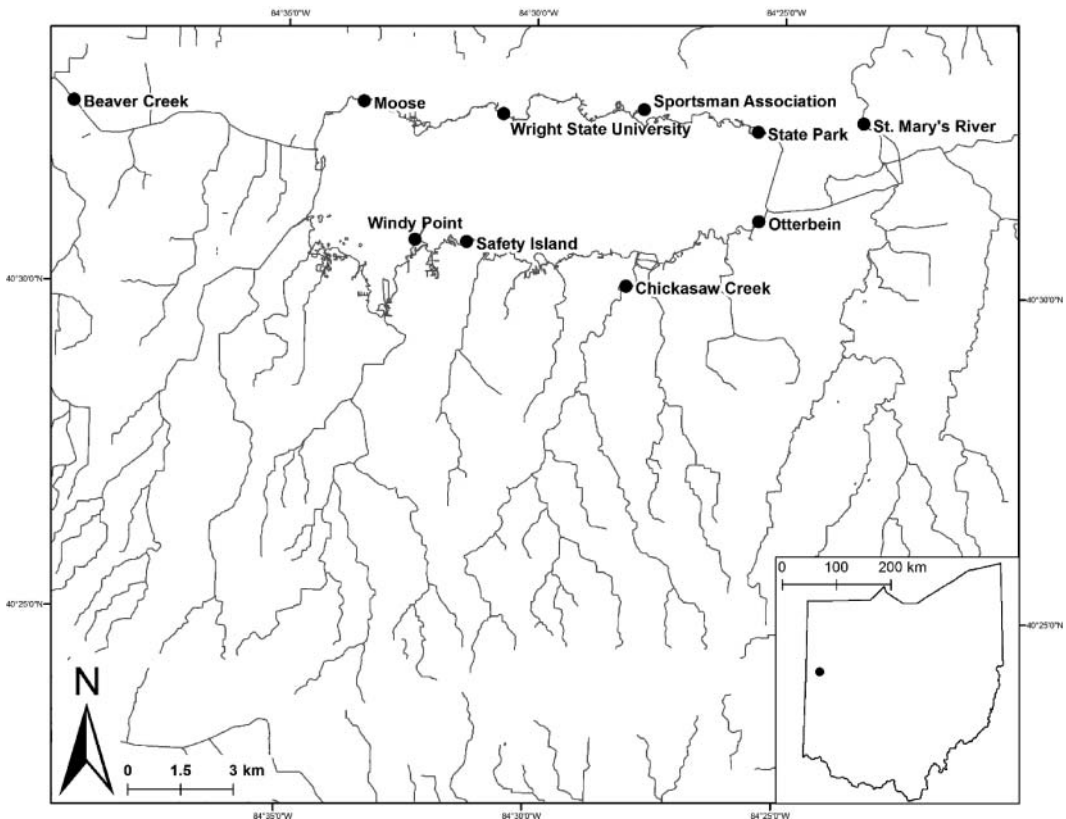


Figure 1. Collecting site locations within the GLSM's watershed.

27 fish species within the lake with as many as 49 species from connected inflow/outflow waterways outside the immediate basin as well as tributaries such as the St Mary's River, Chickasaw Creek, and Beaver Creek (Figure 1; Ohio Environmental Protection Agency sampling efforts 1995–2015). Due to the high levels of natural and anthropogenic connectivity, movement of fishes is theoretically possible between the reservoir, major tributaries, and outflow locations although this phenomenon has not been formally studied.

Habitat characterization and specimen collection

Sites in this study were chosen to represent both lentic and lotic habitats across the GLSM basin and connected waterways (St Mary's River) based on site accessibility and continuity with long term GLSM ecological research (Table 1; Figure 1). Additionally, the potential to show variation in water quality parameters was among the criteria considered in site selection. Each lentic site covered around 100m of shoreline while each lotic site covered approximately 10–15 times the wetted stream width. Habitat at each site was described using dissolved oxygen, pH, temperature, conductivity, average depth, substrate type, turbidity, as well as discharge and fetch length for lotic and lentic sites, respectively. *In situ* water quality parameters (dissolved oxygen, pH, temperature, and conductivity) were taken using a Hach Multimeter (HQ40D Multi) while turbidity measurements (NTU) were gathered using a Hach Turbidimeter (2100P). Average depth and substrate type was determined at each site using a standardized probing approach. A series of 25 probes equally spaced throughout each site were used in characterizing substrate type and depth. Measurements gathered from the longest uninterrupted stretch observed at each lentic site accounted for the maximum fetch length, which was taken from a southwestern direction to coincide with prevailing winds. Specific to lotic sites, discharge rates were gathered from the USGS National Water Information System. Chemical and physical water quality and habitat data taken at each site were described using basic general descriptive statistics.

