Population-Level Responses of Life History Traits to Flow Regime in Three Common Stream Fish Species

Micah G. Bennett

Matt R. Whiles
Southern Illinois University Carbondale

Gregory Whitledge
Southern Illinois University Carbondale, gwhit@siu.edu

Follow this and additional works at: http://opensiuc.lib.siu.edu/fiaq_pubs

Recommended Citation
Population-level responses of life history traits to flow regime in three common stream fish species

Article in Ecohydrology · February 2016
DOI: 10.1002/eco.1734

CITATIONS 0
READS 64

3 authors, including:

Micah G Bennett
United States Environmental Protection Agency
17 PUBLICATIONS 10 CITATIONS

Gregory W Whitledge
Southern Illinois University Carbondale
84 PUBLICATIONS 952 CITATIONS

Some of the authors of this publication are also working on these related projects:

River Ecology View project

All content following this page was uploaded by Micah G Bennett on 10 March 2016.
The user has requested enhancement of the downloaded file. All in-text references underlined in blue are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.
Population-level responses of life history traits to flow regime in three common stream fish species

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Ecohydrology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>ECO-15-0148.R2</td>
</tr>
<tr>
<td>Wiley - Manuscript type:</td>
<td>Research Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>08-Feb-2016</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Bennett, Micah; Southern Illinois University, Department of Zoology and Center for Ecology; U.S. Environmental Protection Agency, Office of Research and Development Whiles, Matt; Southern Illinois University, Department of Zoology and Center for Ecology Whitledge, Gregory; Southern Illinois University, Center for Fisheries, Aquaculture, and Aquatic Sciences; Southern Illinois University, Department of Zoology and Center for Ecology</td>
</tr>
<tr>
<td>Keywords:</td>
<td>flow-ecology relationships, reproduction, functional trait, trait-based</td>
</tr>
</tbody>
</table>

This is the pre-final peer review version of the following article: [Bennett, M.G., M.R. Whiles, and G.W. Whitledge. In press. Population-level responses of life history traits to flow regime in three common stream fish species. Ecohydrology. DOI 10.1002/eco.1734], which has been published in final form at [http://onlinelibrary.wiley.com/doi/10.1002/eco.1734/full]. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Population-level responses of life history traits to flow regime in three common stream fish species

Micah G. Bennett¹*, Matt R. Whiles¹, and Gregory W. Whitledge¹,²

¹ Department of Zoology and Center for Ecology, Southern Illinois University, 1125 Lincoln Drive (Mail Code 6501), Carbondale, Illinois 62901

² Center for Fisheries, Aquaculture, and Aquatic Sciences, Southern Illinois University, 1125 Lincoln Drive (Mail Code 6511), Carbondale, Illinois 62901

*author to whom correspondence should be addressed; email: micahgbenning@yahoo.com;
current address: U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC 20460 USA

Running head: Fish intraspecific flow-life history relationships

Keywords: flow-ecology, reproduction, trait-based, functional trait
ABSTRACT

Trait-based approaches may improve understanding in ecology by linking environmental variation to fitness-related characteristics of species. Most trait-environment studies focus on assemblage-level relationships; yet intraspecific trait variation is important for community, ecosystem, and evolutionary processes and has substantial implications for these approaches. Assessing population-level trait-environment relationships could test the generality of trait models while assessing intraspecific variation. We evaluated the generality of the trilateral life history model (TLHM of Winemiller and Rose 1992: opportunistic, periodic, and equilibrium endpoints) for fishes - a well-studied trait-environment model at the assemblage level - to populations of three stream fishes in the Midwestern United States in relation to flow regime. The TLHM adequately described major trade-offs in traits among populations in all species. Some TLHM flow-based predictions were confirmed, with periodic traits (high fecundity) favored at sites with greater flow seasonality and lower flow variability in two species, and equilibrium traits (large eggs) in more stable flow conditions in two species. Size at maturity was also inversely related to variability in one species. However, relationships contradicting the TLHM were also found. Coupled with the explanatory power of the TLHM for populations, supporting relationships suggest that synthesizing habitat template models with demographic life history theory could be valuable. Trait-environment models that are well-supported at multiple levels of biological organization could improve understanding of the impacts of environmental change on populations and communities and the valuable ecosystem services that they support.
INTRODUCTION

Trait-based approaches are of increasing interest in community ecology because combining information on environmental conditions (i.e., environmental filters; Smith and Powell, 1971; Poff, 1997) with data on species traits that directly influence fitness could generate a more general and mechanistic understanding of community assembly (Poff, 1997; McGill et al., 2006). Such approaches have provided major insights into environmental influences on community assembly in fresh waters (e.g., Poff and Allan, 1995; McManamay and Frimpong, 2015) and can serve as useful frameworks for developing flow-ecology hypotheses and environmental flow standards (McManamay et al., 2014).

The focus of most trait-environment studies to date has been on assemblage-level relationships; yet population-level trait variation also has important implications for these approaches. Intraspecific trait variability can influence community assembly, community stability, and ecosystem function (Luck et al., 2003; Crutsinger et al., 2006; Ackerly and Cornwell, 2007; Albert et al., 2010), as well as the fundamental evolutionary processes of adaptation and speciation (Darwin, 1861). Therefore, assessment of trait-environment relationships at the population level could further test the generality of trait-based models while also estimating intraspecific trait variability (Verberk et al., 2013). Here, we provide a field-based evaluation of the generality of a trait-environment model that has been well-studied at the assemblage level in populations of three widespread stream fishes in the Midwestern United States.
The trilateral life history model (TLHM), a trait-environment model developed for fishes by Winemiller and Rose (1992), reflects trade-offs between juvenile survival, fecundity, and generation time. The endpoints of the TLHM represent life history strategies that are selected for under certain environmental conditions (Figure 1). The opportunistic strategy minimizes generation time, which promotes rapid population growth and should be favored in highly variable environments. The equilibrium strategy maximizes juvenile survival through large eggs or parental care and should be favored under stable environmental conditions that promote investment in few, well-provisioned offspring. The periodic strategy maximizes fecundity by delaying reproduction until a larger size is reached and is favored under predictably variable or highly seasonal environments where reproduction can coincide with favorable conditions.

Studies from across the globe have largely confirmed fish life history trait ordination along these three axes (e.g., Vila-Gispert et al., 2002; Olden and Kennard, 2010) and have documented the utility of the TLHM for predicting assemblage-level life history traits in relation to natural and altered flow regimes (e.g., Olden and Kennard, 2010; Mims and Olden, 2012, 2013).

Although applied at the assemblage level, the TLHM is based on fundamental traits that exhibit trade-offs and determine fitness of individuals and populations (for instance, generation time is a fundamental component of basic population models; and fecundity and egg size often exhibit trade-offs at individual and population levels; Wootton and Smith, 2014), suggesting that it may also apply at the population level; however, few studies have investigated intraspecific trait variation among fish populations in relation to hydrologic variability. The application of simple models like the TLHM or other habitat template approaches (Southwood, 1988) does not reflect the full complexity of factors affecting population life history and does not determine a definitive mechanism for any relationships (Stearns, 1992). Yet such an application could prove
useful in identifying potential selective environmental factors (Endler, 1986) in the absence of
detailed, but difficult-to-acquire, information on age-structured mortality required of
demographic life history theory (Stearns, 1992). Further, using population-level studies to
confirm or refute trends from assemblage-level studies could suggest the relative merits and
generality of the proposed mechanisms or assumptions underlying trait-environment
relationships.

Our specific objectives were to: 1) determine if population-level variation in life history
traits follows assemblage-level TLHM findings; and 2) determine if predictions of the TLHM in
relation to flow regime apply at the population level for three stream fish species. We
hypothesized that: 1) intraspecific variation would be oriented similarly to the assemblage-level
TLHM; and 2) hydrologic variability would influence life history traits of populations as
predicted by the TLHM. Specifically, we hypothesized that flow variability would promote
opportunistic traits (small size at maturity), that flow predictability/stability would promote
equilibrium traits (large egg size), and that flow seasonality would promote periodic traits (high
fecundity) (Table 1).

