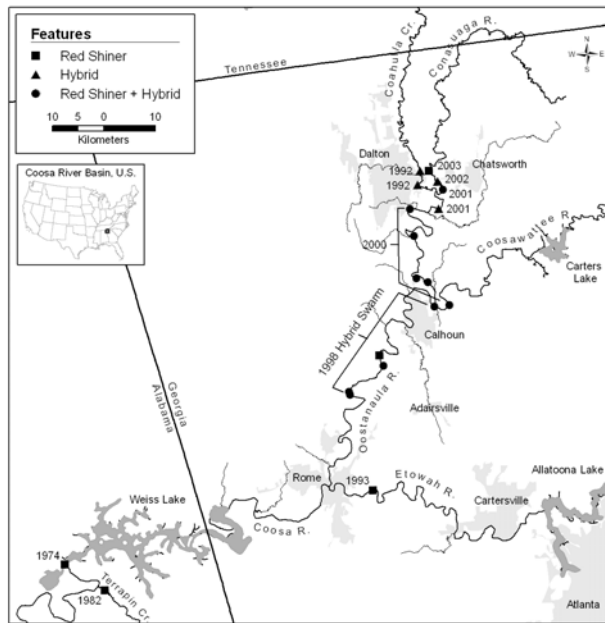


# Red Shiner Invasion of the Upper Coosa River System: Dynamics and Ecological Consequences



blacktail



hybrid



red



RESEARCH AND DEVELOPMENT

# **Red Shiner Invasion of the Upper Coosa River System: Dynamics and Ecological Consequences**

David M. Walters and Michael J. Blum

U.S. EPA Office of Research and Development

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Office of Research and Development  
National Exposure Research Laboratory  
26 West Martin Luther King Jr. Blvd.  
Cincinnati, OH 45268

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## **Executive Summary:**

**Background:** The red shiner (*Cyprinella lutrensis*) has been widely introduced across 11 states outside its native range, presumably through bait-bucket and aquarium releases. Its native range includes Great Plain and Central Lowland tributaries of the Mississippi River and western Coastal Plain drainages of the Rio Grande River. This species thrives under harsh conditions (e.g., low flow, high turbidity, poor water quality) and aggressively colonizes severely degraded habitats. Introduced populations spread rapidly, often displacing native Cyprinids. Red shiners readily hybridize with congeners, sometimes causing widespread displacement of native species. Hybridization is a significant threat to Southeastern *Cyprinella*, as red shiner hybrids have been reported for nine native taxa. NERL scientists, in collaboration with researchers at U.S. Geological Survey (USGS), The University of Georgia, and Duquesne University, conducted three related studies on the dynamics of red shiner invasion and hybridization with native blacktail shiners, *C. venusta stigmatura*, in the upper Coosa River System (UCRS). The overall goals of these studies were to identify environmental drivers of red shiner invasion and to determine genetic and environmental factors promoting hybridization.

**Key Findings:** The first study investigated the role of anthropogenic disturbance in red shiner invasion and hybridization with blacktail shiners in tributary streams draining to mainstem rivers of the UCRS. Human disturbance increases the invasibility of lotic ecosystems and the likelihood of hybridization between invasive and native species. Historical collection records indicated that red shiners and hybrids have rapidly (up to 31 km y<sup>-1</sup>) dispersed in the UCRS via large, mainstem rivers since the mid to late 1990s. We measured the occurrence and abundance of parental species and hybrids near tributary-mainstem confluences and characterized populations at these incipient contact zones by examining variation across morphological traits and molecular markers. Red shiners represented only 1.2% of total catch in tributaries yet introgression was widespread with hybrids accounting for 34% of total catch. Occurrence of red shiners and hybrids was highly correlated with occurrence of blacktail shiners, indicating that streams with native populations are preferentially colonized early in the invasion and that hybridization plays a key role in the establishment and expansion of invasive red shiners and their genome into new habitats. Tributary invasion was driven primarily through advanced backcross (post F<sub>1</sub>) individuals exhibiting asymmetry (in genetic and morphological traits) favoring blacktail shiner. Occurrence of red

shiners and hybrids and the relative abundance of hybrids all significantly increased with measures of human disturbance including turbidity, catchment agricultural land use, and low dissolved oxygen concentration. Red shiners pose a serious threat to southeastern *Cyprinella* species diversity, given that 41% of these species hybridize with red shiner, that five major Southeastern River drainages have been invaded, and that southeastern river systems are increasingly disturbed by urbanization.

A second study focused on population dynamics and genetics of hybrid swarms in the mainstem UCRS in Georgia and Alabama (Conasauga River downstream to Weiss Lake) and the Terrapin Creek/Dead River system in Alabama. A detailed report is in preparation, but preliminary analyses revealed two key results. First, the hybrid swarm is comprised primarily of later generation (post- $F_1$ ) hybrids.  $F_1$  hybrids were rarely collected in sample populations suggesting that interactions between parental red shiner and blacktail shiner are infrequent. In contrast, advanced generation hybrids ( $F_2$  and backcross generations) were common, indicating that hybrids are viable and fertile. Second, the large-scale distribution of parent species and hybrids fit the classic tension zone model (a spatial model of species' distributions describing a clinal transition from species *A* through a hybrid zone to species *B*). Populations in the southern UCRS (near Weiss Lake) had high relative abundance of red shiners, intermediate river reaches of the UCRS (Oostanuala River and lower Conasauga River) were dominated by hybrids, and upstream populations (upper Conasauga River) were dominated by blacktail shiners. At a finer scale, patterns of hybridization depart from the traditional tension zone model due to the dendritic structure of river-stream networks. Data from the Dead River/Terrapin Creek system revealed that (1) hotspots of hybridization occur at mainstem-tributary confluences, and that (2) hybridization markedly attenuates in tributaries above confluences.