Bluegill utilized in this study were collected from GLSM as well as tributary (Chickasaw Creek) and outflow locations (St Mary's River, Beaver Creek; Figure 1) during June of 2015 and 2016. Sampling efforts included a barge electrofishing and beach seines as part of an ongoing project monitoring the biological integrity of GLSM watershed. Electrofishing surveys were conducted using an ETS Electrofishing Systems unit operating under standard duty cycles, pulse frequencies, amperage, and voltage (250 v). Beach seines (5 mm mesh size; 15 × 2 m) were also pulled at each site a total of 5–10 times. Individuals at all stages of maturity were collected where all specimens were enumerated. Total length (to the nearest millimeter) and weight (to the nearest gram) was measured for each individual. Sex was determined through visual inspection of milt or eggs and verified using microscope dissection of gonads using a subset of specimens vouchered for archive in the WSU Biological Collection (Lake Campus, Celina, OH, USA). Individuals not vouchered were released following measurements and data collection. All collection efforts were consistent with Wright State University Animal Care and Use Committee Protocol (IRB – IACUC: AUP 1063) and Ohio Department of Natural Resources Collecting Guidelines.

Morphological data analysis

To characterize morphology of Bluegill, geometric morphometric methods were used. Geometric morphometrics is a contemporary approach to describing morphological variation using multivariate statistical analysis to evaluate the spatial arrangement of designated coordinates in reference to a common shape. Representing a series of manually set homologous landmarks, these coordinates provide effective visualizations of shape variation relative to all individuals (Klingenberg 2015). To describe shape configurations, each fish was first placed on a flat surface immediately upon collection and lateral images of the left hand side of each individual were captured using a digital camera (Nikon Coolpix 7620) adjacent to a reference scale. Images were digitized using the open-source



Table 1. Physiochemical parameters and habitat characterization for sites within the GLSM watershed. All sites were sampled between 1 June 2015 and 1 July 2015.

Site	Dissolved oxygen (mg/L)	pH	Temperature (°C)	Conductivity (µS/cm)	Turbidity (NTU)	Average depth (cm)	Discharge velocity (cm/s)	Prevailing (SW) fetch (km)	Sand (%)	Cobble (%)	Gravel (%)	Boulder (%)	Rip rap (%)	Muck (%)
Lentic ecosystems														
Moose Beach	12.6	8.3	25.6	395	67	50	–	3.8	70	0	10	0	20	0
WSU Beach	11.9	8.9	23.3	397	63	50	–	6.1	50	0	0	50	0	0
Windy Point Beach	11.1	8.9	24.6	385	91	50	–	1.9	50	25	0	25	0	0
State Park Beach	11.2	7.8	24.9	379	122	90	–	6.4	0	0	0	0	20	80
Otterbein Beach	13.4	8.5	24.4	394	84	50	–	0.1	50	0	0	50	0	0
Sportsman Association	7.3	7.9	23.6	412	37	70	–	0.1	0	25	25	0	0	50
Safety Island	12.4	8	23.8	382	84	50	–	0.1	90	0	10	0	0	0
Mean	11.41	8.33	24.31	392	78.3	59	–	2.64	44	7	6	18	6	19
Standard deviation	1.98	0.46	0.81	11.22	26.5	16	–	2.81	34	12	9	24	10	33
Lotic ecosystems														
St Mary's River	5.2	7.1	17.1	736	27	30	54	–	0	50	0	25	25	0
Chickasaw Creek	8.3	7.5	19.2	1090	11	40	4	–	33	33	33	0	0	0
Beaver Creek	11.5	7.1	20.9	762	27	30	43	–	0	50	0	0	40	10
Mean	8.33	7.23	19.07	862	21.67	33	19	–	11	44	11	8	22	3
Standard deviation	3.15	0.23	1.9	197	9.2	6	30	–	19	0.1	19	14	20	6

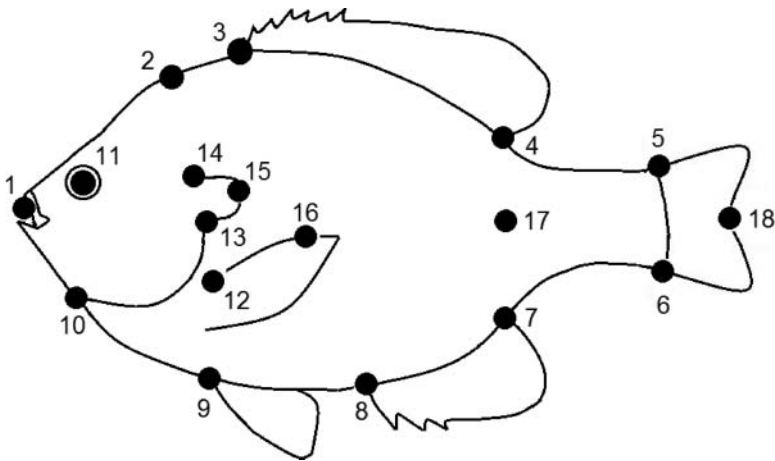


Figure 2. Location of 18 manually set landmarks used for morphological analysis of *Lepomis macrochirus*: 1 tip of snout; 2 nuchal hump (follows angle of operculum to body); 3 anterior insertion of dorsal fin; 4 posterior insertion of dorsal fin; 5 insertion of last dorsal ray on caudal fin; 6 insertion of last ventral ray of caudal fin; 7 posterior insertion of anal fin; 8 anterior insertion of anal fin; 9 insertion of pelvic fin; 10 gill juncture; 11 midpoint of eye; 12 insertion of pectoral fin; 13 ventral insertion of opercular flap; 14 dorsal insertion of opercular flap; and 15 posterior edge of opercular flap. Landmarks numbered 1, 16, 17, and 18 were used to digitally unbend each specimen and thereby not included in the analyses.

