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Conforming to the status flow: The influence of altered habitat on fish body-shape characteristics

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Abstract

1. Anthropogenic stream impoundments often result in altered flow regimes and reservoir formations. Functional fish characteristics may change in response to altered flow conditions, and understanding the underlying mechanisms at work (e.g. natural selection and phenotypic plasticity) is of great importance in both evolutionary and conservation contexts. *Cyprinella lutrensis* is a widely distributed minnow native to stream systems in Missouri, U.S.A. Following impoundment of the Osage River, *C. lutrensis* currently occurs in the resultant Truman Reservoir and surrounding tributaries. To examine the impacts of this habitat alteration on functional fish characteristics, we hypothesised that *C. lutrensis* populations in altered systems with no-flow would show an overall less streamlined body shape as compared to those in flowing systems, assuming body streamlining is beneficial for locomotion in flowing environments.
2. We conducted field and laboratory studies to test effects of flow and no-flow on *C. lutrensis* body shape. Geometric morphometric techniques were used to quantify body shape in field and laboratory individuals, and non-structural lipid extractions were conducted to assess whether fat storage contributes to body shape under flow and no-flow treatments.
3. Morphological comparisons between *C. lutrensis* from Truman Reservoir and surrounding streams yielded significant differences in body shape, with greater body streamlining in stream populations. A laboratory experiment using artificial stream units revealed similar significant differences in body streamlining between flow and no-flow treatments within a single generation. In addition, individuals under no-flow treatment had significantly higher levels of non-structural lipids compared to flow treatment individuals.
4. Our results indicate that changes in flow regime can alter the evolutionary trajectories (i.e. related to morphology) of fish populations. We suggest that body shape alterations are likely to occur within and across generations in a cumulative manner, therefore, it is plausible that both natural selection and plastic response mechanisms play roles in defining minnow body shape following flow alteration.

KEYWORDS

morphology, plasticity, red shiner, reservoir, selective pressures

1 | INTRODUCTION

Anthropogenic alterations of freshwater systems, such as stream impoundments, lead to habitat fragmentation and changes in hydrologic regimes (Nilsson, Reidy, Dynesius, & Revenga, 2005). Changes to aquatic environments due to impoundments and the consequential declines or extirpations of freshwater species have been widely documented (Perkin et al., 2015; Poff & Zimmerman, 2010; Taylor, Knouft, & Hiland, 2001; Vaughn & Taylor, 1999; Winston, Taylor, & Pigg, 1991). However, species capable of persisting following impoundment events may be influenced by novel environmental pressures within reservoirs and possibly undergo alterations in functional morphological traits (e.g. Franssen, 2011; Franssen, Stewart, & Schaefer, 2013), changes in life history strategies (e.g. Mims & Olden, 2013; Perkin & Gido, 2011), and/or changes in physiological factors (e.g. Flodmark et al., 2002). Therefore, examining changes related to species' functionality can lend insights into evolutionary trajectories influenced by anthropogenic disturbances in freshwater systems. To date, mechanisms responsible for changes in population-level functional traits in response to large-scale habitat alterations lack comprehensive understanding (Murchie et al., 2008).

When flowing waters are impounded, they undergo a rapid change from a lotic to a lentic environment. This process creates reservoir systems that are unique from stream or lake systems (Baxter, 1977) and produces novel biotic and abiotic factors, such as altered flow regimes and changed functional community compositions, that may modify selective pressures for aquatic inhabitants (Mims & Olden, 2013). Maintaining natural flow regimes is an important and often required environmental feature for reproduction and longevity of fish populations (Krabbenhoft, Platania, & Turner, 2014). In addition, flow features have been shown to influence the body shape morphology of fishes (e.g. Foster, Bower, & Piller, 2015; Haas, Heins, & Blum, 2015; Langerhans, 2008) and, furthermore, stream impoundment has been recognised as a potentially important evolutionary driver of morphological shifts within fish populations (Franssen, 2011; Franssen, Harris, Clark, Schaefer, & Stewart, 2013; Haas, Blum, & Heins, 2010). Specifically, phenotypic divergence of native fish populations may occur following habitat alteration (i.e. reservoir construction), resulting in deeper-bodied individuals in lentic environments in relation to more streamlined-bodied individuals in lotic environments (Franssen, 2011).

Morphological differences between lentic and lotic fish populations have often been attributed to genetic divergence guided by natural selection mechanisms (e.g. Berner, Adams, Grandchamp, & Hendry, 2008; Collin & Fumagalli, 2011; Langerhans, Layman, Langerhans, & Dewitt, 2003). This is a plausible mechanism when sufficient time (i.e. multiple generations) in a novel environment has passed, allowing for selection to take place. However, some work has suggested that populations may undergo rapid morphological change in novel environments. For example, Kern and Langerhans (2018) found a genetic basis for fish body shape differences between urban and rural stream systems and suggest that this has

resulted from a rapid evolutionary response to urban-related flow alterations. Furthermore, Cureton and Broughton (2014) found that rates of morphological change in small-bodied fish increased rapidly following reservoir construction before drastically decreasing after a decade.

In addition to genetic (selection driven) changes, phenotypic plasticity may also play a role in trait variation following an anthropogenic disturbance (Hendry, Farrugia, & Kinnison, 2008). For example, past studies have documented plastic fish body-shape responses in relation to flow alterations (e.g. Franssen, Stewart, et al., 2013; Istead, Yavno, & Fox, 2015). However, Cureton and Broughton (2014) dismissed the overall role of plasticity in Cyprinid body shape streamlining, as morphological changes in experimentally raised fishes accounted for only a small fraction of the change seen in natural populations. Although a link between fish phenotypic plasticity and flow regime is likely to exist, other environmental pressures including predation (Franssen, 2011) and diet (Andersson, Johansson, & Söderlund, 2005) may also be important morphological influencers. In addition, it is plausible that an adaptive plastic response could allow for canalisation of morphological traits (Debat & David, 2001; Ghalambor, McKay, Carroll, & Reznick, 2007), but this is mostly unexplored. Morphological changes may be affected by numerous environmental drivers and pressures, and examining the relative influences of these factors may provide a more complete understanding of plastic response.

Cyprinella lutrensis is historically a stream-dwelling minnow species (Family: Cyprinidae) native to much of the central U.S.A., including the state of Missouri (Matthews, 1987). The construction of Truman Dam (completed in 1979) in the Osage River drainage within the Missouri River basin has resulted in relatively high abundances of *C. lutrensis* within the reservoir and its surrounding tributaries. This system provides a natural testing ground for examining the influences of an altered flow regime on *C. lutrensis* functional body shape. Previous studies have used similar focal systems to examine fish morphology in relation to stream impoundments (e.g. Franssen, 2011; Haas et al., 2010).