The third study was a laboratory experiment to characterize prezygotic and postzygotic reproductive isolating mechanisms and to measure fitness among parent taxa (red and blacktail shiners) and their hybrid progeny. This information may enable timely management that aids or reinforces pre- and postzygotic isolation to prevent hybridization and curtail the loss of native congeners. We conducted conservative no-choice laboratory trials to measure mating preferences, and raised broods generated from intra- and interspecific crosses to assay hybrid viability through early

juvenile development. Females of both species were significantly more responsive to conspecific versus heterospecific mates, although blacktail shiner females responded more often to heterospecific mates than did red shiner females. Heterospecific crosses resulted in lower fertilization and egg hatching rates, but we found no other evidence of inviability. Rather, we found comparatively low larval mortality of hybrids, which is suggestive of heterosis. Considering prior studies that have linked high water turbidity to the formation of *C. lutrensis* x *C. venusta* hybrid swarms, a prescription of water quality improvement with a focus on reducing turbidity could reinstate sexual isolation and prevent further hybridization between introduced red shiner and endemic blacktail shiner in the Coosa River basin.

Results of these studies provide an integrated picture of the processes driving red shiner invasion in the UCRS that may be applicable to other southeastern rivers. At the basin scale, large mainstem rivers serve as key corridors of dispersal for red shiners and their hybrid progeny. Tributary streams (particularly those with native populations) are subsequently invaded, further dispersing the red shiner genome into the drainage network. Anthropogenic stressors (e.g., detrimental land use practices, elevated turbidity) exacerbate the problem, increasing the vulnerability of streams to invasion and the vulnerability of native populations to hybridization. Effects on native populations are widespread and rapid. Assuming our reconstructed timeline of invasion is reasonable, introgressive hybridization has compromised native populations in hundreds of kilometers of lotic habitats over the course of a single decade. Evidence from tributary and mainstem populations indicates that post-F<sub>1</sub> hybrids are driving the invasion. Most of these hybrids are physically indistinguishable from parental blacktail shiners, complicating efforts to monitor the impact of red shiner on native populations or to assess the efficacy of mitigation efforts. The prevalence of post-F<sub>1</sub> hybrids is indicative of backcrossing, suggesting that hybrids are both viable and fertile. This hypothesis was strongly supported by laboratory spawning experiments showing that heterospecific crosses were commonly successful and that F<sub>1</sub> hybrids demonstrated vigor relative to parental species.

## CHAPTER 1.

Red shiner invasion and hybridization with blacktail shiner in the upper Coosa River,  
USA

D.M. Walters<sup>1\*</sup>, M.J. Blum<sup>1,2</sup>, B. Rashleigh<sup>3</sup>, B.J. Freeman<sup>4</sup>, B.A. Porter<sup>5</sup>, and N.M.  
Burkhead<sup>6</sup>

<sup>1</sup> U.S Environmental Protection Agency, National Exposure Research Laboratory, 26  
West MLK Blvd. Cincinnati, OH. 45268

<sup>2</sup> Department of Ecology and Evolutionary Biology, Tulane University New Orleans, LA  
70118

<sup>3</sup> U.S. Environmental Protection Agency, National Exposure Research Laboratory, 960  
College Station Road, Athens, GA 30605

<sup>4</sup> Georgia Museum of Natural History and Odum School of Ecology, University of  
Georgia, Athens, GA 30602

<sup>5</sup> Duquesne University, Department of Biological Sciences, 600 Forbes Avenue,  
Pittsburgh, PA 15282

<sup>6</sup> U.S. Geological Survey, Florida Integrated Science Center, 7920 NW 71st Street  
Gainesville, Florida 32653

\*corresponding author: [walters.davidm@epa.gov](mailto:walters.davidm@epa.gov); 513-569-7302

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**Abstract:** Human disturbance increases the invasibility of lotic ecosystems and the likelihood of hybridization between invasive and native species. We investigated the role of disturbance in the invasion of red shiner (*Cyprinella lutrensis*) and their hybridization with native blacktail shiner (*C. venusta stigmatura*) in the upper Coosa River System (UCRS). Historical collection records indicated that red shiners and hybrids have rapidly (up to 31 km y<sup>-1</sup>) dispersed in the UCRS via large, mainstem rivers since the mid to late 1990s. We measured the occurrence and abundance of parental species and hybrids near tributary-mainstem confluences and characterized populations at these incipient contact zones by examining variation across morphological traits and molecular markers. Red shiners represented only 1.2% of total catch in tributaries yet introgression was widespread with hybrids accounting for 34% of total catch. Occurrence of red shiners and hybrids was highly correlated with occurrence of blacktail shiners, indicating that streams with native populations are preferentially colonized early in the invasion and that hybridization plays a key role in the establishment and expansion of invasive red shiners and their genome into new habitats. Tributary invasion was driven primarily through advanced backcross (post F<sub>1</sub>) individuals exhibiting asymmetry (in genetic and morphological traits) favoring blacktail shiner. Occurrence of red shiners and hybrids and the relative abundance of hybrids all significantly increased with measures of human disturbance including turbidity, catchment agricultural land use, and low dissolved oxygen concentration. Red shiners pose a serious threat to southeastern *Cyprinella* species diversity, given that 41% of these species hybridize with red shiner, that five major Southeastern River drainages have been invaded, and that southeastern river systems are increasingly disturbed by urbanization.