Thin-Plate Splines (TPS) software tpsDIG (ver. 2.19; Rohlf 2001), according to a series of 15 manually set landmarks (Figure 2). TPS software was also used to produce visualizations of shape and deformations relative to multiple individuals (Requieron et al. 2012; Mayer et al. 2014). Specimens were digitally unbent to account using a series of 4 landmarks placed at the tip of the snout and along the midline of each specimen (Rohlf 2004). Following digitization, geometric morphometric techniques were used to extract, visualize, and test for shape variation consistent with body size, sex, and habitat type.

Generalized Procrustes Analysis (least squares method) was used to align and transpose all individuals along a common scale (Cho et al. 2013). In doing so, comparisons in shape can be made between each specimen (Bartoli et al. 2013). Visualizations of shape variance were performed by TPS software by way of deformation grids produced by tpsRelw (Rohlf 2015) and tpsRegr (Rohlf 2016) software. Relative warp scores were used to summarize shape variation among individuals and were assigned to each specimen in relative warp analysis (RWA) as characterized by a single value which was then used to ordinate and assess differences in shape gradients among all individuals (Demayo et al. 2013). Extracted shape gradients accounting for at least 1% of variation were included in subsequent analyses.

Morphological differentiation as a function of habitat type, sex, and body size was tested for using a combination multivariate analysis of variance (MANOVA) and general linear modeling (GLM) techniques done in the R statistical environment (Shuker 2012). MANOVA using the Wilks' statistic was used to test for morphological differentiation as a function of body size (length to the nearest millimeter), sex (M vs F), and habitat type (i.e. lentic or lotic) within the entire GLSM watershed. Visualizations of these overall trends elicited by MANOVA were done using the tpsRegr software (Rohlf 2016). In this analysis, RWA scores were designated as dependent response variables and body size, sex, habitat type, and interactions between body size \times sex, body size \times flow, and body size \times sex \times flow were designated as independent predictor variables. General linear models (GLM) were then used to further explore the significant effects and slope relationship of body size, sex, and habitat type on each of the primary RWA axes from morphology data of specimens within the GLSM watershed. RWA axes were designated as independent response variables and body size, sex, habitat type and interactions between body size \times sex, body size \times flow, and body size \times sex \times flow

were designated as dependent variables. Reduced models were utilized where interaction terms or variables that were non-significant were excluded from subsequent model analysis (Ho et al. 2007). Coupling these individual models with the overall models facilitated conclusions regarding overall patterns, dominant morphological gradients, slope trajectories, and interaction points among grouping variables.

Functional mechanisms of morphology

To provide potential functional linkages between body shape and size, sex, and habitat, laboratory studies were undertaken to assess relationships between Bluegill body morphology and swimming performance. Since data in the literature is not available regarding swimming performance of Bluegill from different habitats, laboratory tests for swimming performance were conducted to fill in this gap. Swimming performance tests were undertaken using a Blazka style chamber (~100 liter tank with 12 × 30 cm working section) and measured with a U_{crit} protocol across a subset of similarly sized individuals comprising both male and female individuals from lentic (GLSM) and lotic sites (St Mary's River) within the watershed. Collected Bluegill were transported in aerated coolers back to 20 gallon lab aquaria at a maximum density of 4 fish per tank for approximately 7 days. During this time fish were exposed to 12:12 light cycles, climate controlled room temperatures, and fed a combination of brine shrimp and commercial flake meal twice daily. Physiochemical conditions were monitored every 3 days using standard Hach test kits to ensure appropriate water quality parameters were maintained. Individuals were deprived of food 12 hours before the start of testing so as to maintain a post-absorptive state. Prolonged swimming speed tests (critical swimming speed [$^{5/5}U_{crit}$]) were conducted following a 1 hour acclimatization time where flow remained at 0 cm/s for 30 min and was increased to 5 and 10 cm/s at the 45 and 60 min mark. Testing followed incremental increases in flow speeds of 5 cm/s at 5 min intervals. Swimming performance trials tested prolonged swimming speeds using the U_{crit} calculation (see below) for Bluegill, where V_p is the final flow velocity achieved by the specimen before fatigue. V_i corresponds to the velocity increase per time, while T_f and T_i are the final velocity increase and incremental time, respectively (Brett 1964; Wolter & Arlinghaus 2003):

$$U_{crit} = V_p + [V_i(T_f/T_i)]$$

For this experiment, fatigue was defined as a state in which the specimen becomes unable to maintain its position within the chamber followed by impingement with the downstream screen. Swimming performance data will be analyzed using general descriptive statistics. General linear models (GLM) were used to explain significant effects of size, sex, and site on U_{crit} swimming performance metrics for the subset of individuals tested from within the GLSM watershed. Specific U_{crit} values (cm/s) were designated as independent response variables and body size, sex, and site were designated as dependent variables. All tests for significance corresponded with an alpha level of 0.05.