METHODS

Species selection

Three study species were selected based on broad geographic distributions in the Central
Lowlands of North America, potentially high relative abundances, and diverse ecologies: red
shiner (Cyprinella lutrensis; Family Cyprinidae), bluntnose minnow (Pimephales notatus;
Family Cyprinidae), and orangethroat darter (Etheostoma spectabile; Family Percidae). The red
shiner is a habitat generalist most abundant in low-gradient streams and rivers. This species
spawns adhesive eggs in gravel riffles from spring to fall, usually peaking in June-July in the
Midwest (Robison and Buchanan, 1988). Most individuals are mature by their second summer (Boschung and Mayden, 2004). The species is likely a fractional spawner, laying up to 19 clutches/season in captivity (Boschung and Mayden, 2004). The bluntnose minnow occupies mid-water column and near-benthic areas and lays eggs in rock crevices where eggs are protected by males. Females, mature by age one, are fractional spawners and produce up to 4200 eggs/season during late spring-summer (Boschung and Mayden, 2004). The orangethroat darter inhabits runs, pools, and riffle margins and spawns in riffles, with eggs buried in gravel and receiving no parental care; females may spawn with multiple males per season during spring-early summer and are mature by age one (Etnier and Starnes, 1993; Pflieger, 1997). Based on species-level trait data, all three species would be classified as relatively opportunistic strategists within the TLHM, having relatively short generation time, although parental care by bluntnose minnow makes it more closely affiliated with the equilibrium strategy (Winemiller and Rose, 1992; MGB, unpublished).

**Site selection**

We selected a set of ‘reference’ USGS stream gages (Falcone et al., 2010) that coincided with probable locations of target species (see Appendix S1 for details). Final sampling locations containing the target species were all within the Mississippi-Ohio River watershed (Figure 2; Appendix S1). Of the sites sampled, we obtained reproductive individuals from seven populations of red shiner, eight populations of orangethroat darter, and 14 populations of bluntnose minnow (Appendix S1).

**Hydrologic variables and measurement**

Predictions of the TLHM focus on environmental variability, stability, and predictability. Because of the importance of flow regime to stream ecosystems, communities, and populations,
these predictions have largely been operationalized as variability, stability, and
predictability/seasonality of the flow regime in studies of stream fishes. We selected hydrologic
metrics that highlight these aspects based on two recent assemblage-level studies (Mims and
Olden, 2012, 2013) in order to compare results (Table 1). Annual coefficient of variation of daily
flow ($annCV$) and the high pulse count ($HPC$) were used as measures of flow variability.
Colwell’s flow predictability index ($FlowPred$; Colwell, 1974) and the base flow index ($BFI$)
were used as measures of flow predictability/stability. Flow constancy divided by predictability
($ConstPred$; Colwell, 1974) and high pulse duration ($HPD$) were used as measures of flow
seasonality. Additionally, mean daily discharge ($meanQ$) was used to measure flow magnitude
due to previous findings of discharge-life history relationships (Table 1). All indices were
calculated using Indicators of Hydrologic Alteration software (Richter et al., 1996) based on
medians using all years of data with <10 missing days for each gage through 2012, the year
prior to fish sampling (Appendix S1).

We evaluated two variables for additional influences on life history traits due to expected
relationships from the literature: latitude and genetic divergence (Appendix S2). Latitude is
correlated with temperature, ecosystem productivity, and other variables known to influence life
history evolution (e.g., Leggett and Carscadden, 1978; Fleming and Gross, 1990) and served as a
test of broader geographic patterns unrelated to flow regime. We decided whether to include
latitude in our models by assessing individual impact on traits prior to final model testing and
included it if significantly related to at least one life history trait for a species ($p<0.05$). Latitude
was included in bluntnose minnow and red shiner models based on significant regressions
(Appendix S2). Life history traits may also be influenced more by evolutionary history than local
environmental conditions (Stearns, 1992); therefore, we evaluated the effect of genetic
divergence on traits for orangethroat darter using published cytochrome b gene data (Bossu et al., 2013) and a Mantel test between raw genetic and life history distances (cf. Olden and Kennard, 2010; see Appendix S2). Based on this test, genetic divergence was not significantly related to life history differences among populations (Appendix S2). Detailed phylogeographic information was unavailable for the other species so we used stream distance as a potential correlate of genetic divergence. Mantel tests were also non-significant for the minnow species (Appendix S2). Due to this apparent lack of phylogenetic signal, we did not correct for evolutionary history in subsequent analyses (Diniz-Filho et al., 2012).

Fish sampling

Fishes were sampled from a 100 m reach within 1 km of USGS gage locations using a seine (3.05 x 1.22 m) and backpack electrofishing unit (Smith-Root LR-24, Vancouver, WA). Sampling was aimed at study species and ceased when approximately 30 adult females of each species were obtained or when several successive sampling efforts (seine hauls or seine with electrofishing) produced no additional target individuals. Sampling occurred usually once seasonally as appropriate to target peak spawning for study species (spring for orangethroat darter, late summer for cyprinids). Most sites were sampled in 2013 or 2014, and sometimes in both years in an attempt to obtain more mature individuals (Appendix S1). Fish were euthanized using MS-222 (300 mg/L), fixed in 10% formaldehyde, and transferred to 70% ethanol for examination in the laboratory. While ethanol does cause size reduction of ova (Frimpong and Henebry, 2012), the same procedure was conducted on all individuals and thus would not affect comparisons.

Life history trait variables and measurement
A limited number of life history variables were measured on mature females to test TLHM predictions. We measured mean oocyte diameter to capture the parental investment (equilibrium) axis, number of mature oocytes to capture the fecundity (periodic) axis, and smallest size of a mature female to capture the generation time (opportunistic) axis of the TLHM.

Ovaries were extracted and classified according to the external staging method of Heins, which separates females and oocytes into maturity stages based on oocyte size, coloration, yolk condition, and physical location in the ovum (Appendix S3) (e.g., Heins and Rabito, 1986; Heins and Baker, 1993). The diameters of 10 mature, ripening, or ripe oocytes were measured for each individual using digital microscope photography (Leica MZ 9.5 stereomicroscope, Leica DFC 320 camera; Leica Microsystems, Buffalo Grove, IL, USA) and ImageJ software (NIH, Washington, D.C.) at 150x magnification. Because most preserved oocytes were not spherical, large and small axes of each oocyte were averaged (Heins and Rabito, 1988). Based on repeated measurements (n=5) of 10 randomly selected oocytes, measurement variance was low, ranging from $3.8 \times 10^{-6}$ to $2.7 \times 10^{-4}$ mm. The number of mature and ripening oocytes was counted to measure clutch size as an estimate of batch fecundity for each female (Heins and Rabito, 1986; Heins and Baker, 1993); however, ripe females were not included in counts because they could have already released some of the clutch before capture (Heins and Baker, 1993). Because advanced stage oocytes more closely correspond to the size of oocytes released during spawning, we used only ripening and ripe oocytes to estimate egg size for orangethroat darter and bluntnose minnow (Heins and Baker, 1988); however, because not all populations of red shiner had females at these stages, we used mature oocytes in analyses for this species. Females with mature oocytes were identified and the smallest standard length (SL) was recorded as a measure of minimum size at maturity (minSLmat), a proxy for generation time (Winemiller and Rose, 1992;
Wootton, 1998). Although interannual variation in egg size and fecundity are important aspects of life history (Wootton, 1998), for the purposes of this study we combined samples from 2013 and 2014 within populations when available because our focus was on variation among populations. Trait values were divided by SL to correct for differences in size among populations and correlations between size and other traits, with average individual size-corrected values for fecundity and egg size (individual trait/individual SL) and minSLmat divided by average population SL.

**Statistical analyses**

We first evaluated variation in fecundity, egg size, and SL within and among populations using one-way ANOVA (Type III SS) in the R package “car.” Then, for each species, we used multiple linear regression to predict life history traits from flow and latitude (if applicable). We used principal components analysis (PCA) on flow variables to reduce the number of variables for subsequent regression models (Appendix S4), evaluating each principal component (PC) axis based on a broken stick model for interpretability (Jackson 1993) and scree plots. Interpretable PC axes were used in subsequent regression models, which are described below.

The multiple linear regression models, combined with multi-model inference based on small sample size Akaike information criteria (AICc) scores, were used to examine relationships between life history traits and flow variables, with each of the three size-corrected life history traits as the dependent variable and flow PCAs (and latitude, if appropriate) as independent variables (In-transformed). We evaluated all possible combinations of variables in linear models for comparison (including intercept-only model) using the MuMIn package in R. The number of models for each species-trait combination differed based on whether there were two or three flow PCs and whether latitude was included in models (4 models for each life history trait for
For Peer Review

orangethroat darter, 8 per trait for red shiner, and 16 per trait for bluntnose minnow). Models were compared using AICc scores, with models within Δ2 of the lowest AICc considered plausible (Burnham and Anderson, 2002). All size-corrected life history variables were ln-transformed and models were evaluated to ensure they met assumptions of normality and homogeneity of variance. Traits were scaled to a common axis (0-1; with the inverse of minSLmat used to capture the opportunistic axis), and relationships were visualized using ternary plots (SigmaPlot, Systat Software Co., San Jose, CA).