## INTRODUCTION

Invasive species are a primary threat to aquatic biodiversity (Allan & Flecker 1993; Richter et al. 1997). Habitat disturbance facilitates fish invasion in lotic ecosystems (Gido & Brown 1999; Marchetti et al. 2004; Moyle & Light 1996) and increases the likelihood of hybridization between fishes (see studies reviewed in Rhymer & Simberloff 1996). Hybridization is a serious and under-appreciated aspect of species invasion, and can lead to genetic extinction of native species or the loss of locally adapted gene complexes (Hitt et al. 2003; Rhymer & Simberloff 1996). Hybridization can have the perverse effect of enhancing invasion success (Allendorf et al. 2001; Ellstrand & Schierenbeck 2000; Hitt et al. 2003; Rhymer & Simberloff 1996) by mitigating the constraint of propagule pressure, a key factor in successful colonization by invasive fishes and other species (Kolar & Lodge 2001; Ruesink 2005).

Red shiners, *Cyprinella lutrensis*, have been introduced into at least five Southeastern drainages since the 1970s, presumably through bait-bucket or aquarium releases (Fuller et al. 1999). Red shiners thrive under harsh conditions (e.g., low flow, high turbidity, poor water quality) and aggressively colonize severely degraded habitats (Cross & Cavin 1971; Matthews 1985; Matthews & Hill 1977, 1979). Introduced populations spread rapidly, often displacing native congeners and other Cyprinids (Greger & Deacon 1988; Minckley & Deacon 1968; Moyle 2002). They readily hybridize with congeners, sometimes causing widespread displacement of native species (Larimore & Bayley 1996; Page & Smith 1970). Hybridization is a significant threat to Southeastern *Cyprinella* diversity, as red shiner hybrids have been reported for nine native species and subspecies (DeVivo 1996; Hubbs & Strawn 1956; Johnson 1999, W.C.

Starnes personal communication, Burkhead unpublished data; Page & Smith 1970; Wallace & Ramsey 1982).

Prior studies have hypothesized that habitat disturbance increases both the likelihood of red shiner colonization and the likelihood of hybridization with congeners (Hubbs et al. 1953; Larimore & Bayley 1996; Page & Smith 1970). Examples of degraded lotic habitats colonized by red shiners include drainage ditches and severely altered agricultural and urban streams (DeVivo 1996; Moyle 2002; Page & Smith 1970). Likewise, red shiner hybrid swarms in Texas and Illinois were attributed to poor water quality and high turbidity (Hubbs et al. 1953; Hubbs & Strawn 1956; Larimore & Bayley 1996; Page & Smith 1970). However, these prior accounts of habitat disturbance effects on red shiner invasions were observational, and empirical tests of these disturbance hypotheses are lacking.

We investigated red shiner colonization and hybridization with native blacktail shiner, *C. venusta stigmatura*, in the upper Coosa River System (UCRS, Georgia, Alabama and Tennessee). We have observed rapid dispersal of red shiners and hybridization with blacktail shiners in mainstem rivers of the UCRS since the 1990s. Along with establishing a timeline of UCRS invasion, we measured the occurrence and abundance of parental species and hybrids near tributary-mainstem confluences. We then characterized the genetic composition of populations at these incipient contact zones by examining variation across morphological traits and molecular markers. This approach enabled us to determine whether the occurrence of native congeners inhibits (e.g., via competition) or enhances (e.g., via hybridization) dispersal of red shiners into new

habitats. Finally, we tested the hypotheses that disturbance (assessed at the basin and reach scales) promotes red shiner dispersal and hybridization with congeners.

## **METHODS**

### **Invasion History and Site Selection**

We used an extensive database of fish distributions maintained by the Georgia Museum of Natural History (GMNH) to develop a chronology of red shiner and *C. lutrensis* x *C. venusta stigmatura* hybrids (hereafter hybrids) dispersal in the Upper Coosa River system (UCRS). All specimens, including putative hybrids, were identified on the basis of morphological characters (Boschung & Mayden 2003).

Collection records indicated that red shiners or hybrids were distributed in mainstem rivers of the UCRS including Coosa River upstream of Weiss Lake, Oostanaula River, Conasauga River upstream to Dalton, GA, and downstream reaches of the Etowah and Coosawattee rivers (Figure 1). The sample population of tributaries entering these mainstem rivers included 43 second, third, and fourth order streams. Sample reaches within streams were located at the first or second most downstream road crossing, since reaches closer to mainstem populations are most likely to be colonized first. In the instances where neither location was suitable for sampling (e.g., too deep for wading), the stream was excluded. Due to these constraints, only 33 of the 43 available sites were sampled.