Our goal was to understand the effects of flow alteration on *C. lutrensis* body shape and quantify the role that flow-induced phenotypic plasticity plays in any resultant morphological changes. Generally, we hypothesised that populations in altered systems with no-flow would show an overall less streamlined body shape as compared to those in flowing systems (i.e. in the field and the laboratory). This pattern has been documented in field studies by Haas et al., (2010) as well as Franssen (2011), and is based on the assumption that body streamlining is beneficial for swimming in flowing environments (i.e. reduces drag), but a deeper body shape is adaptive in non-flowing environments (i.e. better for burst swimming and/or manoeuvrability). We were also interested in the potential role that non-structural lipid storage plays in these hypothesised shape changes. Much of the predicted transition from a streamlined shape to a less streamlined shape is characterised by a ventral stretch, and therefore, it seemed plausible that some portion of shape change could be due to fat storage. We suggest that this ventral stretch

may be similar to what happens when an active person transitions to a more sedentary lifestyle resulting in increased lipid storage within and surrounding the ventral body cavity. In light of this idea, our intent was to investigate whether flow regime alterations are influential on fish non-structural lipid content, which may contribute to morphological change. The objectives of this study were: (1) to quantify differences in *C. lutrensis* body shape between reservoir and stream populations in the field; (2) to quantify plastic morphological responses in the laboratory using flow and no-flow experimental treatments; and (3) to measure and compare levels of non-structural lipids from individuals temporarily exposed to flow and no-flow conditions to understand impacts of flow regimes on body condition.

2 | METHODS

2.1 | Field collections

To quantify differences in body shape between reservoir and stream populations, *C. lutrensis* were collected from six sites in the Truman Reservoir and six sites in surrounding tributaries via 4.6 m (3 mm mesh) seine between 10 June 2017 and 1 September 2017 (Figure 1). Seining was conducted in wadable areas (≤ 1.5 m depth) at all stream and reservoir sites. Adult male *C. lutrensis* were chosen for analyses based on presence of breeding colours and/or breeding tubercles to account for possible influences of sexual dimorphism and immature individuals (SL range: stream 40–68.3 mm; reservoir 44.8–74.6 mm). Specimens were euthanised using MS-222 and then fixed in the field with 10% formalin before being transferred to 70% ethanol for long-term storage in the University of Central Missouri Research Collection (Warrensburg, Missouri). After transfer to ethanol for long-term storage, specimens were photographed for geometric morphometric analyses.

2.2 | Laboratory experiment

A between-groups laboratory experiment consisting of two treatments, flow and no-flow, was used to test for plasticity-driven morphological divergence related to flow regime and compare these findings to results from the field. Eight artificial stream units were set up in the aquatics laboratory at the University of Central Missouri, and each unit consisted of a rectangular (213 cm \times 56 cm \times 30 cm) 363-L fibreglass aquaculture tank (model FRT-25 Red Ewald, Inc.) equipped with a 15.25-cm standpipe on one end that emptied into a drop-tank. Water was recirculated into each unit from the drop-tank back to the opposite (i.e. upstream) end via a pump (Supreme Aqua Mag model 9.5, Danner Manufacturing Inc.) fitted to a ball valve and 1.9-cm diameter return hose. The return hose was fitted to a submerged T-shaped PVC flow bar spanning the width of the upstream end of the tank. The bottoms of the tanks were filled with c. 2.5-cm diameter Ozark gravel to simulate natural conditions in the region. Four Odyssey 122-cm t5 quad aquarium lights were supported 1.5 m above the tanks (one light per two tanks) and set to 12-hr light cycles to replicate summer breeding conditions. One 189 L aquarium heater (Penn-Plax CH10200, Penn-Plax, Inc.) was placed in each tank to regulate water temperature at 23°C. This temperature was determined based on general water temperature measures from Missouri waters during previous field collections. In September of 2017, adult *C. lutrensis* (both male and female) were collected via 4.6 m (3-mm mesh) seine from Post Oak Creek—a tributary to the Blackwater River, a neighbouring catchment to the Osage system, laying approximately 50 km north of our field study collection sites—just north of Warrensburg, Missouri and transported via aerated coolers to the UCM aquatics laboratory. This system is not impounded; therefore, the results of our laboratory experiment may be similar to the response of a population experiencing a novel impoundment. In total, 160 sub-adult and adult individuals

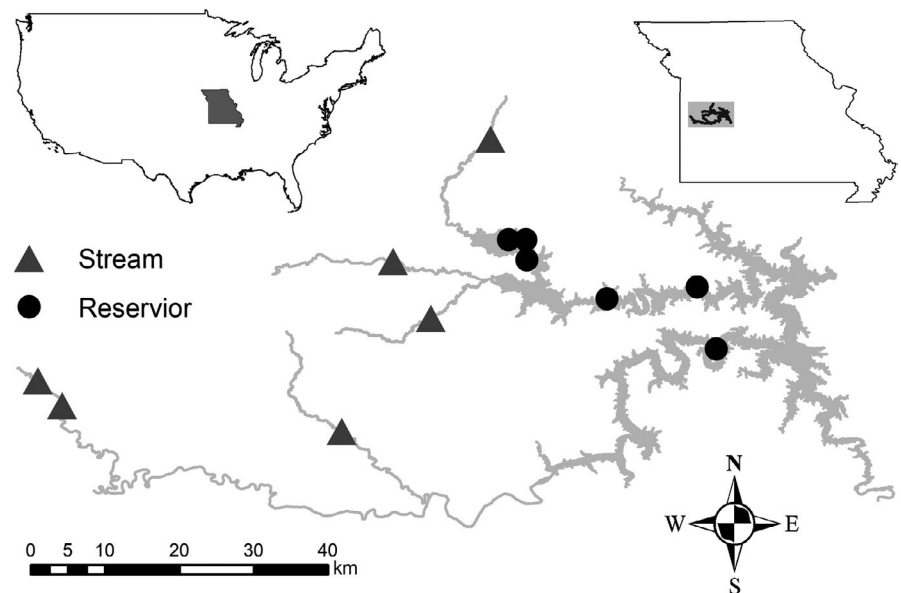


FIGURE 1 Map depicting the 12 sites of collection in the Osage River drainage

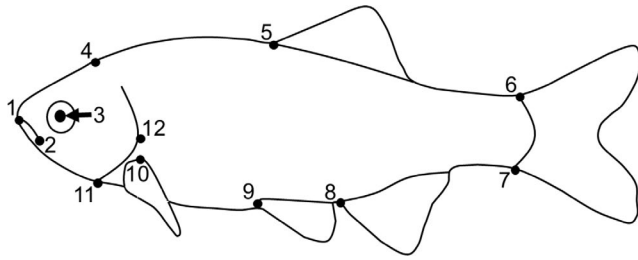


FIGURE 2 The 12 homologous landmarks examined in field and laboratory studies: the anterior tip of the mouth (1), the posterior gape of mouth (2), the center of the eye (3), the nape (4), the anterior insertion of the dorsal fin (5), the dorsal and ventral insertions of the caudal fin (6 and 7), the anterior insertion of the anal (8), pelvic (9), and pectoral fins (10), the anterior operculum insertion at the base of the isthmus (11), and the most posterior edge of the operculum (12)