Results

A total of 336 individuals were collected from GLSM, as well as several tributaries and outflow locations (St Marys River, Beaver Creek, and Chickasaw Creek). Individuals ranged from 23 to 207 mm in total length and included a total of 189 females and 146 males. RWA retained 12 axes which explained a total of 95% of the morphological variability, weighed most heavily along the first 3 which explained 65% of morphological variation of Bluegill in the GLSM watershed area.

MANOVA (η^2) of RWA scores revealed significant differences in shape attributed to length (mm TL), sex (male vs female), and habitat (lentic vs lotic). Significant interactions between length × sex and length × habitat were also present in the MANOVA (Table 2; Figure 3). Among all

Table 2. MANOVA results of relative warp analyses of all individuals collected from the GLSM watershed. Length is the primary predictor of morphology in Bluegill.

Variable	η^2	Test Statistic	F	df	p
Length	0.90	0.86	668.07	328	<0.01
Sex	0.25	0.16	20.76	328	<0.01
Flow	0.11	0.03	2.31	328	0.05
Length \times sex	0.20	0.11	13.16	328	<0.01
Length \times flow	0.09	0.04	4.74	328	<0.01

individuals sampled, length was the primary variable explaining morphological variation while sex and interactions between length \times sex and length \times flow were secondary (Table 2; Figure 3).

General linear mixed models (GLMM) were used to determine morphological variation and directionality attributed to length, sex, and habitat (i.e. flow) across the three primary axes (Table 3). In RWA1 (40%), gradients were consistent with shifts in body shape from more robust morphologies (i.e. pronounced nuchal humps and increased caudal-peduncle area; positive loading) to more streamlined counterparts (i.e. increased streamlining and decreased nuchal hump; negative loading; Figure 4). Among the variables tested in this model, length accounted for the largest amount of variation explained (Table 3 and Figure 5). More specifically, individuals developed a more robust morphology as body size increased. This relationship was also relevant relative to sex as an interaction between sex \times length was also recovered. Morphological variation extracted from RWA2 (17%) evaluated length, sex, flow and interactions between length \times flow using GLMM. Shifts from anterior (positive loading) to posterior (negative loading) placement and orientation of the operculum, pelvic, and pectoral fins were observed (Figure 4). Such shifts were consistent with increasing size as larger individuals exhibited a more posterior placed operculum relative to smaller individuals. Among the variables tested, the interaction between length and sex as well as length \times flow emerged as significant variables indicating differential responses between length and sex and habitat among individuals (Table 3 and Figures 5–9). Finally, shape variation retained in RWA3 (8%) evaluated length, sex, and flow (Table 3). Results therein indicated shifts in dorsal fin placement, body

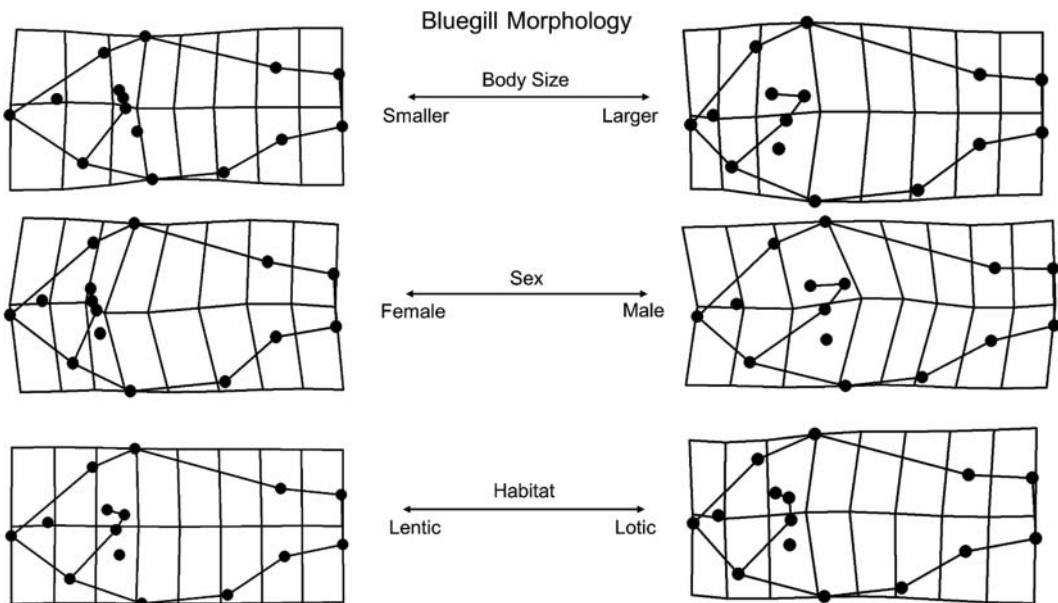


Figure 3. Overall visualizations for morphological variation in the GLSM watershed area. Note: Figures magnified 3x in tpsR software.