We also conducted PCA for size-corrected, ln-transformed life history traits to evaluate trade-offs in relation to the TLHM. For the life history PCAs, we selected the first two PCs for all species to capture the major variation and trade-offs in traits even if the second axis did not explain more variation than a single variable according to a broken stick model. We examined life history PCAs to determine if trait relations in multivariate space corresponded to the TLHM (Winemiller and Rose, 1992).

RESULTS

Population-level and species-level trait variability

Fecundity differed significantly among populations for orangethroat darter (F7,194=31.70, P<0.001; raw site means ± SE: 50.2±4.3 – 163.7±12.8), red shiner (F6,61=2.54, P=0.03; means: 221.7±24.7 – 475.0±132.0), and bluntnose minnow (F13,84=2.60, P<0.01; means: 161±17.5 – 433±82.5; Appendix S5). Despite the smaller differences among populations, egg size also differed significantly among populations for orangethroat darter (F7,166=20.64, P < 0.001; population means: 1.02±0.008 – 1.17±0.008 mm), red shiner (F6,25=2.74, P=0.03; means: 0.82±0.02 – 0.90±0.005 mm), and bluntnose minnow (F13,69=4.94, P < 0.001; means: 0.92±0.02 – 1.14±0.13 mm; Appendix S5). Differences in standard length of mature individuals were
borderline among populations of red shiner ($F_{6,61}=2.15$, $P=0.06$; population means: 36.7±1.16 – 45.1±1.4 mm) but did differ for orangethroat darter ($F_{7,194}=9.44$, $P<0.0001$; means: 32.3±0.59 – 38.7±0.54 mm) and bluntnose minnow ($F_{13,84}=6.05$, $P<0.0001$; means: 36.8±0.90 – 55.7±1.05 mm) populations (Appendix S5). Size-corrected fecundity and egg size also differed significantly ($p < 0.05$) among populations for all species, except that differences in fecundity among bluntnose minnow populations were borderline ($P=0.06$; Appendix S5).

**Life history relationships with flow regime**

Flow PCs explained moderate to substantial portions of variation in some life history traits for all three species examined based on the most informative models ($R^2=0.15-0.78$; Tables 2-4; only models within $\Delta AIC_c=2$ of lowest AICc are discussed). For fecundity in red shiner, the intercept-only model was ranked highest based in AICc; however, a model showing a negative relationship with flow PC2 (negative: HPD, HPC) was ranked second and within $\Delta AIC_c=2$ of the intercept model (Table 2; Figure 3). The top models for egg size (within $\Delta AIC_c=2$) showed positive relationships with flow PC1 (positive: BFI, FlowPred; negative: annCV) and latitude, with both models explaining >70% of the variation in egg size. For minSLmat, the intercept-only model was ranked best, but a model showing a negative relationship with flow PC1 (positive: BFI, FlowPred; negative: annCV) was ranked second (Table 2; Figure 3).

In bluntnose minnow, fecundity was positively related to latitude (best model) and negatively related to flow PC1, higher values of which represented less seasonal flows (lower HPD, higher ConstPred), and flow PC2 (positive: annCV; negative: BFI; Table 3; Figure 4). For egg size, the intercept-only model was ranked best but two models within $\Delta AIC_c=2$ showed a positive relationship with flow PC2 (positive: annCV; negative: BFI) and a negative relationship with latitude (Table 3; Figure 4). For minSLmat, the intercept-only model was also ranked as
best, but a model with a positive relationship with flow PC3 (positive: meanQ) was within
\( \Delta AIC_c=2 \) (Table 3; Figure 4).

In orangethroat darter, fecundity was positively related to flow PC2 (positive: HPD; negative: annCV); this model had substantially lower weight than the intercept-only model but was within \( \Delta AIC_c=2 \) of the best-ranked model (Table 4; Figure 5). The intercept-only model also had the best ranking among models for egg size, with the second-ranked model showing a positive relationship with flow PC1 (positive: BFI; negative: HPC, FlowPred; Table 4; Figure 5) that also had substantially lower weight but was within \( \Delta AIC_c=2 \) of the best-ranked model.

Among models for minSLmat, the intercept-only model was the best-ranked, but the model showing a positive relationship with flow PC2 (positive:HPD; negative: annCV) had similar weight (0.45) to the intercept-only model (0.51) and was within \( \Delta AIC_c=2 \) of the best-ranked model (Table 4).

Life history trait variability and relationships

Life history traits along the first two PC axes largely mirrored established life history trade-offs across fish species based on the TLHM (Figure 6; Appendix S4). We interpreted PC1 as an equilibrium-to-periodic axis for all species, with the periodic traits of high fecundity and large minSLmat loading at the positive end and the equilibrium trait of large egg size at the negative end of the axis (Figure 6). We interpreted PC2 as an opportunistic to periodic axis for all species, with the opportunistic trait of small minSLmat loading on the negative end of the axis, and the periodic traits of high fecundity or large minSLmat loading on the positive end (Figure 6).

DISCUSSION
Our results suggest that the trait-based life history trade-offs and strategies of the TLHM may apply at the population level for three common fish species and that some of the predicted effects of flow regime variability and predictability mirror those found at the assemblage level (Mims and Olden, 2012, 2013), suggesting consistent mechanisms. Trade-offs between periodic (large body size, high fecundity) and opportunistic traits (small size at maturity) and between opportunistic and equilibrium traits (large egg size) largely described the life history investment of populations as predicted by the TLHM. While we did select a small number of traits to coincide with the TLHM, there was no *a priori* reason for these traits to ordinate in a similar fashion.

Although the population-level life history traits examined in this study aligned well with the assemblage-based TLHM, the predicted relationships between hydrology and life history traits had variable support. As predicted by the TLHM, populations of bluntnose minnow and orangethroat darter that experience more seasonal (high HPD) and less variable (low CV, HPC) flows had higher fecundity. Also as predicted, sites with greater flow stability (BFI) had populations of red shiner and orangethroat darter with larger eggs. Orangethroat darter populations from low-variability sites and red shiner populations from more stable sites also had larger minSLmat, as predicted. However, counter to TLHM predictions, seasonality and variability measures were correlated in among red shiner sites, leading to a positive relationship between fecundity and HPD/HPC. Higher variability was also associated with larger eggs in bluntnose minnow populations, counter to TLHM predictions. Overall, these relationships suggest that, in many cases, flow regime (or some correlated variable) is associated with life history traits of populations in some ways as predicted by the TLHM and assemblage-level studies (Mims and Olden, 2012).
Support for TLHM flow-trait associations varied by species, suggesting species-level differences in behavior could influence how organisms experience flow variation. Both minnow species have adhesive eggs, with red shiner often described as crevice spawning but utilizing various substrates and bluntnose minnow attaching eggs to the underside of rocks where they are actively guarded. The orangethroat darter buries eggs in gravel interstices and also does not defend the clutch (Boschung and Mayden, 2004). The different responses of egg size to flow regime in these species (bluntnose minnow: larger eggs with more variable flow; others: larger eggs with more stable flow;) could be a result of different spawning behavior, with more physical protection afforded to bluntnose minnow clutches and eggs of the other two species left at risk of reduced hatching in variable flows (Durham and Wilde, 2006). Larger eggs, which produce larger offspring may be favored under variable flows if mortality at the egg stage is reduced through protection. Flow variables for future trait-environment prediction should be carefully selected as species experience flow variability differently based on life history and behavioral differences (Poff and Ward, 1990; Beachum et al., 2015).

Identifying mechanisms underlying flow-trait relationships is made difficult by trade-offs among traits, and determining which traits could be under selection, or the role of phenotypic plasticity in trait responses, is quite challenging (Wootton and Smith, 2014). For instance, egg size appears inversely correlated with fecundity in our study species, as is commonly the case due to physiological and geometric limits; thus responses of one trait may be the result of selection on another (Wootton and Smith, 2014). The fact that egg size and fecundity generally did not respond to the same flow regime components (except in bluntnose minnow, Table 3) suggests that our patterns may not be merely the result of trade-offs between these traits. Recent studies of fish life history traits have documented substantial local adaptation with a genetic
basis, but particular traits such as egg size may exhibit extreme plasticity (Torres Dowdall et al., 2012; Wootton and Smith, 2014).

Our estimates of life history traits and flow relationships were inevitably influenced by several factors including small sample sizes and assumptions about measured traits. Small sample sizes are typical for multi-site life history studies of small-bodied fishes over broad geographic regions, and timing sampling effort to spawning at many of sites was difficult, especially for cyprinids. Further, the small size of the streams sampled and the reliance on USGS gages limited the number of available sampling locations. Small sample sizes could influence trait estimates and increase sample variability. Minimum SL at maturity is likely to change with increased sampling because of its dependence on capturing the smallest reproductive individual in a population; however, this trait was correlated with median SL in all species, suggesting that it might be more resilient to sample size effects. The consistency of slopes between the full dataset and the dataset with small samples (<4 individuals) removed in all but one case suggests that our results may be somewhat robust to small sample sizes (Appendix S6). However, related to issues of small sample size, the presence of outliers, particularly in two of the three important models for red shiner (population RCNE in both cases), appeared to drive relationships and should be considered.