(SL range post-experimentation: flow 37.1–58.8 mm; no-flow 40.4–61.9 mm) were randomly parsed out into eight artificial stream units (20 per unit), four of which were assigned to a flowing water treatment, and four were assigned to a non-flowing water treatment. Flow conditions were achieved by having three holes facing downstream (i.e. towards the stand pipe on the opposite end) on the PVC flow bar and running the pumps at full capacity. No-flow conditions were achieved by having 20 holes pointing up and down on the flow bar to disperse outflow while the pump ball valve was nearly closed off. This allowed for circulation without occurrence of measurable flow. To maintain equal dissolved oxygen levels between flow and no-flow treatments, each unit was aerated using an aquarium air pump recommended for 100–200-L tanks (Azoo Air Pump 5500, TaiKong Corp.). Tanks were provided with commercial pellet food (0.75 g/day) and all other conditions were held constant, with flow versus no-flow being the only variable altered for the two treatments. After 60 days, specimens were euthanised using MS-222 and fixed with 10% formalin. Specimens were then photographed for geometric morphometrics analyses using the following procedures.

2.3 | Geometric morphometric analyses

Specimens from both the field and laboratory studies were photographed using the following procedures; however, field and laboratory data were analysed separately. A Pentax K-S2 DSLR digital camera (Ricoh Co., Ltd.) was used to photograph the left side of all specimens. A scale bar was included in each photograph so size could be removed in later analyses of body shape. Using the software TPSDig version 2.30 (Rohlf 2017a), 12 homologous landmark points were digitised and assigned to each specimen (Figure 2). These points were identified a priori to represent a comprehensive coverage of lateral form, consisted of anatomical locations on the same plane that do not affect the location of other landmarks, and are easily replicable (Zelditch, Swiderski, Sheets, & Fink, 2004). All landmarks for all photographs were added by the same researcher

for consistency. Digitised images were then appended using TPSUtil version 1.74 (Rohlf 2017b) to create combined TPS data files for each of our two studies independently (field and laboratory studies).

To superimpose data onto a common plane, a Generalised Procrustes Alignment was used to rotate, translate, and scale TPS data to a best-fit model. This procedure was done using the *geomorph* package in R (Adams, Collyer, & Kaliontzopoulou, 2018; R Development Core Team, 2008). A principal component (PC) analysis was then used to simplify the 12 landmark variables into linearly uncorrelated variables that explain the greatest amount of variance among the data. Principal components that accounted for $\geq 5\%$ of the total variance were retained (Zelditch et al., 2004). For the field study, PCs were used as response variables in permutational multivariate analysis of variance (PERMANOVA) to test for significant differences in body shape between environments (stream versus reservoir). Prior to analysis field data were pooled by treatment (stream or reservoir) due to differing numbers of individuals collected from localities within each treatment. Additionally, pooling generated a more balanced model (i.e. stream $n = 61$; reservoir $n = 59$). The PERMANOVA was based on Euclidean distance and used 9,999 unrestricted permutations. For the laboratory experiment, PERMANOVA was used to test for significant differences between treatments (flow versus no-flow) with effects of replicate tanks nested within treatment. The analysis was based on Euclidean distances among individuals and used a reduced model with 9,999 permutations of residuals. All models were run using PERMANOVA + in Primer 7 (Anderson, Gorley, & Clarke, 2008).

2.4 | Lipid analyses

Analysis of body condition vis-à-vis lipid extractions was implemented to assess average body condition of a random subset of specimens from the laboratory experiment. This allowed us to test for non-structural lipid content as a quantifiable explanation for shape divergence. Five specimens from each tank ($n = 40$, flow = 20, no-flow = 20) were placed in a drying oven for 17 days at 42°C to obtain individual dry masses. Dried specimens were given six 24-hr baths in petroleum ether to dissolve and extract non-structural lipids and then placed in a drying oven again for 37 days at 42°C to evaporate residual moisture and ether before recording the new dry mass. The difference between the new mass and the old mass indicates the mass of the removed non-structural lipids. This methodology was derived from Kerr, Ankney, and Millar (1982) and Dobush, Ankney, and Krementz (1985), and days required to obtain stable dry masses were determined by previously conducted tests. Proportional difference (i.e. percentage change) of dry mass before and after petroleum ether baths was used to remove potential effects of size differences among individuals. Due to sample size constraints, data for each of the two treatments were pooled, and differences in mean lipid content between the two treatments were tested using a Welch's two-sample *t*-test.

3 | RESULTS

3.1 | Field study

Of all *C. lutrensis* individuals collected, 61 adult males were collected from six stream sites and 59 adult males were collected from six reservoir sites (Table 1). Following PC analysis, the first five PC scores were retained for use in the PERMANOVA, and they accounted for 76.33% of total body shape variation (PC1 = 36.02%, PC2 = 13.7%, PC3 = 11.52%, PC4 = 8.96%, and PC5 = 6.13% respectively). Difference in *C. lutrensis* body shape between the reservoir and stream sites was significant ($p < 0.001$; see Table 2). Principal component 1 mostly explained variation in dorso-ventral compression and PC2 explained an increased head size relative to the rest of the body (Figure 3). Both traits described were more exaggerated in the stream group when compared to the reservoir group and are characteristic of individuals being more streamlined (Langerhans, 2008).

3.2 | Laboratory experiments

All individuals that survived the full duration of the experiment (60 days) were included in body shape analyses with the exception of one outlier from the no-flow treatment (i.e. the individual had a PC2 score = 0.042, which was over three standard deviations greater than the mean; $n = 150$, flow = 76, no-flow = 74). The first five PC scores retained for the PERMANOVA accounted for a combined 76.59% of the total body shape variation (PC1 = 37.79%, PC2 = 16.05%, PC3 = 10.09%, PC4 = 7.08%, and PC5 = 5.60%, respectively). The PERMANOVA showed a significant difference in individual body shape between the flow and no-flow treatments ($p = 0.015$) and no significant influence of tank within each treatment ($p = 0.163$; see

Table 2). Principal component 1 displayed overlap between both treatments and described a large portion of body shape streamlining, but a clear divergence in mid-body depth between the flow and no-flow treatments is shown along PC2 (Figure 4a). Additionally, PC3 displayed a divergence in head size relative to the rest of the body (Figure 4b). The deeper bodies and smaller head-size-to-body-size ratios are both characteristics of less streamlined individuals (Langerhans, 2008) and were expressed by the no-flow treatment.