Table 3. GLM results of relative warp analyses of all individuals collected from the GLSM watershed.

Source	Estimate	Standard error	<i>T</i>	<i>p</i>
RWA1				
Length	0.00071	0.000022	32.32	<0.05
Sex	-0.0029	0.0041	-0.72	0.47
Flow	0.0013	0.0019	0.699	0.49
Length × flow	0.000072	0.000032	2.258	<0.05
RWA2				
Length	0.00014	0.000037	3.85	<0.05
Sex	0.014	0.0066	2.14	<0.05
Flow	0.021	0.000052	2.73	<0.05
Length × sex	-0.00026	0.000052	-5.10	<0.05
Length × flow	-0.0002	0.000077	-2.56	<0.05
RWA3				
Length	0.000014	0.000023	0.62	0.54
Sex	0.0025	0.0020	1.26	0.21
Flow	-0.0092	0.0058	-1.58	0.12
Length × flow	0.00016	0.000058	2.74	<0.05

Bluegill Morphology

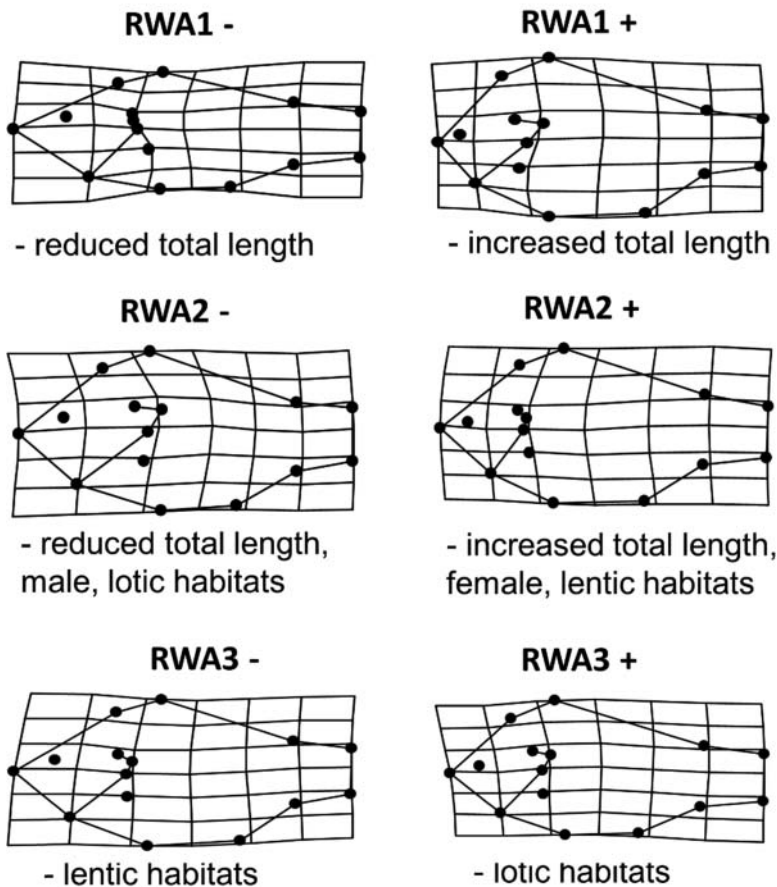


Figure 4. RWA for three primary axes which explained 65 percent of morphological variation in the GLSM watershed area, including significant relationships with parent terms as shown in general linear models.

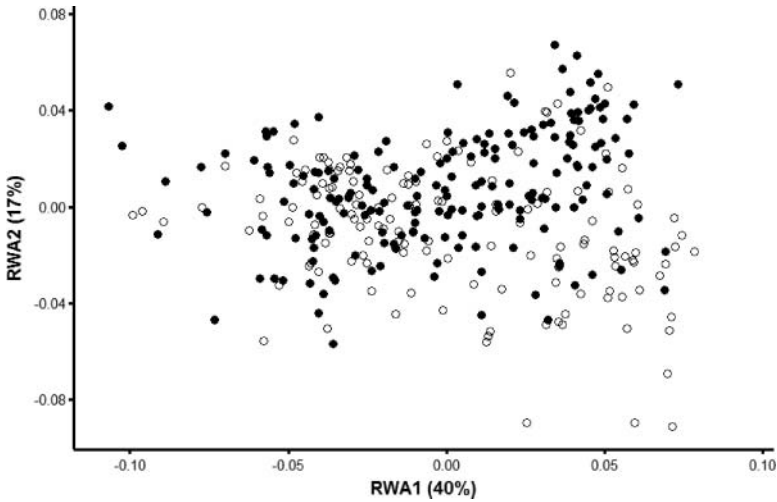


Figure 5. Scatterplot of RWA1 and RWA2 for both male and female individuals in the GLSM watershed. Closed circles indicate females and open circles indicate males.

orientation, and reduction nuchal regions. More specifically, shifts were observed from increased nuchal regions (positive loading) to a posteriorly placed dorsal fin and upturned body orientation (negative loading; [Figure 4](#)). Along this third primary axis, flow, particularly the interaction with length emerged as the most significant predictors in morphological variation in RWA3 ([Figure 9](#)).