### **Collection and characterization of fishes**

Reach-length for fish sampling was scaled to 25X stream width, and all reaches included both riffle and pool habitats. Representative habitats were sampled using a total of 30 kick-sets and/or seine hauls along with a backpack electrofisher. Samples were

collected between June and August 2005, and all *Cyprinella* were anesthetized and preserved in 95% ethanol for genetic analyses.

### **Morphological Characterization**

We characterized all individuals  $\geq 30$ mm standard length (SL) using three traits that differentiate red from blacktail shiners (Boschung & Mayden 2003): 1) caudal spot intensity, 2) the number of lateral line scales, and 3) the ratio of body standard length to depth (length:depth). The caudal spot is large and intense in blacktail shiners but absent in red shiners. Blacktail shiners have more lateral line scales (36-48 versus 33-36 for red shiners), and a higher length:depth ratio. Caudal spot intensity was assessed on a phenetic scale scored from zero (absent) to two (intense), with a faint or muted spot scored as a one. Specimens classified as hybrids exhibited obvious morphological intermediacy or incongruency (i.e., morphological traits of both parental species). For example, an incongruent specimen had a high lateral line scale count and length:depth ratio (blacktail shiner traits), but lacked a caudal spot (red shiner trait). We did not assess morphology of individuals  $< 30$ mm SL ( $n = 10$ , 2.4% total catch) due to the difficulty of counting scales on juveniles.

### **Genetic Characterization**

*DNA extraction and mtDNA RFLP assay:* Genomic DNA was extracted from  $\sim 0.05$  g of preserved fin tissue from each specimen using DNeasy kits (Qiagen, Inc., Valencia, CA). Approximately 10-50 ng of DNA was then used as template for 15  $\mu$ l polymerase chain reaction (PCR) mixtures that also included 2.5 mM  $MgCl_2$ , 2.5 mM each dNTP, 0.5 units *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), 0.5  $\mu$ M each of a pair of oligonucleotide primers and PCR buffer (Invitrogen, Carlsbad, CA) to a final 1X

concentration. The complete cytochrome *b* gene (1140bp) was amplified with primers *HA* and *LA* as described in Schmidt et al. (1998) under a thermal regime of 35 cycles of 94°C for 30 seconds, 49°C for 30 seconds, and 72°C for 90 seconds, followed by a final extension stage at 72°C for 5 minutes with a MJ Dyad thermocycler (MJ Research, Inc., Waltham, MA).

Prior to PCR amplification of the cytochrome *b* gene from specimens in this study, we examined cytochrome *b* sequence variation in each species from an alignment of sequences previously obtained from 34 blacktail shiners from the upper Conasauga River (TN), Raccoon Creek (Etowah River, GA) and Sipsey Creek (Black Warrior River, AL) as well as sequences from 26 red shiners from Peachtree Creek (Chattahoochee River, GA), and from the Canadian River (OK) (Blum et al., unpublished data). These data revealed that *HinfI* restriction sites of cytochrome *b* amplified from blacktail shiners generate ~ 130bp, 480bp and 530bp fragments versus ~ 95bp, 130bp, 350bp and 570bp fragments from red shiners. These differences formed the basis for a PCR-RFLP approach to establish species-level mtDNA ancestry of specimens in this study. The PCR-RFLP approach involved *HinfI* restriction digestion of each cytochrome *b* PCR amplicon as recommended by the enzyme manufacturer and scoring fragment size profiles by agarose gel electrophoresis of the restricted amplicons.

*Microsatellite PCR amplification and analysis:* Individuals were genotyped at seven polymorphic microsatellite markers developed for other target species. We used the following loci (modified annealing temperature given in parentheses): Can6EPA (53°C) developed for *Camptostoma anomalum* (Dimsoski et al. 2000); *Nme* 25C8.208 (55°C), *Nme* 18C2.178 (52°C), *Nme* 24B6.191 (57°C), and *Nme* 24B6.211 (57°C) developed for

*Notropis mekistocholas* (Burrige & Gold 2003); and Rhca20 (54°C ) and Rhca24 (52°C ) developed for *Rhinichthys cataractae* (Girard & Angers 2006). PCR mixtures for amplifying microsatellite loci were identical to those designed for amplifying the cytochrome *b* gene. The PCR regime for all loci was 25 cycles of 60 seconds at 95°C, 60 seconds at the locus-specific annealing temperature, and 90 seconds at 72°C, followed by a final extension stage of 7 minutes at 72°C. All reactions were run on an MJ Research Dyad with fluorescently labeled forward primers. Labeled PCR amplicons were characterized using a MJ Research Basestation Genetic Analyzer and Cartographer© software.