Although most of the study species lay multiple clutches in a season, we were limited to using batch fecundity due to difficulty in measuring number of clutches per season (Winemiller and Rose, 1992). Batch fecundity is thought to closely correlate with lifetime fecundity (Roff, 1992) and is frequently used to characterize fecundity and life history patterns (Winemiller and Rose, 1992); however, the actual relationship for the study species is unknown. If batch fecundity is not strongly correlated with annual or lifetime fecundity in the study species, the
relationships we report may not represent important life history trade-offs or environmental variation. We also used egg size to represent parental investment, and thus an assumption of our study is that parental care behavior remains constant across populations. Increasing parental care behavior may be a more viable evolutionary strategy than increasing fecundity or egg size due to evolutionary constraints of small body size in many stream fishes (Winemiller and Rose, 1992); however, parental care may be less evolutionarily labile than the life history traits in our study (Sternberg and Kennard, 2014). Among the study species, bluntnose minnow exhibits parental care, and this could confound attempts to evaluate investment per progeny in relation to flow regime using only egg size.

Our findings combined with those of other studies suggest – whether due to phenotypic plasticity, natural selection, or a combination – intraspecific trait variation in fishes is high (Blanck and Lamouroux, 2006; Frimpong and Angermeier, 2010). Although we were unable to undertake a full assessment of intra- versus inter-specific trait variability, at least one trait per species exhibited substantial variation at the population level that rivaled variability at the family or congeneric level (e.g., compare data in FishTraits, Frimpong and Angermeier, 2009). Such high variability within species could greatly affect community-level trait studies, which normally assign mean trait values to species (Albert et al., 2010; Frimpong and Angermeier, 2010). While the three species studied are known to be widely-distributed and might therefore be expected to exhibit more trait variation than restricted-range species, they are common members of fish assemblages in eastern and central North America. Therefore, while not necessarily suggestive of intraspecific trait variability in fishes as a whole, such variation could have significant bearing on trait models and should be investigated more broadly in fishes.
The potential for high intraspecific trait variation, coupled with the fact that the TLHM explained life history trait variation at the population level, suggests that efforts to synthesize environmental filtering or habitat template models with demographic life history theory, which is currently lacking, could be valuable (Wootton, 1998). This could be accomplished by linking age- or size-specific mortality or its correlates to key environmental features like flow regime or habitat variability from major template theories like the TLHM or River Habitat Template (Townsend and Hildrew, 1994). Once environmental factors are known to be selective, demographic theory could be used to make new predictions about the effects of the environment on life history traits. With testing and refinement of these predictions, knowledge of how and why individual species respond to environmental conditions and gradients could then improve understanding of community assembly and change (Matthews, 1998).

The ability to predict trait responses to environmental variation has major implications for species and community persistence in the face of continuing habitat destruction and climate change. As functional traits, the life history parameters of the TLHM largely determine individual performance and population viability. For example, there is evidence that larger eggs produce larger offspring (Gale, 1986; Marsh, 1986; Kamler, 2005), which can be stronger and have higher survival under a variety of conditions (Marsh, 1986; Harvey, 1987). Our results suggest that larger eggs and offspring are associated with more stable flow conditions in some species (red shiner, orangethroat darter); thus, increasing flow variability from climate change, as predicted for the Midwestern U.S. (Wuebbles and Hayhoe, 2004), could promote smaller offspring with reduced survival. Trait-environment models that are well-supported at multiple levels of biological organization could help us understand how changing flow or temperature regimes will impact population and community viability and the sustainability of the valuable
ecosystem services that they, and their natural variability, support (Daily, 1997; Luck et al., 2003).

ACKNOWLEDGMENTS

The SIU NSF-IGERT Program in Watershed Science and Policy and the Graduate Professional Student Council supported this research. Thanks to J. Adams, K. Baumann, A. Beattie, T. Egdorf, J. Fulgoni, H. Rantala, L. Shoup, B. Zuñiga-Céspedes for field help. Sampling was conducted with permits from IL, IN, KS, MN, MO, NE, and OH and IACUC. K. Gido provided useful discussions that greatly improved the manuscript; and the Freshwater Ecology Lab at SIU provided helpful comments and edits on an earlier draft of the manuscript.

REFERENCES


Table 1. Hydrologic metrics and predicted impacts of their increase on life history strategy and traits used in this study (modified from Mims & Olden 2012). Letters after metrics refer to flow regime component measured by metric: V = variability, P = predictability, S = seasonality. + = predict positive relationship between flow variable and traits; - = predict negative relationship between flow variable and traits; 0 = predict no relationship between flow variable and traits.

<table>
<thead>
<tr>
<th>Hydrologic parameter</th>
<th>Abbreviation</th>
<th>Description</th>
<th>Opportunistic (small size at maturity)</th>
<th>Periodic (high fecundity)</th>
<th>Equilibrium (large egg size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual coefficient of variation (V)</td>
<td>AnnCV</td>
<td>Standard deviation of all daily flow values divided by mean annual flow</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>High pulse count (V)</td>
<td>HPC</td>
<td>Number of flows &gt;75th percentile each year</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Base flow index (P)</td>
<td>BFI</td>
<td>7-day minimum flow/mean flow for year</td>
<td>-</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Flow predictability (P)</td>
<td>FlowPred</td>
<td>Constancy/(measure of temporal invariance) + contingency (measure of periodicity)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Constancy/predictability (S)</td>
<td>Const/Pred</td>
<td>Constancy/(constancy + contingency)</td>
<td>0</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>High pulse duration (S)</td>
<td>HPD</td>
<td>Median duration of flows &gt;75th percentile</td>
<td>-</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Mean discharge¹</td>
<td>MeanQ</td>
<td>Average of daily flows over study period</td>
<td>-?</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

¹ Predictions for meanQ are based on other literature sources (Heins and Baker, 1987; Heins and Rabito, 1988; Heins, 1991; Machado et al., 2002; Cattaneo, 2005a, 2005b; Morrongiello et al., 2012). Prediction for Opportunistic strategy based on correlation with periodic traits (larger size and size at maturity).
Table 2. Regression model selection results for fecundity, egg size, and minimum SL at maturity (minSLmat) for all species. Bold models indicate those within ΔAICc=2 of lowest AICc. For red shiner and bluntnose minnow, only four best-ranked models shown for each trait of a possible eight and 16 models, respectively.