To specifically quantify the potential effect of flow differences on swimming performance, a subset of 21 similarly sized individuals ranging from 95 to 164 cm total length were used to compare GLSM to St Marys River populations. One individual was found to be a non-performer (e.g. exhibited erratic or atypical behavior during swimming tests) and was excluded from subsequent analyses. GLMM were used to determine swimming performance variation attributed to size, sex, and habitat (GLSM; St Mary’s River). Not surprisingly, given the specific size class used, fish size was found to be insignificant ($p < 0.05$). In addition to size, sex was also found to be an insignificant predictor of swimming performance ($p < 0.05$). However, habitat was found to be an overall indicator of

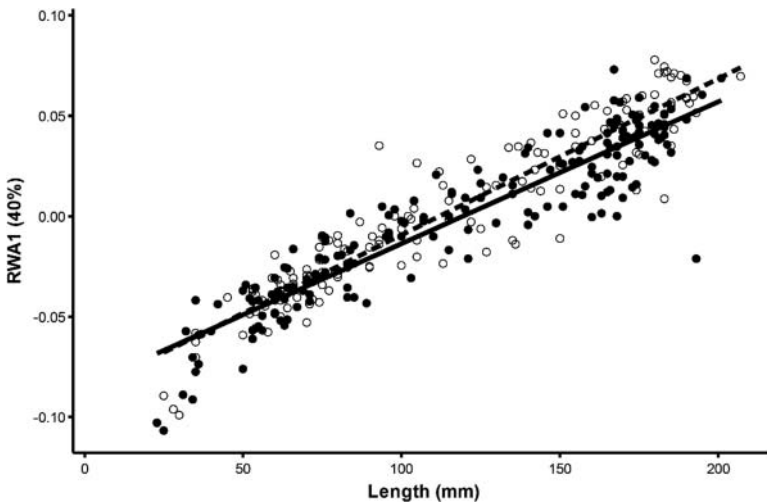


Figure 6. Scatterplot of RWA 1 and total length with regressions for Bluegill. Closed circles and solid regression line indicate females and open circles and dashed regression line indicate males.

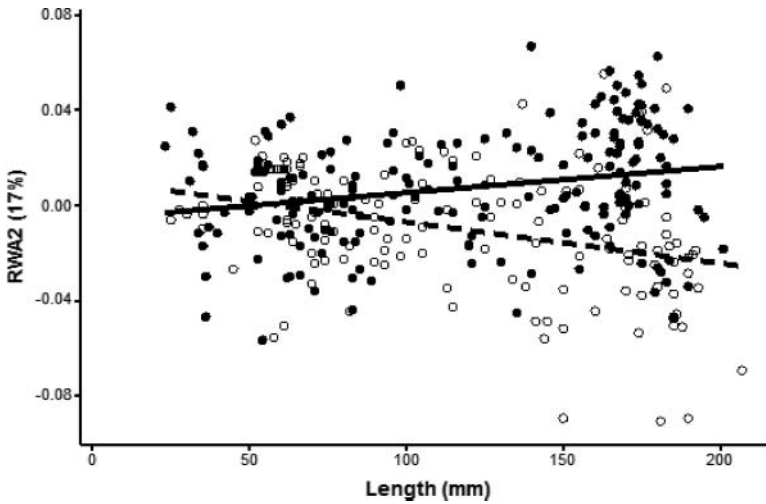


Figure 7. Scatterplot of RWA2 and total length (mm) with regressions for Bluegill individuals. Closed circles and solid regression line indicate females and open circles and dashed regression line indicate males.

swimming performance (Est = 5.9, SE = 2.4, $t = 2.4$, $p = 0.03$), where individuals from lotic sites (mean U_{crit} 51.5 cm/s^{-1} ; SD U_{crit} 9.7 cm/s^{-1}) swam approximately 20% faster and exhibited less variation relative to those from the lentic site (mean U_{crit} 39.6 cm/s^{-1} ; SD U_{crit} 11.9 cm/s^{-1}).

Discussion

This study provides strong support for morphological covariation with body size, sex, and habitat within a local area and contributes to existing ecomorphological research by using field collections to highlight these key corollaries and laboratory methods to identify functional effects and suggest mechanisms that may drive these patterns. By exploring the interactions between morphology,