3.3 | Lipid analyses

Forty specimens were analysed for non-structural lipid content; however, one individual was identified a priori as being gravid and removed from the data set ($n = 19$ flow, $n = 20$ no-flow). The Welch's two-sample *t*-test yielded a significant difference in lipid proportion ($t = -2.1087$, $df = 36.74$, $p = 0.0419$) between individuals from the two treatments (Figure 5). Individuals from no-flow treatments showed greater difference between before and after lipid extraction dry masses as compared to the flow treatment individuals, and this was indicative of the significantly greater non-structural lipid content recovered in individuals of the no-flow treatment (Figure 5).

The general association between non-structural lipid content and PC2 scores for experimental individuals is presented as a visualisation of the lipid/body shape relationship (Figure 6). Although no significant correlation was found between lipid content and PC2, the two convex hulls that represent our categorical variables (flow and no-flow) provide a general visualisation of the *positive relationship* between body depth and lipid content (Figure 6).

4 | DISCUSSION

Overall, *C. lutrensis* in flowing environments were found to have more streamlined body shapes compared to individuals in non-flowing environments. This supports our assumption that this native stream species benefits from being less streamlined in lentic habitats. This general trend has been noted in other anthropogenically altered river systems (Franssen, 2011; Franssen, Harris, et al., 2013; Haas et al., 2010), but by incorporating both field and laboratory studies we were able to take one step further in understanding the rate and mechanisms through which body shape changes occur in response to anthropogenic habitat alteration. In general, our findings suggest that these observed morphological changes result from phenotypic plasticity; however, we acknowledge the possibility of additional, selection-based processes that may further influence fish body shape (e.g. Berner et al., 2008; Franssen, 2011).

We observed deeper bodies, shallower heads, and larger caudal peduncle areas in the reservoir population compared to stream populations. These traits are generally associated with acceleration (Webb, 1984) and are more typical of fishes in lentic environments (Langerhans, 2008). Shifts toward smaller head size (in proportion to the body) and a more anterior eye location, were also observed

TABLE 1 Collection site information and number of male *C. lutrensis* individuals in breeding form used for field study data collection

	Latitude (°N)	Longitude (°W)	No. individuals	Field No.
Reservoir	38.3075	-93.7370	15	ADG015
	38.2749	-93.5307	2	ADG016
	38.2000	-93.5078	9	ADG017
	38.2603	-93.6400	7	ADG024
	38.3318	-93.7380	21	ADG026
	38.3318	-93.7591	5	ADG027
Stream	38.1623	-94.3285	6	ADG020
	38.1321	-94.2989	36	ADG021
	38.1011	-93.9609	6	ADG022
	38.4540	-93.7811	2	ADG028
	38.3063	-93.8990	8	ADG029
	38.2372	-93.8531	3	ADG030

Field numbers correspond to collections housed at the University of Central Missouri.

	df	SS	MS	Pseudo-F	p (perm)	Unique permutations
Field study						
Environment	1	0.0046	0.0046	7.2568	0.0003*	9,947
Pooled	118	0.0747	0.0006			
Total	119	0.0792				
Laboratory experiment						
Treatment	1	0.0026	0.0026	3.3121	0.0147*	6,265
Tank (treatment)	6	0.0047	0.0008	1.3205	0.1625	9,909
Residual	142	0.0835	0.0006			
Total	149	0.0907				

Significant *p*-values are indicated by an asterisk.