Microsatellite allelic variation was analyzed using Structure v2.2 (Falush et al. 2007) to construct a multi-locus admixture profile for all specimens. Few putative *C. lutrensis* specimens were collected, so admixture profiles were based on an expanded dataset that included additional “learning samples” (Montana & Pritchard 2004) of 50 *C. lutrensis* from Proctor Creek (Chattahoochee River) and genotyped for a related study (Blum et al. unpublished data). After several intermediate-length trial runs, we chose a burn-in period of 30,000 iterations and collected data from an additional 10<sup>6</sup> iterations for five replicate runs where *K* (the number of populations) was set at two, which is representative of the two parental species potentially contributing to the ancestry of each specimen. Each run was parameterized following a model of admixture and correlated allele frequencies, and average assignment values to each cluster were subsequently calculated for all specimens. Admixture categories reflected average assignment values to the first cluster as follows: (i) red shiner for values 0.90-1.0; (ii) backcross to red shiner ( $H_r$ ) for values 0.76-0.89; (iii)  $F_2$  hybrids for values of 0.26-0.39 and 0.61-0.75;

(iv) F1 hybrids for values 0.40-0.6; (v) backcrosses to blacktail shiner ( $H_{bt}$ ) for values 0.11-0.25; and (vi) blacktail shiner for values 0-0.10.

### **Integrative characterization of specimens**

Summary determinations of mixed ancestry for individuals  $\geq 30$ mm SL reflected incongruency or intermediacy of an individual's morphology, mtDNA haplotype and microsatellite genotype. Characterization of individuals  $<30$ mm SL was restricted to comparison of mtDNA haplotype and nuclear genotype. Comparison of phenotypic variation to mtDNA haplotype and nuclear genotype enabled us to differentiate 14 hybrid categories from red to blacktail shiner (e.g., Costedoat et al. 2007). For example, individuals were classified as hybrid if they exhibited blacktail shiner morphology and multi-locus microsatellite genotype, but expressed a red shiner mtDNA haplotype. Similarly, hybrid classification extended to individuals with a hybrid phenotype, but with blacktail mtDNA profile and microsatellite genotype. Hybrids were categorized as either putative  $F_1$  hybrids, or later generation hybrids with asymmetry favoring red shiner (i.e., red shiner backcross) or blacktail shiner traits.

### **Environmental Characterization**

*Water Quality and Geomorphology:* Environmental variables and summary values are provided in Table 1. Baseflow turbidity was measured on three occasions using a hand-held turbidity meter (Hach 2100P). Baseflow conditions were met if no rain was observed in the region for the preceding 72 hours and if USGS gauges on nearby streams indicated stable, low flow. Turbidity measurements were made at least two weeks apart from late May through early October, 2005, which overlaps with the spawning season for

red and blacktail shiner. Conductivity, pH and dissolved oxygen concentration were measured once per site using a Hydrolab Datasonde 4a.

Depth, velocity, particle size, and large woody debris (LWD, >10 cm diameter) were measured along the centerline of the stream at intervals equal to 0.25 times the channel width (Walters et al. 2003b). The centerline transect length was 25X channel width, with n=100 observations per site. Mean width was calculated from five randomly selected locations within the first 100 m of the reach. Velocity was measured at 60% depth using a top-setting wading rod and velocity meter (Marsh-McBirney Flo-Mate). Maximum velocity was also measured in the area of highest flow observed within the reach. Substrate particle size was determined by visually estimating the dominant particle size class in a 50 cm diameter patch at each sample point (Walters et al. 2003b). Size classes were based on the *phi* scale (Gordon et al. 1992) and values were recorded as whole *phi* intervals ( $-\log_2$  of intermediate axis in mm). These size classes were used to calculate the percentage of major substrate types (e.g., sand) for the reach. Each sampling point was visually estimated as either erosional (as in riffles) or depositional (as in pools) habitat. The presence of LWD in the cross section of the stream perpendicular to the centerline at the sampling point was also noted. Percentage open canopy was measured using a spherical densiometer at every 20th sampling location. Stream gradient (average gradient projected through the tops of riffles in the reach) was measured using an electronic total station.

### **Spatial Analysis**

Land use for the basin upstream of sites was calculated from a 1998, 18-class land-use layer (NARSAL 2005a). Classifications were further grouped into 6 classes:



open water, urban (including high and low intensity urban and transportation areas), forest (including evergreen, deciduous and mixed forests), cleared land (including clear cuts, bare rock and quarries), agriculture (including pasture and row crops), and wetlands (including forested and non-forested wetlands). Only the three dominant land use types, urban, forest, and agriculture, were considered for analyses. For basins that extended beyond the Georgia border, data were patched in from the 1992 National Land Cover Dataset (Multi-Resolution Land Characteristics Consortium 1992). Percent of impervious surface area (ISA) in a 1 km radius surrounding the sampling location was also measured to provide an indicator of local land use conditions. ISA was calculated from 2001 color infrared photos with 1m resolution (NARSAL 2005b). Basin area and distance from the sample reach to mainstem tributaries were calculated using ArcMap 9.0 (ESRI, Redlands, CA).

### **Statistical Analysis of Distributional Patterns**

Exploratory analysis was conducted to prepare data for regression analysis. We used SAS (SAS Institute, Cary, NC) to examine variables for normality and skewness prior to regression analysis, and non-normal variables were transformed accordingly (Table 1). Boxplots were used to identify extreme outliers (>3 interquartile ranges away from either the sample 25th or 75th percentiles) (Jongman et al. 1995). Three variables (pH, CV-V, IA) had extreme outliers and were excluded from analyses. We calculated Spearman correlations among variables and retained only those correlated at  $|\rho| < 0.80$  in order to reduce multicollinearity (Glantz & Slinker 1990). Drainage area (DA) and width (W) were highly correlated ( $\rho = 0.89$ ), and W was retained. Particle size (PS) was highly correlated with percent sand (SAND,  $\rho = -0.85$ ), and SAND was retained.