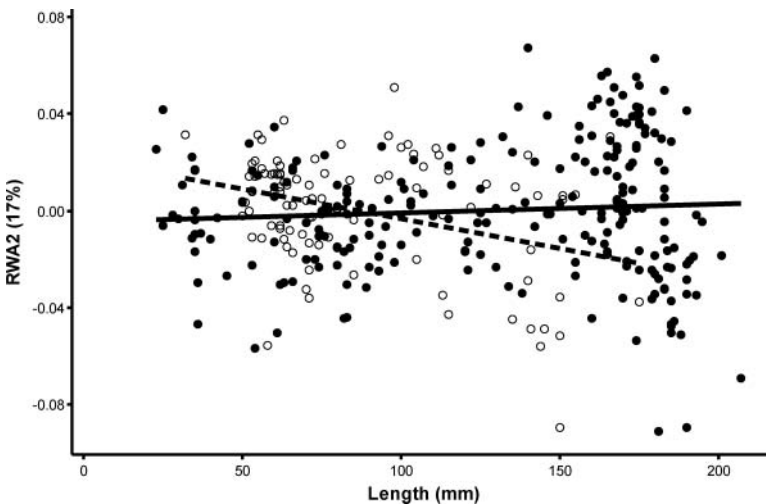


Figure 8. Scatterplot of RWA2 and total length (mm) with regressions for Bluegill individuals. Closed circles and solid regression line indicate individuals from lentic habitats and open circles and dashed regression line indicates individuals from lotic habitats.

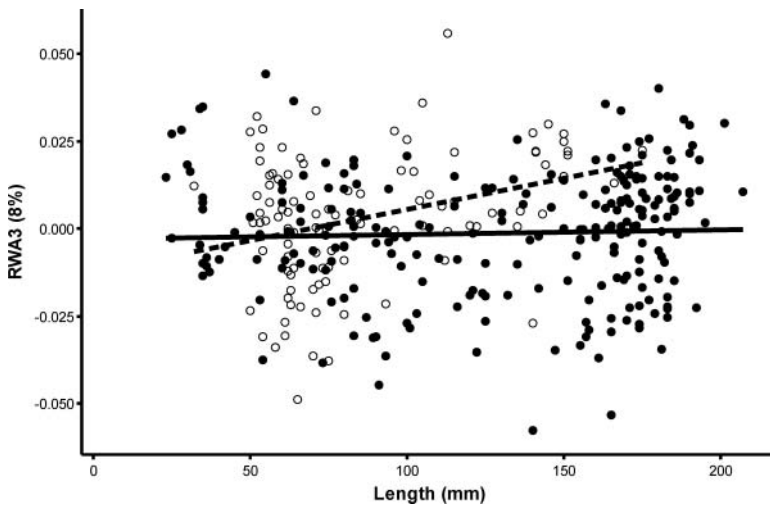


Figure 9. Scatterplot of RWA3 and total length (mm) with regressions for Bluegill individuals. Closed circles and solid regression line indicate individuals from lentic habitats and open circles and dashed regression line indicates individuals from lotic habitats.

physiological, and environmental gradients, biologists can better predict mechanisms that influence both the morphology of organisms and their functional role in the ecosystem (Norton et al. 1995; Langerhans & Reznick 2010). Here, evidence is provided that body size is the primary contributing factor to morphological variation in Bluegill while sex and habitat are secondary. These results suggest that a combination of factors ranging from ontogeny, sex, and environment may drive variation in shape, likely as a result of selection for differences in diet, reproductive role, and flow regime of habitat.

Allometric growth is expected to covary with ontogenetic changes as few fish actually exhibit isometric patterns (Wainwright & Richard 1995). This is largely a reflection of resource use and physiological changes that coincide with life history (Wainwright 1996; Svanbäck & Ecklöv 2002). The analysis of body shape supports previous findings suggesting that Bluegill growth is not isometric and thus certain morphological characters do not grow proportionally to the rest of the body. Similar to other studies that have observed morphological allometry in Bluegill (Ehlinger 1991; Yokogawa 2013) individuals in this study were found to have developed a more robust morphology (i.e. pronounced nuchal hump, stouter/increased caudal peduncle area) coinciding with increasing size (i.e. age). Interestingly, several studies have identified key dietary shifts (zooplankton to benthic invertebrates) in Bluegill linked to trade-offs in prey selectivity between individuals of different age classes that occur at some point between 25 mm (Showalter et al. 2016) and 50 mm (Werner & Hall 1988). Other members of the genus *Lepomis* have also been shown to exhibit variation in body shape, including gill rakers and pharyngeal jaw morphology, in response to dietary variation (Hegrenes 2001). Linking the ontogenetic variation in shape described in this study with past studies delving into dietary and morphological variation provides a unique explanation for mechanisms that can potentially explain shape variability among size classes. These functional variations likely extend to variation between male and female individuals or individuals from different microhabitats (Layzer & Clady 1987; Ehlinger 1990) as well. Building on this, one can also infer a degree of plasticity that may be present as Bluegill could potentially adjust or be selected for various morphologies. Thus, these ontogenetic trends may be more broadly linked with aspects of optimal foraging theory, sexual selection, or phenotypic plasticity.