TABLE 2 PERMANOVA results from the field study and laboratory experiment

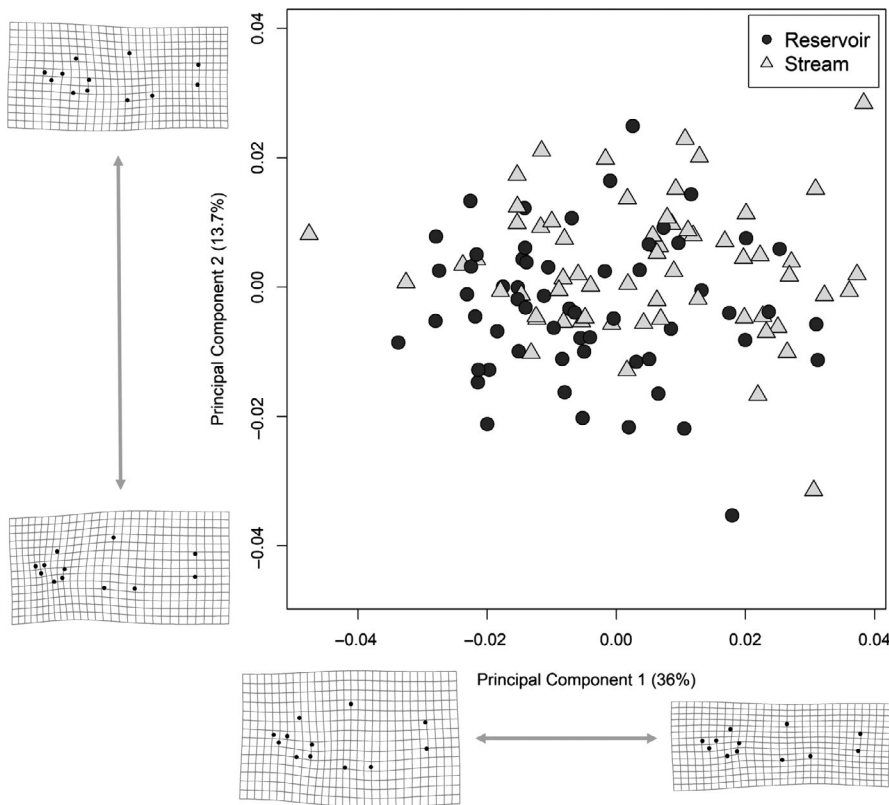


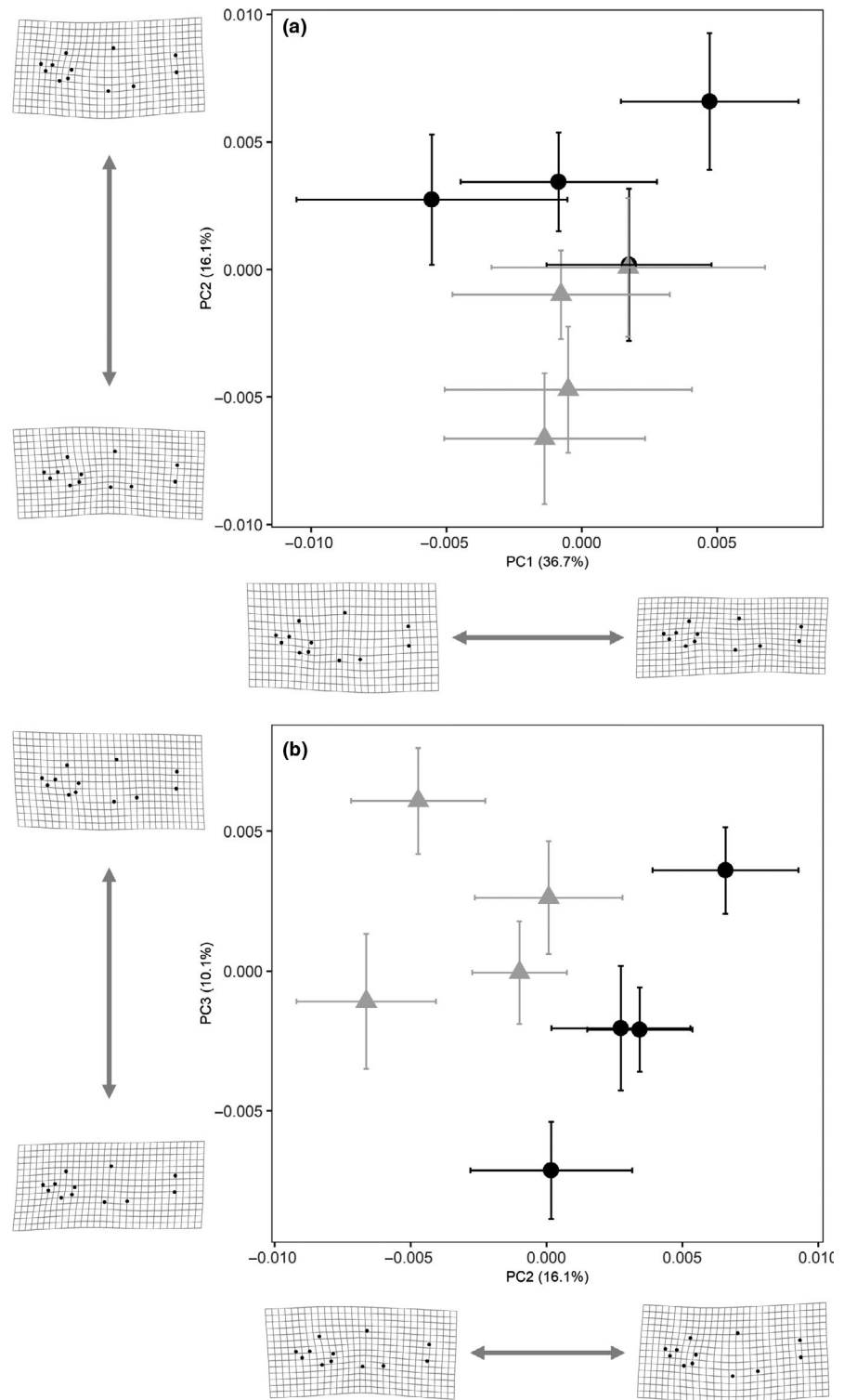
FIGURE 3 The body shape relationships between principal components 1 and 2 for individuals in stream versus reservoir sites. Warped grids depict the extreme body forms of each axis and are magnified 2× to emphasise shape differences

in the reservoir individuals (although these features were less pronounced than body-depth related variables). These traits may be linked to trophic position (Franssen, Harris, et al., 2013), and therefore variability in features of the head could be related to differences in predation pressures or food availability between stream and reservoir habitats.

The body depth differences quantified from populations in the field (which represented a snapshot in time) demonstrated significant change in body shape between stream and reservoir environments. However, it is difficult to accurately pinpoint the relative influences of mechanistic processes (e.g. natural selection and phenotypic response) that may have led to these body shape differences using these field data alone. Environmental selection

pressures that have acted across multiple generations probably explain some degree of difference in observed body shapes. Although, without direct knowledge of *C. lutrensis* body-shape trait heritability (e.g. high versus low heritability) in stream and reservoir populations, it is impossible to truly quantify the relative role that natural selection has played in the observed stream-reservoir differences. Cureton and Broughton (2014) found evidence of directional selection for deeper body shape in *Pimephales* species following the creation of the Lake Texoma reservoir (Red River at the Oklahoma-Texas border, U.S.A.), and this suggests that natural selective pressures may have contributed to our field study findings from the Osage River/Truman Reservoir system in a similar manner. However, experimentally testing *C. lutrensis*

FIGURE 4 The body shape relationships between principal component (PC)1 and PC2 for experimental individuals from the two treatments (a). Depicts the body shape relationships between PC2 and PC3 for experimental individuals from the two treatments (b). Each tank is represented by a single data point depicting mean body shape. Grey triangles represent flow tank centroids, black circles represent no-flow tank centroids, and standard error bars are depicted. Warped grids depict the extreme body forms of each axis and are magnified 2× to emphasise shape differences



phenotypic response in relation to altered flow regime allowed us to tease apart rates and processes of the plastic responses specific to our system, while removing any influences of heritability (i.e. natural selection).

In our experimental study, PC1 did not seem to describe variation specifically related to our two treatments. This was somewhat expected considering we did not limit sex of individuals in our experiment as we were able to do in the field study (i.e. it is nearly

impossible to sex individuals outside of breeding season). Therefore, we were not surprised to find a good portion of morphological variation not related to our flow and no-flow treatments directly. Experimental components that seemed to visually corroborate the significant body shape difference between treatments (i.e. based on PERMANOVA) included PC2 and PC3. Specifically, PC2 determined deeper-bodied individuals in the no-flow treatment and PC3 indicated that the no-flow treatment resulted in individuals with smaller

head sizes relative to body sizes. Morphologic plasticity in response to the alteration of flow regime is supported by these changes occurring within one cohort generation. These differences generally reflected the results of our field study; however, in our experiment, not all trait divergences observed in the field were replicated in the laboratory. Franssen, Stewart, et al. (2013) indicated that attributing all change in a reservoir to flow and predation pressures is an oversimplification of a much more complex system. Considering this, the lack of replicating a few specific trait disparities was generally

unsurprising. The field study, but not the laboratory study, depicts the addition of a deeper caudal peduncle in reservoir individuals. Caudal peduncle depth is generally attributed to predator avoidance due to a high aspect ratio allowing for quick bursts of speed (Webb, 1984) and is consistently seen in low flow environments (Langerhans, 2008). Additionally, our results from the field study indicated that reservoir individuals have more anteriorly located eyes. This change in eye position was not recovered in the laboratory experiment, but this could be due to eye location being indicative of trophic position (Franssen, Harris, et al., 2013).