We used the remaining 20 environmental variables to develop multiple logistic regression models predicting the probability of occurrence for red shiner, blacktail shiner, and hybrids. We considered all models containing  $\leq 3$  variables to avoid overfitting models relative to sample size (Burnham & Anderson 2002). Models were constructed using variables within each of three variable classes (landscape, reach, water quality) both alone and in combination, for a total of seven categories of models. We used Akaike's Information Criterion corrected for small sample size (AICc) to assess model goodness-of-fit, where smaller AICc indicates a more parsimonious model (Burnham & Anderson 2002). For each model  $i$ , difference ( $\Delta_i$ ) was calculated between the model's AICc and the minimum AICc value within the set. Values of  $\Delta_i < 2$  are considered the most parsimonious (Burnham & Anderson 2002). A weight ( $w_i$ ) was calculated for each model according to Burnham and Anderson (2002). The weight can be interpreted as the probability that model  $i$  is the best model within the set of models considered. We calculated the percent of correctly predicted presence, absence, and overall occurrences. In order to examine the intensity of hybridization relative to environmental variables, we used multiple linear regression to relate hybrid shiner abundance to environmental variables for the subset of sites that contained hybrids, blacktail shiners, or both ( $n=18$ ). We used the AIC approach for model selection and reported the  $r^2$  of the final model.

## **RESULTS**

### **Time-line of red shiner invasion of the Upper Coosa River System (UCRS)**

Red shiners were first collected in Weiss Lake in 1974 (Figure 1A) with additional populations collected in Terrapin Creek, a tributary of the "Dead River" arm of the Coosa River, in 1982. The earliest records upstream of Lake Weiss were two hybrids

collected in 1992 in Coahulla Creek and a single red shiner collected in 1993 from the lower Etowah River. Collections in the Oostanaula River in 1998 revealed an extensive hybrid swarm extending upstream from Lake Weiss to the confluence with the Conasauga River. The hybrid swarm rapidly dispersed into the Conasauga River between 2000-2003. We conducted annual surveys of the Conasauga River from 2000 and 2003 to map the upstream extent of the hybrid swarm and documented that it extended 31 river km upstream between August 2000 and August 2001 alone. Four fish collections were made in the Oostanaula and lower Conasauga rivers between 1993 and 1997 (Figure 1B). Blacktail shiners were collected at three of these sites, but neither red shiners nor hybrids were present, suggesting that most of the upstream dispersal of red shiners in the UCRS has occurred since the mid-to-late 1990s. A 2005 survey confirmed that a hybrid swarm currently extends from Lake Weiss north to Dalton (M. Blum and B. Porter, unpublished data).

The absence of red shiners and hybrids from localities between Coahulla Creek and Lake Weiss from 1974 to 1992 suggests the potential for separate introductions in the southern and northern parts of the UCRS. Samples collected from 1993 to 1998 from Coahulla Creek, Conasauga River, and their tributaries failed to uncover additional specimens in the northern part of the UCRS prior to the upstream expansion of the swarm from the Oostanaula River in 2000 (Figure 1B). We draw four conclusions from historical records: 1) the spread of red shiners and hybrids in the upper Coosa River system likely began from the southern part of the UCRS in the vicinity of Weiss Lake; 2) red shiners and hybrids are dispersing upstream in the system via large, mainstem rivers;

3) much of the dispersal has occurred since the mid-to-late 1990s; and 4) the rate of dispersal is high (up to 31 km y<sup>-1</sup>).

It is important to note that red shiners and hybrid were previously collected from Cane Creek, a tributary of the lower Coosa River system, in 1970 and 1971 (R.D. Suttkus, Tulane University, retired, personal communication). Cane Creek is a tributary of Lake Logan Martin, a Coosa River impoundment. Lake Logan Martin and Weiss Lake are separated by another impoundment, Lake H. Neely Henry. The occurrence of red shiners in 1970 suggests that they could have been introduced to the UCRS earlier than museum records indicate. It is unlikely that red shiners from Cane Creek traversed two dams and two reservoirs spanning 97 river km (between Cane Creek and Weiss Lake) in four years. Rather, the association of red shiners with different reservoirs suggests multiple introductions within the Coosa River system, probably through bait-bucket releases.

### **Genetic and morphological assessment of populations**

Red shiners, blacktail shiners, or hybrids were collected at 18 of 33 sites (Table 2, Figure 2). Red shiners were rarely collected, occurring at 5 sites and accounting for only 1.2% of the total catch. They only occurred at sites that contained both hybrids and blacktail shiners. Hybrids and blacktail shiners each occurred at sixteen sites. Hybrid occurrence was significantly correlated with blacktail shiner occurrence ( $\chi^2 = 18.9$ ,  $p < 0.0001$ ) with hybrids occurring at all but two of the sites occupied by blacktail shiners and vice-versa. Overall, hybrids accounted for approximately 36% of the total catch, with the vast majority exhibiting asymmetry favoring blacktail shiners ( $H_{bt}$ , Table 2).

Hybrids were well distributed among sites, with no clear trend in relative abundance with respect to upstream distance from Weiss Lake (Figure 2).