<table>
<thead>
<tr>
<th>Model</th>
<th>R²</th>
<th>df</th>
<th>logLik</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>weight</th>
<th>Intercept</th>
<th>Latitude</th>
<th>flowPC1</th>
<th>flowPC2</th>
<th>flowPC3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red Shiner</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecundity (intercept)</td>
<td>0.00</td>
<td>2</td>
<td>-0.063</td>
<td>7.1</td>
<td>0.00</td>
<td>0.62</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecundity ~ FlowPC2</td>
<td>0.56</td>
<td>3</td>
<td>2.795</td>
<td>8.4</td>
<td>1.28</td>
<td>0.327</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>-0.747</td>
</tr>
<tr>
<td>Fecundity~FlowPC1</td>
<td>0.13</td>
<td>3</td>
<td>0.413</td>
<td>13.2</td>
<td>6.05</td>
<td>0.030</td>
<td>0</td>
<td></td>
<td></td>
<td>-0.357</td>
<td></td>
</tr>
<tr>
<td>Fecundity~Latitude</td>
<td>0.01</td>
<td>3</td>
<td>-0.043</td>
<td>14.1</td>
<td>6.96</td>
<td>0.019</td>
<td>0</td>
<td></td>
<td></td>
<td>-0.076</td>
<td></td>
</tr>
<tr>
<td>Egg size~FlowPC1</td>
<td>0.78</td>
<td>3</td>
<td>11.119</td>
<td>-8.2</td>
<td>0.00</td>
<td>0.638</td>
<td>0</td>
<td></td>
<td>0.885</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg size~Latitude</td>
<td>0.72</td>
<td>3</td>
<td>10.209</td>
<td>-6.4</td>
<td>1.82</td>
<td>0.257</td>
<td>0</td>
<td></td>
<td>0.848</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg size (intercept)</td>
<td>0.00</td>
<td>2</td>
<td>5.763</td>
<td>-4.5</td>
<td>3.71</td>
<td>0.100</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg size~FlowPC2</td>
<td>0.00</td>
<td>3</td>
<td>5.772</td>
<td>2.5</td>
<td>10.70</td>
<td>0.003</td>
<td>0</td>
<td></td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MinSLmat (intercept)</td>
<td>0.00</td>
<td>2</td>
<td>3.724</td>
<td>-0.4</td>
<td>0.00</td>
<td>0.476</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MinSLmat~FlowPC1</td>
<td>0.60</td>
<td>3</td>
<td>6.950</td>
<td>0.1</td>
<td>0.55</td>
<td>0.362</td>
<td>0</td>
<td></td>
<td>-0.776</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MinSLmat~Latitude</td>
<td>0.49</td>
<td>3</td>
<td>6.048</td>
<td>1.9</td>
<td>2.35</td>
<td>0.147</td>
<td>0</td>
<td></td>
<td>-0.697</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MinSLmat~FlowPC2</td>
<td>0.02</td>
<td>3</td>
<td>3.778</td>
<td>6.4</td>
<td>6.89</td>
<td>0.015</td>
<td>0</td>
<td></td>
<td>-0.124</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bluntnose Minnow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecundity~Latitude</td>
<td>0.43</td>
<td>3</td>
<td>-0.428</td>
<td>9.3</td>
<td>0.00</td>
<td>0.325</td>
<td>0</td>
<td></td>
<td>0.657</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecundity~FlowPC1+2</td>
<td>0.54</td>
<td>4</td>
<td>1.111</td>
<td>10.2</td>
<td>0.97</td>
<td>0.200</td>
<td>0</td>
<td></td>
<td>-0.422</td>
<td>-0.604</td>
<td></td>
</tr>
<tr>
<td>Fecundity~FlowPC2</td>
<td>0.37</td>
<td>3</td>
<td>-1.196</td>
<td>10.8</td>
<td>1.54</td>
<td>0.150</td>
<td>0</td>
<td></td>
<td>-0.604</td>
<td>-0.604</td>
<td></td>
</tr>
</tbody>
</table>
### Fecundity ~Lat+FlowPC2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coef 1</th>
<th>Coef 2</th>
<th>Coef 3</th>
<th>Coef 4</th>
<th>Coef 5</th>
<th>Coef 6</th>
<th>Coef 7</th>
<th>Coef 8</th>
<th>Coef 9</th>
<th>Coef 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity (intercept)</td>
<td>0.00</td>
<td>2</td>
<td>14.540</td>
<td>-24.0</td>
<td>0.00</td>
<td>0.283</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg size ~FlowPC2</td>
<td>0.17</td>
<td>3</td>
<td>15.823</td>
<td>-23.2</td>
<td>0.74</td>
<td>0.195</td>
<td>0</td>
<td></td>
<td></td>
<td>0.409</td>
</tr>
<tr>
<td>Egg size ~Latitude</td>
<td>0.15</td>
<td>3</td>
<td>15.688</td>
<td>-23.0</td>
<td>1.01</td>
<td>0.171</td>
<td>0</td>
<td></td>
<td></td>
<td>-0.389</td>
</tr>
<tr>
<td>Egg size ~FlowPC1</td>
<td>0.09</td>
<td>3</td>
<td>15.173</td>
<td>-21.9</td>
<td>2.04</td>
<td>0.102</td>
<td>0</td>
<td></td>
<td></td>
<td>0.294</td>
</tr>
<tr>
<td>MinSLmat (intercept)</td>
<td>0.00</td>
<td>2</td>
<td>17.167</td>
<td>-29.2</td>
<td>0.00</td>
<td>0.300</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MinSLmat ~FlowPC3</td>
<td>0.16</td>
<td>3</td>
<td>18.355</td>
<td>-28.3</td>
<td>0.93</td>
<td>0.188</td>
<td>0</td>
<td></td>
<td></td>
<td>0.395</td>
</tr>
<tr>
<td>MinSLmat ~FlowPC2</td>
<td>0.07</td>
<td>3</td>
<td>17.654</td>
<td>-26.9</td>
<td>2.34</td>
<td>0.093</td>
<td>0</td>
<td></td>
<td></td>
<td>-0.259</td>
</tr>
<tr>
<td>MinSLmat ~FlowPC1</td>
<td>0.06</td>
<td>3</td>
<td>17.620</td>
<td>-26.8</td>
<td>2.40</td>
<td>0.090</td>
<td>0</td>
<td></td>
<td></td>
<td>-0.250</td>
</tr>
</tbody>
</table>

#### Orangethroat Darter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coef 1</th>
<th>Coef 2</th>
<th>Coef 3</th>
<th>Coef 4</th>
<th>Coef 5</th>
<th>Coef 6</th>
<th>Coef 7</th>
<th>Coef 8</th>
<th>Coef 9</th>
<th>Coef 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity (intercept)</td>
<td>0.00</td>
<td>2</td>
<td>-2.519</td>
<td>11.4</td>
<td>0.00</td>
<td>0.681</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecundity ~FlowPC2</td>
<td>0.38</td>
<td>3</td>
<td>-0.625</td>
<td>13.3</td>
<td>1.81</td>
<td>0.275</td>
<td>0</td>
<td></td>
<td></td>
<td>0.614</td>
</tr>
<tr>
<td>Fecundity ~FlowPC1</td>
<td>0.00</td>
<td>3</td>
<td>-2.514</td>
<td>17.0</td>
<td>5.59</td>
<td>0.042</td>
<td>0</td>
<td></td>
<td></td>
<td>0.033</td>
</tr>
<tr>
<td>Fecundity ~FlowPC1+2</td>
<td>0.38</td>
<td>4</td>
<td>-0.618</td>
<td>22.6</td>
<td>11.13</td>
<td>0.003</td>
<td>0</td>
<td></td>
<td></td>
<td>0.033</td>
</tr>
<tr>
<td>Egg size (intercept)</td>
<td>0.00</td>
<td>2</td>
<td>10.650</td>
<td>-14.9</td>
<td>0.00</td>
<td>0.661</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg size ~FlowPC1</td>
<td>0.37</td>
<td>3</td>
<td>12.513</td>
<td>-13.0</td>
<td>1.87</td>
<td>0.259</td>
<td>0</td>
<td></td>
<td></td>
<td>0.610</td>
</tr>
<tr>
<td>Egg size ~FlowPC2</td>
<td>0.14</td>
<td>3</td>
<td>11.241</td>
<td>-10.5</td>
<td>4.42</td>
<td>0.073</td>
<td>0</td>
<td></td>
<td></td>
<td>-0.371</td>
</tr>
<tr>
<td>Egg size ~FlowPC1+2</td>
<td>0.51</td>
<td>4</td>
<td>13.501</td>
<td>-5.7</td>
<td>9.23</td>
<td>0.007</td>
<td>0</td>
<td></td>
<td></td>
<td>0.610</td>
</tr>
<tr>
<td>MinSLmat (intercept)</td>
<td>0.00</td>
<td>2</td>
<td>-13.836</td>
<td>-21.3</td>
<td>0.00</td>
<td>0.511</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MinSLmat ~FlowPC2</td>
<td>0.49</td>
<td>3</td>
<td>16.510</td>
<td>-21.0</td>
<td>0.25</td>
<td>0.450</td>
<td>0</td>
<td></td>
<td></td>
<td>0.698</td>
</tr>
<tr>
<td>MinSLmat ~FlowPC1</td>
<td>0.02</td>
<td>3</td>
<td>13.916</td>
<td>-15.8</td>
<td>5.44</td>
<td>0.034</td>
<td>0</td>
<td></td>
<td></td>
<td>0.141</td>
</tr>
<tr>
<td>MinSLmat ~FlowPC1+2</td>
<td>0.51</td>
<td>4</td>
<td>16.667</td>
<td>-12.0</td>
<td>9.27</td>
<td>0.005</td>
<td>0</td>
<td></td>
<td></td>
<td>0.141</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. The trilateral life history framework of Winemiller and Rose (1992). Modified from Winemiller (2005), and Mims and Olden (2012).

Figure 2. Map of study sites where mature (mature, ripening, or ripe) specimens of target species were found. For site codes and information, see Appendix S1.

Figure 3. Population life history strategies and flow-trait relationships for red shiner. (a) Ternary plot illustrating trade-offs in traits among populations. Axes scaled between 0 and 1 for the three traits (bottom axis: fecundity; right axis: egg size; left axis: inverse(minSLmat)). Solid circles represent study sites (see Appendix S1). (b) Scatterplots of flow PC-trait relationships. Asterisk indicates important relationship from multiple regression results (Table 2). Higher values of flow PC1 (lower CV), in left panels, were associated with larger eggs and smaller size at maturity. Higher values of flow PC2 (lower HPC, HPD, higher CV), on right panels, were associated with lower fecundity. Illustration of male red shiner copyright and used with permission of Joseph R. Tomelleri.