In addition to ontogenetic variation, a high degree of sexual dimorphism was also detected among individuals. Variation in sexually dimorphic traits have been well documented in teleosts and have been related to feeding mechanism, color, mating system, behavior, and size traits (Newman 1907;

Kodric-Brown 1990; Parker 1992; Kazancioglu et al. 2009; Mcgee & Wainwright 2013). In this species, male bluegill exhibit increased growth rates and larger maximum size compared to females (Ehlinger 1997; Yokogawa 2013) which when coupled with ontogenic morphological variation, results in variation at particular age classes. Similar to other studies that have observed sexual variation in Bluegill morphology (Ehlinger 1991; Colborne et al. 2011; Yokogawa 2013), male individuals in this study (while controlling for body size) were found to have developed increased dorsal height and overall streamlining relative to the more ventrally deeper bodied females. This difference was found to be highly size dependent and divergence between male and female body shapes did not occur until approximately 100 mm in length. It seems probable that this major difference is a result of female biased sexual sized dimorphism where selection has targeted phenotypic traits that contribute to female body size and by relation correlate to increased fecundity. These trends may also suggest that alternative reproductive strategies could be functionally supported in Bluegill (Gross & Charnov 1980; Gross 1991; Ehlinger et al. 1997)). Alternative reproductive strategies (e.g. sneaker males) have likely evolved in competitive systems and as a result, given the smaller sizes and shape relationship present, a complete generalization of male and female Bluegill shape may be problematic (Colborne et al. 2011). Future work in this area should focus on the identification and inclusion of individuals exhibiting alternative life history strategies into morphological analyses at this scale.

Finally, this study indicated significant habitat-specific variations in Bluegill morphology where lotic ecosystems yielded shallow bodied individuals and lentic ecosystems yielded deeper bodied individuals. Past research in Bluegill has indicated microhabitat level variation that has been tied primarily to prey selection, predation avoidance, and/or competition (Werner & Hall 1977; Layzer & Clady 1987; Ehlinger 1990; Wilson et al. 1996). Beyond Bluegill, morphological variation attributed to habitat type often corresponds to resource use and has been well documented in freshwater fishes (Svanbäck & Ecklöv 2002; Langerhans et al. 2003; Franssen 2011; Santos & Araújo 2014). In this study, fish collected from lotic ecosystems experienced greater flow velocities and variation in both physical and chemical habitat parameters relative to the deeper bodied fish from lentic ecosystems. To further investigate potential mechanisms which could explain this pattern and given the paucity of swimming performance studies on *Lepomis*, laboratory swimming performance tests were conducted on individuals from lotic and lentic sites. Past works have investigated factors influencing swimming performance in other taxa and have centered on both fish morphology as well as aspects of locomotor ability (Videler 1993; Blake 2004). Both of which are intimately linked through a general morphology, swimming performance, and fitness paradigm. As part of this system, the evolution of swimming performance is investigated at the lower level (i.e. fish morphology), while also considering the potential impact of an organisms shape or abilities on higher levels (i.e. fitness; Arnold 1983). Our analysis of swimming performance support prior findings in other taxonomic groups which suggest that individuals with streamlined morphologies display increased locomotor abilities favoring steady swimming in flow (Langerhans et al. 2003). Notwithstanding the role of other selective agents, the evolution of swimming performance does not occur independently of other adaptations and other factors influencing locomotor abilities – albeit evolutionary or ecological – may interact with one another influencing responses to selection (Langerhans & Reznick 2010). Similar analyses posit that such mechanisms may serve as a priori predictors of morphological variation and, by extension, swimming performance in fishes, a task which becomes particularly important in the face of widespread landscape alterations (Langerhans et al. 2007; La Croix et al. 2011).

The environmental and genetic variation that underlie morphological variation between organisms at the individual and population level have been fundamentally linked to trade-offs in swimming performance (Gerry et al. 2012), predator –prey dynamics (Brönmark & Miner 1992; Langerhans & Makowics 2009), and foraging efficiency (Svanbäck & Ecklöv 2002). Morphological variation observed in Bluegill within the GLSM watershed area can be attributed to a number of mechanisms. Here, it is shown that body size, and by relation ontogenetic growth, is a primary factor in driving morphological variation. Other mechanisms, namely genetic components, are known to

contribute to morphological variation at the population level (Lee et al. 2015). While there is no genetic evidence included in this study, it seems likely that this mechanism would be secondary to plasticity given the lack of physical barriers to gene flow present between sites.

Ecomorphological research posits that morphological patterns reflect differences in functional mechanisms where variation is detected in physiology, environment, or diet composition and covaries with body size (Norton et al. 1995; Hüseyin et al. 2012). Further work is necessary to investigate other mechanisms driving morphological variations described in the present study. While a growing base of ecomorphological literature provides evidence of other causal mechanisms, further work is needed to identify an underlying factor which accounts for between-habitat morphological variation. The current study as well as other studies moving to quantify such responses can prove useful to natural resource managers as well as conservation workers. More specifically, such experiments may serve as a priori predictors for morphological variation – a task which becomes particularly important in the face of widespread landscape alterations. Though landscape alterations, specifically those that alter flow regimes, are certain, modifications and restoration strategies which will better maintain the biological integrity should be implemented. Morphological analyses allow biologists to assess the implications associated with structures that impact our natural resources, and in doing so, provide a way to better predict ecological communities in the face of variation in habitat.

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Disclosure statement

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