Morphological traits distinguished between red shiners, blacktail shiners and several categories of hybrids (Figure 3). Red shiners and hybrids with asymmetry favoring red shiners ( $H_r$ ) had fewer lateral line scales, lower length:depth, and a less intense or absent caudal spot. Values for all three measurements were higher for  $H_r$  individuals than red shiner, which is likely a reflection of blacktail shiner genomic contributions.  $F_1$  hybrids were intermediate for all morphological measurements, but showed more phenotypic overlap with blacktail shiners than red shiners. Blacktail shiners and hybrids showing asymmetry favoring blacktail shiners ( $H_{bt}$ ) overlapped in all morphological measurements and were indistinguishable from one another based on the selected traits.

### **Environmental regulation of occurrence and hybridization**

Overall percent correctly predicted occurrences was >75% for the best-fit models (Table 3), which is considered satisfactory model performance (Hurley 1986). Models constructed using variables from multiple environmental categories (i.e., landscape, reach geomorphology, and water quality) consistently outperformed single category models. Land use variables were generally absent from best-fit models, except for the negative relationship between hybrid occurrence and relative abundance with basin agriculture. Turbidity (NTU) was consistently selected in occurrence models that included water quality variables. However, NTU was not selected for models of hybrid relative abundance. Stream size and water velocity were consistent predictors of parental species and hybrids. All shiners were more likely to occur in larger, higher velocity streams.

The most parsimonious model for blacktail shiners showed that they were more likely to occur in wider, more turbid streams with higher velocity (Table 4). None of the confidence intervals for the model parameters contained zero. Despite the similarity of the distribution patterns of blacktail shiners and hybrids, their best-fit models differed. Hybrids were more likely to occur at sites with higher turbidity and agricultural land cover that were closer to mainstem rivers. Only the confidence intervals for the intercept parameter contained zero (Table 3). Hybrid relative abundance was positively related to agriculture and negatively related to dissolved oxygen concentration. Relative abundance was also positively associated with gradient in this model. However, the confidence interval for this parameter contained zero, so there is uncertainty about this relationship. The AIC approach identified parsimonious models for red shiner occurrence, but even the most parsimonious model had poor explanatory power and confidence intervals on all model parameters contained zero. Low power for this model was related to infrequent occurrence of red shiners (n = 5 sites).

## **DISCUSSION**

### **Dispersal of invasive red shiner**

Biological invasions generally follow a pattern of introduction, permanent establishment, and range expansion (Kolar & Lodge 2001). Prior reports of red shiner introductions compared pre- and post-introduction data collected decades apart (Moyle 2002; Page & Smith 1970), and thus provide limited information on early stages of invasion and factors contributing to establishment and spread. We identified four patterns of red shiner dispersal in the upper Coosa River system that may be generalized to other red shiner invasions in other systems with native congeners. Red shiners and

hybrids rapidly disperse (up to 31 river km  $y^{-1}$ ) via large, mainstem rivers with subsequent invasion of tributaries. Tributaries with populations of native congeners were the first to be colonized and the incipient invasion of tributary streams was driven primarily by hybrids. The strong affinity of red shiners and hybrids for streams with blacktail shiner populations suggests that hybridization plays a key role in the establishment and expansion of red shiners and their genome into new habitats, supporting prior findings that introgressive hybridization facilitates the establishment and spread of invasive species (Allendorf et al. 2001; Ellstrand & Schierenbeck 2000; Hitt et al. 2003; Rhymer & Simberloff 1996). This mechanism of dispersal is particularly relevant for invasive fishes, which are more prone to hybridization than other vertebrates (Allendorf et al. 2001; Rhymer & Simberloff 1996).

Hybridization often complicates taxonomic identification, hindering the assessment of native and introduced populations (Allendorf et al. 2001; Rhymer & Simberloff 1996). Populations in the UCRS expressed a mosaic of parental phenotypes and genotypes and are best characterized as hybrid swarms (Allendorf et al. 2001). The majority of hybrids exhibited asymmetry favoring blacktail shiners ( $H_{bt}$ ). These were morphologically indistinguishable from parental species, suggesting that the degree of hybridization could be underestimated without complementary data from multi-locus genetic analyses. Thus, our reconstructed timeline conservatively estimates the geographic extent and expansion of the hybrids, since it depended on morphological identification of specimens.

The nature of pre- and postzygotic reproductive isolation between red and blacktail shiners may influence patterns in phenotypic and genetic variation observed in

the UCRS. Artificial crosses (where eggs and sperm were mixed to produce hybrids) and backcrosses of laboratory reared hybrids to red and blacktail shiners have produced viable and fertile hybrids (Hubbs and Strawn 1956). The UCRS hybrid swarm is dominated by later generation hybrids. F<sub>1</sub> hybrids are relatively uncommon, suggesting that interactions between red and blacktail shiners are infrequent. If so, then prezygotic reproductive isolation is stronger than postzygotic isolation between red and blacktail shiners (Mendelson 2003). The predominance of H<sub>bt</sub> individuals among hybrids is also suggestive of asymmetric pre- or postzygotic barriers that promote introgression. The comparatively low abundance of red shiners likely augments asymmetric backcrossing between hybrids and blacktail shiners (Taylor and Hastings 2005). Thus, undiminished hybrid fitness and persistent backcrossing under demographic conditions that favor introgression could be driving progressive genetic assimilation of blacktail shiners with few (if any) external indications of hybridization.