Figure 4. Population life history strategies and flow-trait relationships for bluntnose minnow. (a) Ternary plot illustrating trade-offs in traits among populations. Axes scaled between 0 and 1 for the three traits (bottom axis: fecundity; right axis: egg size; left axis: inverse(minSLmat)). Solid circles represent study sites (see Appendix S1). (b) Scatterplots of flow PC-trait relationships. Asterisk indicates important relationship from multiple regression results (Table 2). Higher values of flow PC1 (lower HPD, higher ConstPred), in left panels, were associated with lower
fecundity. Higher values of flow PC2 (higher CV, HPC; lower BFI), in middle panels, were associated with lower fecundity and larger egg size. Higher values of flow PC3 (higher meanQ), right panels, were associated with larger size at maturity. Illustration of male bluntnose minnow copyright and used with permission of Joseph R. Tomelleri.

Figure 5. Population life history strategies and flow-trait relationships for orangethroat darter. (a) Ternary plot illustrating trade-offs in traits among populations. Axes scaled between 0 and 1 for the three traits (bottom axis: fecundity; right axis: egg size; left axis: inverse(minSLmat)). Solid circles represent study sites (see Appendix S1). (b) Scatterplots of flow PC-trait relationships. Asterisk indicates important relationship from multiple regression results (Table 2). Higher values of flow PC1 (higher BFI, lower HPC) were associated with larger eggs. Higher values of flow PC2 (higher HPD, lower CV) were associated with higher fecundity and larger size at maturity. Illustration of male orangethroat darter (*E. spectabile pulchellum*) copyright and used with permission of Joseph R. Tomelleri.

Figure 6. Principal component plots for life history traits for (left to right) red shiner, bluntnose minnow, and orangethroat darter. We interpreted PC1 as an equilibrium (negative) to periodic (positive) axis for all species. We interpreted PC2 as an opportunistic (around zero) to periodic (positive or negative) axis for all species. Red text associated with arrows identifies trait vectors (ln-transformed, size-corrected traits). Uppercase black text identifies site scores (see Appendix S1). Fish illustrations copyright and used with permission of Joseph R. Tomelleri.
Supplementary Material

Appendix S1. Sample and gage locations and collecting information

Appendix S2. Additional environmental and other data for study sites and evaluation for inclusion in final model sets

Appendix S3. Ovary and oocyte stages and descriptions

Appendix S4. Principal components results for flow and life history trait variable reduction

Appendix S5. Data on life history trait variability among populations

Appendix S6. Comparison of results from full datasets with results after small samples removed
Population-level responses of life history traits to flow regime in three common stream fish species

Micah G. Bennett¹*, Matt R. Whiles¹, and Gregory W. Whitledge¹,²

Electronic supporting material

¹ Department of Zoology and Center for Ecology, Southern Illinois University, 1125 Lincoln Drive (Mail Code 6501), Carbondale, Illinois 62901

² Center for Fisheries, Aquaculture, and Aquatic Sciences, Southern Illinois University, 1125 Lincoln Drive (Mail Code 6511), Carbondale, Illinois 62901

*corresponding author

email: micahgbennett@yahoo.com

phone: 618-453-4125
The following Supporting Information is available for this article:

Appendix S1. Sample and gage locations and collecting information

Appendix S2. Additional environmental and other data for study sites and evaluation for inclusion in final model sets

Appendix S3. Ovary and oocyte stages and descriptions

Appendix S4. Principal components results for flow and life history trait variable reduction

Appendix S5. Data on life history trait variability among populations

Appendix S6. Comparison of results from full datasets with results after small samples removed
Appendix S1. Sample and gage location selection and collecting information.

To limit the influence of anthropogenic flow modification, potential study reaches were selected based on proximity to “reference” USGS stream gages from the GAGES (Gages for Evaluating Stream Flow) database (Falcone et al., 2010). This database contains USGS stream gages with discharge data from 1950-2007 and determines “reference” gage designations based on anthropogenic modification of stream flow in the gaged watersheds, including human population and urban/housing density and presence of dams and channelization (Falcone et al., 2010).

Gages within the Central Lowlands physiographic province were further selected to limit variability based on ecoregion while still maintaining a large number of potential gages from which to select.

Reference gages were narrowed by selecting those with comparable temporal coverage (≥15 years of flow data prior to 2013; > 40% overlap in period of flow record) and excluding gages with >10 days of missing data per year (Kennard et al., 2010; Mims and Olden, 2012).

Finally, the partitioning around medioids (PAM) procedure (“pam” command) in the package “cluster” in R (R Core Team, 2012) was used to group gages based on similarity in drainage size. PAM is a non-hierarchical clustering method that searches for representative medioids (point nearest a multivariate centroid) among observations and assigns each observation to one of these medioids, forming clusters (Borcard et al., 2011). Three groupings were best as judged by average silhouette width (degree of membership of an observation to a cluster) (Borcard et al., 2011), and the largest group was selected (n=31). Particular gage sites were then selected which spanned the range of variation in the hydrologic metrics and were likely to contain the target species based on information from fish collections (FishNet2, Illinois Natural History Survey, U. of Kansas, Ohio State U., Indiana Dept. of Environmental Management) and the Global
Biodiversity Information Facility (www.gbif.org). Final sampling locations containing the target
species were all within the Mississippi-Ohio River watershed (Figure 2; Appendix S1). Of the
sites sampled, we obtained reproductive individuals from seven populations of red shiner, eight
populations of orangethroat darter, and 14 populations of bluntnose minnow (Appendix S1).

References


evaluating natural and altered flow conditions in the conterminous United States. *Ecology*
91(6): 621 DOI: 10.1890/09-0889.1.

Kennard MJ, Mackay SJ, Pusey BJ, Olden JD, Marsh N. 2010. Quantifying uncertainty in
estimation of hydrologic metrics for ecohydrological studies. *River Research and
Applications* 26: 137–156

Mims MC, Olden JD. 2012. Life history theory predicts fish assemblage response to hydrologic
regimes. *Ecology* 93: 35–45

for Statistical Computing: Vienna, Austria.
Table S1. Species columns contain number of individuals for fecundity and egg size estimates, respectively. Mature and ripening oocytes were combined to estimate fecundity, but only ripening and ripe oocytes were used to estimate egg size when available (except for Red Shiner; see text and Appendix S3).