Results from lipid analysis showed that *C. lutrensis* from the no-flow treatment were significantly fatter than individuals of the flow treatment. This was of great interest concerning the documented plastic response in body shape, as the most drastic difference between treatments was a ventral stretch in the mid-section of the body (see PC2 laboratory experiment), which favoured the no-flow treatment. Adipogenesis in teleost fishes can occur at the visceral organs (Salmerón, 2018), which leads us to believe that the increase of lipid content seen in our no-flow treatment may be contributing to the ventral stretch depicted by PC2 in our laboratory experiment. We explored the relationship between lipid content and body shape (i.e. PC2) of experimental individuals and found no statistically significant relationship. However, our lack of statistical support for this positive correlation may be partially due to the limited sample size included in the lipid analyses ($n = 39$). Due to the specimen preparation requirements used in the lipid extraction procedure, adding more individuals to our analysis was an impossibility (lipids cannot be extracted from individuals once preserved in ethanol). Furthermore, differences in age and sex may have bearing on plastic morphologic responses (e.g. Meuthen, Ferrari, Lane, & Chivers, 2019), and it is plausible that these factors may also influence lipid storage and content. Examining the influences of flow regime on fish body depth/lipid content responses with greater focus on individual age and sex is warranted in the future to more precisely explicate the relative role of

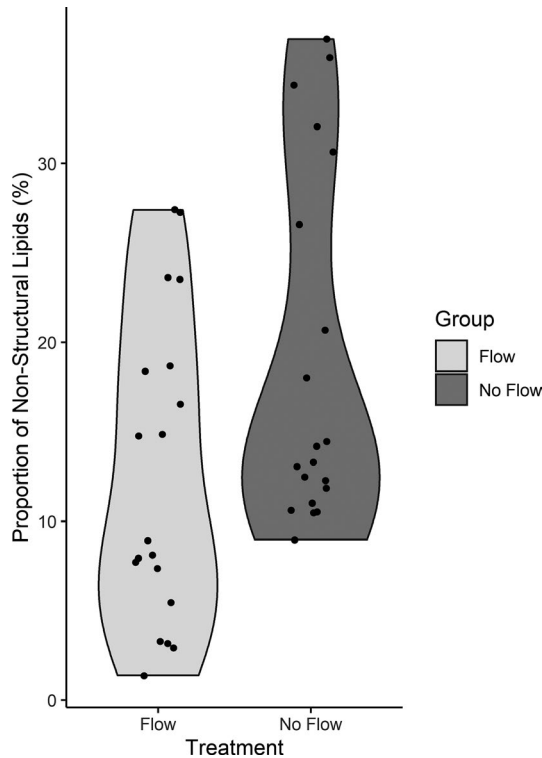


FIGURE 5 Violin plot comparing mass differences between individuals from flow and no-flow treatments. Proportion of non-structural lipids determined by percentage change

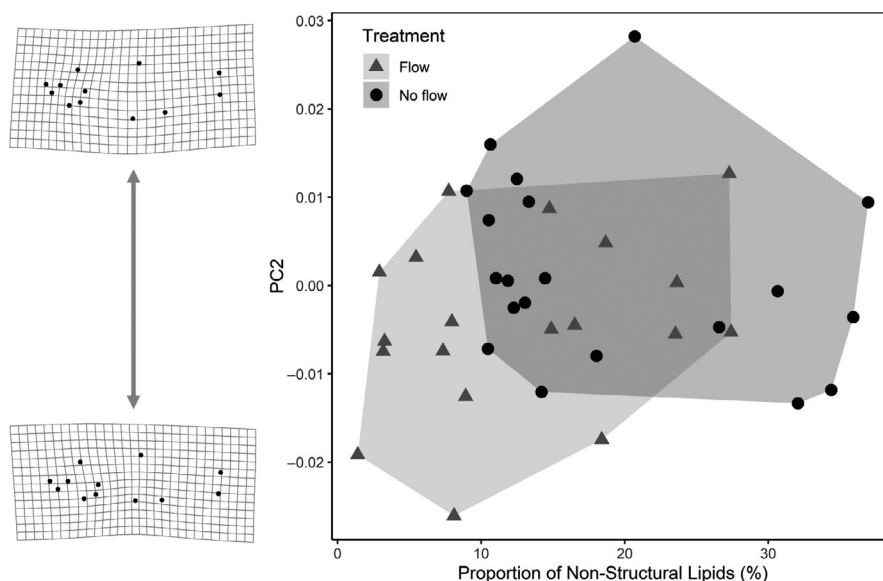


FIGURE 6 Scatterplot depicting the relationship between principal component (PC)2 scores (experimental study) and non-structural lipid content of individuals. Convex hulls are used to distinguish treatments

plasticity in impounded habitats. Additionally, it should be noted that directly comparing PC2 and non-structural lipid content may be an oversimplification of a potentially complex relationship due to the possibility that stored lipids account for other shape variation besides the variation explained by PC2 (e.g. lateral thickness when lipids are stored in subcutaneous tissue or muscle tissue). Despite this, the results from our experiment and lipid comparisons between treatments show that *C. lutrensis* introduced to a lentic system store greater amounts of non-structural lipids than *C. lutrensis* introduced to a lotic system. Pairing this finding with the positive trend between lipid content and experimental PC2, we suspect that lipid storage may in turn alter body shape morphology to some degree. This may be occurring through a physiological change, or perhaps there is no change and rapid storage of lipids—which may seem maladaptive in slower waters—is actually adaptive as it produces a beneficial body shape.