<table>
<thead>
<tr>
<th>Gage No</th>
<th>Code</th>
<th>Site Name</th>
<th>Drainage</th>
<th>Lat</th>
<th>Long</th>
<th>Flow Years</th>
<th>Red Shiner (fecundity; egg size)</th>
<th>Bluntnose Minnow (fecundity; egg size)</th>
<th>Orangetheat Darter (fecundity; egg size)</th>
<th>Sample Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>5556500</td>
<td>BBIL</td>
<td>Big Bureau Cr., Princeton, IL</td>
<td>Illinois R.</td>
<td>41.37</td>
<td>-89.50</td>
<td>1938-2012</td>
<td>0;0</td>
<td>15;15</td>
<td>18;29</td>
<td>2013, 2014</td>
</tr>
<tr>
<td>3340800</td>
<td>BRIN</td>
<td>Big Racoon Cr., Fincastle, IN</td>
<td>Wabash R.</td>
<td>39.81</td>
<td>-86.95</td>
<td>1958-2012</td>
<td>-</td>
<td>10;9</td>
<td>-</td>
<td>2014</td>
</tr>
<tr>
<td>3364500</td>
<td>CCIN</td>
<td>Clifty Cr., Hartsville, IN</td>
<td>E. Fk. White R.</td>
<td>39.27</td>
<td>-85.70</td>
<td>1949-2012</td>
<td>-</td>
<td>1;2</td>
<td>-</td>
<td>2014</td>
</tr>
<tr>
<td>7180500</td>
<td>CCKS</td>
<td>Cedar Cr., Cedar Point, KS</td>
<td>Cottonwood/upper Neosho R.</td>
<td>38.20</td>
<td>-96.82</td>
<td>1939-2012</td>
<td>6;5</td>
<td>8;6</td>
<td>38;17</td>
<td>2013, 2014</td>
</tr>
<tr>
<td>5444000</td>
<td>ECIL</td>
<td>Elkhorn Cr., Penrose, IL</td>
<td>Rock R.</td>
<td>41.90</td>
<td>-89.70</td>
<td>1940-2012</td>
<td>-</td>
<td>14;11</td>
<td>-</td>
<td>2013, 2014</td>
</tr>
<tr>
<td>7184000</td>
<td>LCKS</td>
<td>Lightning Cr., McCune, KS</td>
<td>Neosho R.</td>
<td>41.90</td>
<td>-89.70</td>
<td>1960-2012</td>
<td>2;2</td>
<td>2;2</td>
<td>16;20</td>
<td>2014</td>
</tr>
<tr>
<td>5317200</td>
<td>LCMN</td>
<td>Little Cottonwood R., Courtland, MN</td>
<td>Minnesota R.</td>
<td>37.28</td>
<td>-95.03</td>
<td>1974-2009</td>
<td>-</td>
<td>2;1</td>
<td>-</td>
<td>2013</td>
</tr>
<tr>
<td>5507600</td>
<td>LCMO</td>
<td>Lick Cr., Perry, MO</td>
<td>Salt R. (upper Mississippi R.)</td>
<td>44.25</td>
<td>-94.34</td>
<td>1980-2012</td>
<td>0;0</td>
<td>2;1</td>
<td>20;40</td>
<td>2014</td>
</tr>
<tr>
<td>7167500</td>
<td>OCKS</td>
<td>Otter Cr., Climax, KS</td>
<td>Fall/Verdigris R.</td>
<td>37.71</td>
<td>-96.22</td>
<td>1947-2012</td>
<td>2;2</td>
<td>6;6</td>
<td>21;11</td>
<td>2014</td>
</tr>
<tr>
<td>Site Code</td>
<td>Site</td>
<td>Site Name</td>
<td>River System</td>
<td>Date Range</td>
<td>M</td>
<td>V</td>
<td>Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
<td>-----------</td>
<td>--------------</td>
<td>------------</td>
<td>---</td>
<td>---</td>
<td>------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3327520</td>
<td>PCIN</td>
<td>Pipe Cr., Bunker Hill, IN</td>
<td>Wabash R.</td>
<td>1969-2003</td>
<td>1;1</td>
<td>-</td>
<td>2013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6803530</td>
<td>RCNE</td>
<td>Rock Cr., Ceresco, NE</td>
<td>Salt Cr./ Platte R.</td>
<td>1941-2012</td>
<td>24;5</td>
<td>-</td>
<td>2013, 2014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6889160</td>
<td>SCKS</td>
<td>Soldier Cr., Circleville, KS</td>
<td>Kansas R.</td>
<td>1965-2000</td>
<td>12;4</td>
<td>9;7</td>
<td>20;18</td>
<td>2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3272700</td>
<td>SCOH</td>
<td>Sevenmile Cr., Camden, OH</td>
<td>Great Miami R.</td>
<td>1971-2000</td>
<td>-</td>
<td>2;2</td>
<td>-</td>
<td>2013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix S2. Additional environmental and other data for study sites and evaluation for inclusion in final model sets.

Many environmental factors that can influence life history such as growing season, temperature, and climate patterns are correlated with latitude, and several studies have found significant latitudinal clines in life history traits (Leggett and Carscadden, 1978; Fleming and Gross, 1990; Johnston and Leggett, 2002). Latitude was calculated for each site from Google Earth (Google, Inc., Mountain View, CA) and included in final models for Bluntnose Minnow and Red Shiner based on significant regressions for some traits (p < 0.05).

Recent molecular data suggest that our sampling sites for Orangethroat Darter include three distinct phylogenetic clades reflective of ancient drainage configurations, glacial refugia, and dispersal events (Bossu et al., 2013): a western clade (including sites CCKS, LCKS, OCKS, SCKS) that included Etheostoma spectabile pulchellum and E. s. squamosum, which separated from populations east of Kansas approximately 4.5 Ma during the Pliocene (Bossu et al. 2013); two sister clades from the Osage and Marais des Cygnes rivers (including site MdCKS) and lower portions of middle Mississippi River and tributaries; and a clade including upper Mississippi River tributaries (including the Illinois River and presumably site BBIL in the Illinois drainage and presumably sites CCMO and LCMO in the Salt River, but this drainage was not sampled). Because life history traits could be influenced more by evolutionary history than local environmental conditions (Harvey and Pagel, 1991; Stearns, 1992), we evaluated the effect of genetic divergence on life history traits using published data on the cytochrome b (cyt b) gene (Bossu et al., 2013). We obtained 90 cyt b sequences used by Bossu et al. (2013) from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) representing all available cyt b sequences from the clades listed above. Similar to Olden and Kennard (2010), we conducted a Mantel test (phytools
package in R with 999 permutations) between raw genetic and life history distances (ln-transformed, size-corrected values of fecundity, minSLmat, and egg diameter) based on Euclidean distances calculated in the vegan package in R. Based on this test, life history differences were not significantly related to genetic divergence among populations ($R^2=0.01$, $P=0.49$).

Detailed phylogeographic information was not available for the other study species; however, because our study area encompasses mostly formerly-glaciated streams, we used pairwise stream distances as a potential corollary of genetic divergence due to dispersal limitation and isolation-by-distance. This is a feature of phylogeographic patterns in portions of the range of some minnow species (e.g., Echelle et al. 2014) but ignores the likelihood of dispersal from several glacial refugia (e.g., Berendzen et al. 2003; Berendzen et al. 2008) and resulting patterns that would contradict simple isolation-by-distance. We calculated pairwise stream distances (km) among all populations of Red Shiner and Bluntnose Minnow in ArcMap 10.2 (Network Analyst Tool) and used them in Mantel tests with trait distances among all populations calculated as with Orangethroat Darter. For Red Shiner ($R^2=0.001$, $P=0.80$) and Bluntnose Minnow ($R^2=0.005$, $P = 0.55$), stream distances did not significantly explain differences in life history traits.

References


John Wiley & Sons, Ltd


Appendix S3. Ovary and oocyte stages and descriptions\(^a\).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latent (LA)</td>
<td>Ovaries transparent-translucent and thin; maturing oocytes without visible yolk or with nucleus still visible</td>
</tr>
<tr>
<td>Early Maturing (EM)</td>
<td>Ovaries translucent to opaque and small-moderate sized; maturing oocytes are small-moderate, translucent to opaque, and with nucleus obscured by yolk</td>
</tr>
<tr>
<td>Late Maturing (LM)</td>
<td>Ovaries white to cream and small to greatly enlarged; maturing oocytes are moderate to large and white-cream or yellow</td>
</tr>
<tr>
<td>Mature (MA)</td>
<td>Ovaries cream to yellow and moderately to very enlarged; two distinct groups of oocytes present including a group of small oocytes (translucent to opaque) and a group of larger oocytes that are opaque and cream to yellow but without vitelline membrane (membrane surrounding yolk) separated from yolk</td>
</tr>
</tbody>
</table>
| Ripening (MR)       | Ovaries cream to yellow and moderately to very enlarged; two distinct groups of oocytes present including a group of small oocytes and a group of larger oocytes that are translucent or transparent with the vitelline membrane obviously separated from yolk |}

\(^a\) (Heins and Rabito 1986; Heins and Baker 1993; Ross 2013)

References


Appendix S4. Principal components results for flow and life history trait variable reduction.

Flow variable reduction

Many of the hydrologic variables were highly correlated based on PCA (Table 1; Figures 1-3). Flow variation across orangethroat darter populations was best explained by the first two PC axes (75% of the variation). We interpreted the first PC as a contrast between flow variability (high negative loadings of HPC) and predictability (high positive loadings of BFI); however, the other measure of predictability (FlowPred) also loaded strongly positively. We interpreted the second PC as a contrast between variability (high negative loadings of annCV) and both discharge and seasonality (high positive loadings of meanQ and HPD, negative loadings for ConstPred). Variables across red shiner populations were best explained by the first two PC axes (84% of variation; Appendix S4). We interpreted the first axis as a contrast between variability (high negative loadings of annCV) and predictability (high positive loadings of BFI and FlowPred). We interpreted the second axis as contrasting variability and seasonality (high negative loadings of HPC and HPD) with predictability (less negative or near zero loadings of BFI and FlowPred). Variables across bluntnose minnow populations were best explained by the first three PC axes (77% of variation; Table 1). We interpreted the first axis as contrasting seasonality from other metrics (high negative loadings of HPD, positive loadings for ConstPred); the second axis as contrasting predictability (high negative loadings of BFI and FlowPred) and variability (high positive loadings of annCV and HPC); and the third axis as a discharge axis (high positive loadings meanQ).
Table 1. Principal component loadings for flow variables. Letters after variables refer to flow regime components (see Table 1): v = variability; s = seasonality; p = predictability.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Red Shiner</th>
<th>Bluntnose Minnow</th>
<th>Orangethroat Darter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC1 (60.9%)</td>
<td>PC2 (23.3%)</td>
<td>PC1 (31.5%)</td>
</tr>
<tr>
<td>meanQ</td>
<td>-0.329</td>
<td>-0.357</td>
<td>-0.139</td>
</tr>
<tr>
<td>annCV (v)</td>
<td>-0.471</td>
<td>0.145</td>
<td>0.356</td>
</tr>
<tr>
<td>HPC (v)</td>
<td>-0.606</td>
<td>0.362</td>
<td>0.302</td>
</tr>
<tr>
<td>BFI (p)</td>
<td>0.477</td>
<td>0.250</td>
<td>-0.616</td>
</tr>
<tr>
<td>FlowPred (p)</td>
<td>0.450</td>
<td>-0.283</td>
<td>0.365</td>
</tr>
<tr>
<td>HPD (s)</td>
<td>-0.199</td>
<td>-0.629</td>
<td>-0.461</td>
</tr>
<tr>
<td>Const/Pred (s)</td>
<td>0.447</td>
<td>0.573</td>
<td>-0.113</td>
</tr>
</tbody>
</table>