We would like to highlight two possible scenarios describing how increased lipid storage in stream fishes might lead to an adaptive body shape in altered flow environments. Both scenarios begin with generation zero, which represents a recently impounded stream fish population. In scenario one, which we dub the *treadmill effect*, the deep-bodied phenotype may simply be the result of increased lipid storage. Generation zero cultivates mass but has an adaptive body shape which allows this generation to be resistant to the impoundment event. Future generations follow suit with overall body shape resulting from flow alteration and lipid storage. If any future generation (e.g. whether it be generation 1, 13, or 55) were to experience an impoundment removal in which the original stream flow is restored, we would expect these fish to get back on the flow *treadmill*, lose their stored lipids, and return to generation zero's pre-impoundment body shape. However, in scenario two we acknowledge the possibility of canalisation, or cryptic genetic changes that may act on the robust shape of future generations (Debat & David, 2001). In this scenario, generation zero cultivates mass, which alters body shape, and then over several generations the robust structure of some individuals (i.e. skeletal and muscular) becomes deeper and excessive lipid storage becomes less necessary. These individuals are most adapted and drive selective processes influential on body depth of future generations. Specifically, selection for deeper underlying body structures across generations might end up replacing the role of short-term lipid storage. In scenario two if the impoundment is removed, we expect this structurally deep-bodied population to be maladapted to a lotic environment.

Although we acknowledge the potential influences of natural selection on body depth and the possible underlying processes of plastic response related to lipid storage, our finding that fish get fatter in environments of lower metabolic cost (no-flow) seems parsimonious. The complete understanding of population-level changes in form and function in resilient fishes adapting to life in reservoirs remains elusive due to the complexity and sheer number of variables that are altered during the construction of these systems. A potential starting point for rapid change could be storage of lipids immediately altering phenotype, which may then possibly lead to

directional selection and canalisation of genes (Debat & David, 2001; Ghalambor et al., 2007). We recorded divergence in *C. lutrensis* morphology after only 60 days of treatment, but it would be impossible to definitively say selective pressures on genotype are sure to follow (without inclusion of multiple generations under experimental treatment). However, our documented time period of morphological response could greatly increase understanding of rate of change facilitated by plasticity in relation to genetics. In stream systems where flow regime is altered and/or manipulated, understanding whether changes in functional morphological characters are facilitated slowly over time by selective pressures or rapidly through plastic responses may prove crucial for proper conservation practices. Rudolfsen, Watkinson, and Poesch (2017) determined that adaptive morphological features of Rocky Mountain sculpin were influenced by both flow regime and genetic isolation; however, under rapidly changing habitat conditions (e.g. stream flow alterations and/or seasonal flow regimes), understanding the rate of adaptive morphological response is likely to be paramount for the conservation of the species. Work documenting and assessing adaptive morphological responses in rapidly changing (or stochastic) aquatic environments is warranted and will provide baseline information used to inform future conservation efforts for small-bodied fishes.

We demonstrated that flow regime alone can have a major impact on contemporary and potentially evolutionary trajectories of stream fish functional morphology. Based on the integrative field and experimental approaches employed, we found deeper-bodied individuals in no-flow conditions (i.e. reservoir populations and artificial no-flow streams) and more streamlined individuals in flow conditions (i.e. stream populations and artificial flow streams). We suggest that morphological changes related to flow alterations result from both phenotypic plasticity as well as previously acknowledged selection-based processes. Moreover, the plastic response rate of fish body depth to flow alteration is rapid (≤ 60 days), and this response may begin through the accumulation of mass due to increased lipid storage in no-flow environments. Although flow regime can shape fish morphological divergence, it is facile to attribute all morphological change to flow regime alone. Future work examining the ecology and physiology of stream populations subjected to lentic and lotic environmental conditions will aid in further illumination of mechanisms influential on functional features of freshwater fishes.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

- Adams, D. C., Collyer, M. L., & Kaliontzopoulou, A. (2018). Geomorph: Software for geometric morphometric analyses. *R Package Version*, 3, 6. <https://cran.r-project.org/package=geomorph>.
- Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to software and statistical methods* (pp. 214). Plymouth: PRIMER-E.
- Andersson, J., Johansson, F., & Söderlund, T. (2005). Interactions between predator and diet-induced phenotypic changes in body shape of crucian carp. *Proceedings of the Royal Society B: Biological Sciences*, 273(1585), 431–437. <https://doi.org/10.1098/rspb.2005.3343>
- Baxter, R. M. (1977). Environmental effects of dams and impoundments. *Annual Review of Ecology and Systematics*, 8, 255–283. <https://doi.org/10.1146/annurev.es.08.110177.001351>
- Berner, D., Adams, D. C., Grandchamp, A. C., & Hendry, A. P. (2008). Natural selection drives patterns of lake–stream divergence in stickleback foraging morphology. *Journal of Evolutionary Biology*, 21(6), 1653–1665. <https://doi.org/10.1111/j.1420-9101.2008.01583.x>
- Collin, H., & Fumagalli, L. (2011). Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). *Molecular Ecology*, 20(21), 4490–4502. <https://doi.org/10.1111/j.1365-294X.2011.05284.x>
- Cureton, J. C., & Broughton, R. E. (2014). Rapid morphological divergence of a stream fish in response to changes in water flow. *Biology Letters*, 10(6), 20140352. <https://doi.org/10.1098/rsbl.2014.0352>
- Debat, V., & David, P. (2001). Mapping phenotypes: Canalization, plasticity and developmental stability. *TRENDS in Ecology and Evolution*, 16, 555–561. [https://doi.org/10.1016/S0169-5347\(01\)02266-2](https://doi.org/10.1016/S0169-5347(01)02266-2)
- Development Core Team, R. (2008). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org>. ISBN 3-900051-07-0.
- Dobush, R., Ankney, C. D., & Kremetz, D. G. (1985). The effect of apparatus, extraction time, and solvent type on lipid extractions of Snow Geese. *Canadian Journal of Zoology*, 63, 1917–1920. <https://doi.org/10.1139/z85-285>
- Flodmark, L. E. W., Urke, H. A., Halleraker, J. H., Arnekleiv, J. V., Vøllestad, L. A., & Poléo, A. B. S. (2002). Cortisol and glucose responses in juvenile brown trout subjected to a fluctuating flow regime in an artificial stream. *Journal of Fish Biology*, 60(1), 238–248. <https://doi.org/10.1111/j.1095-8649.2002.tb02401.x>
- Foster, K., Bower, L., & Piller, K. (2015). Getting in shape: Habitat-based morphological divergence for two sympatric fishes. *Biological Journal of the Linnean Society*, 114(1), 152–162. <https://doi.org/10.1111/bij.12413>
- Franssen, N. R. (2011). Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish. *Evolutionary Applications*, 1752–4571. <https://doi.org/10.1111/j.1752-4571.2011.00200.x>
- Franssen, N. R., Harris, J., Clark, S. R., Schaefer, J. F., & Stewart, L. K. (2013). Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. *Proceedings of the Royal Society B*, 280, 201222715. <https://doi.org/10.1098/rspb.2012.2715>
- Franssen, N. R., Stewart, L. K., & Schaefer, J. F. (2013). Morphological divergence and flow-induced phenotypic plasticity in a native fish from anthropogenically altered stream habitats. *Ecology and Evolution*, 3(14), 4648–4657. <https://doi.org/10.1002/ece3.842>
- Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21, 394–407. <https://doi.org/10.1111/j.1365-2435.2007.01283.x>
- Haas, T. C., Blum, M. J., & Heins, D. C. (2010). Morphological responses of a stream fish to water impoundment. *Biology Letters*, 6, 803–806. <https://doi.org/10.1098/rsbl.2010.0401>
- Haas, T. C., Heins, D. C., & Blum, M. J. (2015). Predictors of body shape among populations of a stream fish (*Cyprinella venusta*, Cypriniformes: Cyprinidae). *Biological Journal of the Linnean Society*, 115(4), 842–858.
- Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. (2008). Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology*, 17, 20–29. <https://doi.org/10.1111/j.1365-294X.2007.03428.x>
- Istead, A. E., Yavno, S., & Fox, M. G. (2015). Morphological change and phenotypic plasticity in response to water velocity in three species of Centrarchidae. *Canadian Journal of Zoology*, 93(11), 879–888. <https://doi.org/10.1139/cjz-2015-0096>
- Kern, E. M., & Langerhans, R. B. (2018). Urbanization drives contemporary evolution in stream fish. *Global Change Biology*, 24(8), 3791–3803. <https://doi.org/10.1111/gcb.14115>
- Kerr, D. C., Ankney, C. D., & Millar, J. S. (1982). The effect of drying temperature on extraction of petroleum ether-soluble fats of small birds and mammals. *Canadian Journal of Zoology*, 60, 470–472. <https://doi.org/10.1139/z82-064>
- Krabbenhoft, T. J., Platania, S. P., & Turner, T. F. (2014). Interannual variation in reproductive phenology in a riverine fish assemblage: Implications for predicting the effects of climate change and altered flow regimes. *Freshwater Biology*, 59(8), 1744–1754. <https://doi.org/10.1111/fwb.12379>
- Langerhans, R. B. (2008). Predictability of phenotypic differentiation across flow regimes in fishes. *Integrative and Comparative Biology*, 48, 750–768. <https://doi.org/10.1093/icb/icn092>
- Langerhans, R. B., Layman, C. A., Langerhans, A. K., & Dewitt, T. J. (2003). Habitat-associated morphological divergence in two Neotropical fish species. *Biological Journal of the Linnean Society*, 80(4), 689–698. <https://doi.org/10.1111/j.1095-8312.2003.00266.x>
- Matthews, W. J. (1987). Geographic Variation in *Cyprinella lutrensis* (Pisces: Cyprinidae) in the United States, with Notes on *Cyprinella lepida*. *Copeia*, 3, 616–637. <https://doi.org/10.2307/1445654>
- Meuthen, D., Ferrari, M. C. O., Lane, T., & Chivers, D. P. (2019). Predation risk induces age- and sex-specific morphological plastic responses in the fathead minnow *Pimephales promelas*. *Scientific Reports*, 9, 15378. <https://doi.org/10.1038/s41598-019-51591-1>
- Mims, M. C., & Olden, J. D. (2013). Fish assemblages respond to altered flow regimes via ecological filtering of life history strategies. *Freshwater Biology*, 58(1), 50–62. <https://doi.org/10.1111/fwb.12037>
- Murchie, K. J., Hair, K. P. E., Pullen, C. E., Redpath, T. D., Stephens, H. R., & Cooke, S. J. (2008). Fish response to modified flow regimes in regulated rivers: Research methods, effects and opportunities. *River Research and Applications*, 24(2), 197–217. <https://doi.org/10.1002/rra.1058>
- Nilsson, C., Reidy, C. A., Dynesius, M., & Revenga, C. (2005). Fragmentation and flow regulation of the world's large river systems. *Science*, 308, 405–408. <https://doi.org/10.1126/science.1107887>
- Perkin, J. S., & Gido, K. B. (2011). Stream fragmentation thresholds for a reproductive guild of Great Plains fishes. *Fisheries*, 36(8), 371–383. <https://doi.org/10.1080/03632415.2011.597666>

- Perkin, J. S., Gido, K. B., Cooper, A. R., Turner, T. F., Osborne, M. J., Johnson, E. R., & Mayes, K. B. (2015). Fragmentation and dewatering transform Great Plains stream fish communities. *Ecological Monographs*, 85(1), 73–92. <https://doi.org/10.1890/14-0121.1>
- Poff, N. L., & Zimmerman, J. K. (2010). Ecological responses to altered flow regimes: A literature review to inform the science and management of environmental flows. *Freshwater Biology*, 55(1), 194–205. <https://doi.org/10.1111/j.1365-2427.2009.02272.x>
- Rohlf, J. F. (2017a). *TPS Dig. Ecology and Evolution*, SUNY at Stony Brook.
- Rohlf, J. F. (2017b). *TPS Utility Program. Ecology and Evolution*, SUNY at Stony Brook.
- Rudolfson, T., Watkinson, D. A., & Poesch, M. (2017). Morphological divergence of the threatened Rocky Mountain sculpin (Cottus sp.) is driven by biogeography and flow regime: Implications for mitigating altered flow regime to freshwater fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28, 1–9.
- Salmerón, C. (2018). Adipogenesis in fish. *The Journal of Experimental Biology*, 221, jeb161588. <https://doi.org/10.1242/jeb.161588>
- Taylor, C. A., Knouft, J. H., & Hiland, T. M. (2001). Consequences of stream impoundment on fish communities in a small North American drainage. *Regulated Rivers: Research & Management*, 17, 687–698. <https://doi.org/10.1002/rrr.629>
- Vaughn, C. C., & Taylor, C. M. (1999). Impoundments and the decline of freshwater mussels: A case study of an extinction gradient. *Conservation Biology*, 13(4), 912–920. <https://doi.org/10.1046/j.1523-1739.1999.97343.x>
- Webb, P. W. (1984). Form and function in fish swimming. *Scientific American*, 251(1), 72–83. <https://doi.org/10.1038/scientificamerican0784-72>
- Winston, M. R., Taylor, C. M., & Pigg, J. (1991). Upstream extirpation of four minnow species due to damming of a prairie stream. *Transactions of the American Fisheries Society*, 120(1), 98–105. [https://doi.org/10.1577/1548-8659\(1991\)120<0098:UEOFMS>2.3.CO;2](https://doi.org/10.1577/1548-8659(1991)120<0098:UEOFMS>2.3.CO;2)
- Zelditch, M. L., Swiderski, D. L., Sheets, H. D., & Fink, W. F. (2004). *Geometric morphometrics for biologists: A primer*, San Diego, CA: Elsevier Academic Press.

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