Table 2. Principal component loadings for life history variables. All life history variables were size-corrected and ln-transformed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Red Shiner</th>
<th>Bluntnose Minnow</th>
<th>Orangethroat Darter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC1 (69.4%)</td>
<td>PC2 (24.5%)</td>
<td>PC1 (42.2%)</td>
</tr>
<tr>
<td>Ln(Fecundity)</td>
<td>0.45307</td>
<td>0.88082</td>
<td>0.469583</td>
</tr>
<tr>
<td>Ln(Egg Size)</td>
<td>-0.61532</td>
<td>0.420524</td>
<td>-0.68203</td>
</tr>
<tr>
<td>Ln(min SL @ maturity)</td>
<td>0.645063</td>
<td>-0.21752</td>
<td>0.560647</td>
</tr>
</tbody>
</table>
Figure 1. Flow PCA for Orangethroat Darter. Black text shows site scores. Red arrows and text highlight flow variable vectors.
Figure 2. Flow PCA for Red Shiner. Black text shows site scores. Red arrows and text highlight flow variable vectors.
Figure 3. Flow PCAs for Bluntnose Minnow. (a) Flow PC1 (x axis) versus flow PC2 (y axis); (b) Flow PC2 (x axis) versus flow PC3 (y axis). Black text shows site scores. Red arrows and text highlight flow variable vectors.
Appendix S5. Data on life history trait variability among populations.

Figure S1. Boxplots showing variation in key traits among populations of orangethroat darter. (a) un-transformed fecundity, (b) size-corrected fecundity, (c) ln-transformed egg size, (d) ln-transformed size-corrected egg size, (d) ln-transformed standard length (SL). Box represents lower and upper quartiles (25% and 75%, respectively) and black line represents median (50% quartile). Whiskers represent 1.5x the box length, with outliers shown.
Figure S2. Boxplots showing variation in key traits among populations of red shiner. (a) un-transformed fecundity, (b) size-corrected fecundity, (c) ln-transformed egg size, (d) ln-transformed size-corrected egg size, (d) ln-transformed standard length (SL). Box represents lower and upper quartiles (25% and 75%, respectively) and black line represents median (50% quartile). Whiskers represent 1.5x the box length, with outliers shown.
Figure S3. Boxplots showing variation in key traits among populations of bluntnose minnow. (a) un-transformed fecundity, (b)size-corrected fecundity, (c) ln-transformed egg size, (d) ln-transformed size-corrected egg size, (d)ln-transformed standard length (SL). Box represents lower and
upper quartiles (25% and 75%, respectively) and black line represents median (50% quartile). Whiskers represent 1.5x the box length, with outliers shown.
**Appendix S6.** Comparison of slopes of linear trait-environment regressions for full datasets (data from all sites for each species included) and for datasets in which sites with small sample sizes (<4 individuals available for estimating trait values for that population) were excluded. Variables from the top ranked models (within ΔAICc=2 of lowest AICc) and were selected for comparison of full datasets to those with small sample size sites removed. Slopes of relationships were consistent in direction and generally similar in magnitude compared to results from the full dataset except for a positive relationship between flow PC1 (negative: seasonality) and fecundity in bluntnose minnow in the dataset with small samples removed.

<table>
<thead>
<tr>
<th>Species</th>
<th>Life history trait</th>
<th>Environmental variable</th>
<th>Slope: Full dataset</th>
<th>Slope: Small removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red shiner</td>
<td>Fecundity</td>
<td>Flow PC2</td>
<td>-0.14</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td>Egg size</td>
<td>Flow PC1</td>
<td>+0.05</td>
<td>+0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Latitude</td>
<td>+0.08</td>
<td>+0.11</td>
</tr>
<tr>
<td>MinSLmat</td>
<td></td>
<td>Flow PC1</td>
<td>-0.05</td>
<td>-0.04</td>
</tr>
<tr>
<td>Bluntnose minnow</td>
<td>Fecundity</td>
<td>Flow PC1</td>
<td>-0.09</td>
<td>+0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flow PC2</td>
<td>-0.14</td>
<td>-0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Latitude</td>
<td>+0.12</td>
<td>+0.10</td>
</tr>
<tr>
<td></td>
<td>Egg size</td>
<td>Flow PC2</td>
<td>+0.02</td>
<td>+0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Latitude</td>
<td>-0.02</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>MinSLmat</td>
<td>Flow PC3</td>
<td>+0.03</td>
<td>+0.02</td>
</tr>
</tbody>
</table>
Figure 1. The trilateral life history framework of Winemiller and Rose (1992). Modified from Winemiller (2005) and Mims and Olden (2012).

Modified from Winemiller 2005, Mims & Olden 2012

Figure 1. The trilateral life history framework of Winemiller and Rose (1992). Modified from Winemiller (2005) and Mims and Olden (2012).

254x190mm (96 x 96 DPI)
Figure 2. Map of study sites where mature (mature, ripening, or ripe) specimens of target species were found. For site codes and information, see Appendix S1.

111x74mm (600 x 600 DPI)
Figure 3. Population life history strategies and flow-trait relationships for red shiner. (a) Ternary plot illustrating trade-offs in traits among populations. Axes scaled between 0 and 1 for the three traits (bottom axis: fecundity; right axis: egg size; left axis: inverse(minSLmat)). Solid circles represent study sites (see Appendix S1). (b) Scatterplots of flow PC-trait relationships. Asterisk indicates important relationship from multiple regression results (Table 2). Higher values of flow PC1 (lower CV), in left panels, were associated with larger eggs and smaller size at maturity. Higher values of flow PC2 (lower HPC, HPD, higher CV), on right panels, were associated with lower fecundity. Illustration of male red shiner copyright and used with permission of Joseph R. Tomelleri.

175x86mm (300 x 300 DPI)
Figure 4. Population life history strategies and flow-trait relationships for bluntnose minnow. (a) Ternary plot illustrating trade-offs in traits among populations. Axes scaled between 0 and 1 for the three traits (bottom axis: fecundity; right axis: egg size; left axis: inverse(minSLmat)). Solid circles represent study sites (see Appendix S1). (b) Scatterplots of flow PC-trait relationships. Asterisk indicates important relationship from multiple regression results (Table 2). Higher values of flow PC1 (lower HPD, higher ConstPred), in left panels, were associated with lower fecundity. Higher values of flow PC2 (higher CV, HPC; lower BFI), in middle panels, were associated with lower fecundity and larger egg size. Higher values of flow PC3 (higher meanQ), right panels, were associated with larger size at maturity. Illustration of male bluntnose minnow copyright and used with permission of Joseph R. Tomelleri.

156x69mm (300 x 300 DPI)
Figure 5. Population life history strategies and flow-trait relationships for orangethroat darter. (a) Ternary plot illustrating trade-offs in traits among populations. Axes scaled between 0 and 1 for the three traits (bottom axis: fecundity; right axis: egg size; left axis: inverse(minSLmat)). Solid circles represent study sites (see Appendix S1). (b) Scatterplots of flow PC-trait relationships. Asterisk indicates important relationship from multiple regression results (Table 2). Higher values of flow PC1 (higher BFI, lower HPC) were associated with larger eggs. Higher values of flow PC2 (higher HPD, lower CV) were associated with higher fecundity and larger size at maturity. Illustration of male orangethroat darter (E. spectabile pulchellum) copyright and used with permission of Joseph R. Tomelleri.

166x79mm (300 x 300 DPI)
Figure 6. Principal component plots for life history traits for (left to right) red shiner, bluntnose minnow, and orangethroat darter. We interpreted PC1 as an equilibrium (negative) to periodic (positive) axis for all species. We interpreted PC2 as an opportunistic (around zero) to periodic (positive or negative) axis for all species. Red text associated with arrows identifies trait vectors (ln-transformed, size-corrected traits). Uppercase black text identifies site scores (see Appendix S1). Fish illustrations copyright and used with permission of Joseph R. Tomelleri. 177x79mm (300 x 300 DPI)