### **The role of disturbance in red shiner invasion and hybridization**

Regression models supported prior hypotheses that disturbance increases colonization by red shiners and hybridization with congeners (e.g., Hubbs et al. 1953; Larimore & Bayley 1996; Page & Smith 1970) and other studies linking disturbance with fish invasion of lotic ecosystems (Gido & Brown 1999; Marchetti et al. 2004; Moyle & Light 1996). Best-fit models indicated that hybrid and red shiner occurrence increased with turbidity and agricultural land use. These findings have two limitations. First, red shiner models had low power due to small sample size, limiting their interpretation. Second, we cannot separate the role of disturbance from the presence of blacktail shiners in determining the distribution of red shiners and hybrids. That is, red shiners and



hybrids both had strong affinity for blacktail shiner streams, but blacktail shiners were also positively correlated with turbidity, an indicator of stream disturbance. Hybrid relative abundance was also positively related to disturbance, particularly lower dissolved oxygen concentration and increased agricultural land use. This suggests that rates of hybridization or hybrid survival increase with disturbance, thereby facilitating the spread of red shiner genome into native populations. Occurrence and hybrid relative abundance models using variables from multiple environmental categories (i.e., landscape, reach geomorphology, and water quality) consistently outperformed single-category models. Superior performance of these models indicate that red shiner colonization and hybridization are influenced by factors operating at multiple spatial scales and that physical habitat and water quality are both influential at the reach-scale.

We expected positive correlations between turbidity and hybrid relative abundance since turbidity has been associated with other red shiner hybrid swarms (Hubbs et al. 1953; Hubbs & Strawn 1956; Larimore & Bayley 1996). Turbidity is thought to weaken prezygotic reproductive barriers by impairing visual recognition and assortative mating among *Cyprinella* species (Page and Smith 1970, Hubbs et al. 1956). This hypothesis is consistent with other studies demonstrating that turbidity can weaken sexual selection and promote hybridization (Candolin et al. 2007; Seehausen et al. 1997). Contrary to our expectations, turbidity was not a leading predictor of hybrid relative abundance. The influence of turbidity on hybridization rates may be obscured in the UCRS because blacktail shiners appear to be moderately tolerant to elevated turbidity (i.e., blacktail shiner occurrence was positively correlated with turbidity in this study). Alternatively, other aspects of mating behavior unaffected by turbidity could

counterbalance the negative effects of turbidity on assortative mating. Controlled spawning experiments under different turbidity regimes are needed to test the hypothesis that light limitation increases hybridization between red shiners and congeners.

### **Conservation implications for Southeastern Rivers and *Cyprinella* diversity**

Hybridization with red shiners is a serious threat to Southeastern *Cyprinella* diversity. Twenty-two *Cyprinella* occur in the Southeast (Warren et al. 2000), and nine (41%) hybridize with red shiners in the wild (*C. analostana*, *C. camura*, *C. callitaenia*, *C. spiloptera*, *C. venusta cercostigma*, *C. v. stigmatura*, *C. v. venusta*, and *C. whipplei*; (Hubbs & Strawn 1956; Johnson 1999, and W.C. Starnes personal communication; Page & Smith 1970; Wallace & Ramsey 1982)) or under laboratory conditions (*C. caerulea*, Burkhead, unpublished data). Southeastern rivers colonized by red shiners include the Pee Dee, Roanoke, Mobile, Apalachicola, and Altamaha river drainages (Fuller et al. 1999), and hybrids have been documented in three of these systems (DeVivo 1996, W.C. Starnes personal communication, this study; Wallace & Ramsey 1982). Southeastern fishes are characterized by high rates of endemism and imperilment, and nine *Cyprinella* are endemic to a single southeastern river drainage (Warren et al. 2000). These species are particularly vulnerable to red shiner invasion considering their limited distribution and relatively small populations (Allendorf et al. 2001; Rhymer & Simberloff 1996).

While the blacktail shiner is not a protected species, their displacement via hybridization in the Coosa system underscores the conservation challenges facing other southeastern *Cyprinella* species. Hybridization is difficult to stop if, as our data suggest, hybrids are fertile and capable of backcrossing with parental taxa (Allendorf et al. 2001; Rhymer & Simberloff 1996). Native populations decline rapidly under these conditions

as hybrid swarms form within a few generations and the proportion of parental individuals progressively declines in successive generations (Allendorf et al. 2001). Through this process, native species are lost or relegated to isolated parts of their former range. Our data support this model of native species decline, with widespread introgression of populations in lower reaches of mainstem tributaries.

Urbanization will likely play an increasing role in red shiner invasions of Southeastern rivers. The Southeast is among the most rapidly developing regions of the U.S. (U.S. Department of Agriculture 2000), and the UCRS epitomizes development pressures facing these river systems. Human population in the UCRS increased 37% (270000 to 330000) from 1990 to 2006 with 7400 residential building permits issued from 2000-2006 in one county (Bartow) centrally located in the UCRS (U.S. Census Bureau 2007). Red shiners thrive in urban streams (DeVivo 1996) whereas endemic and other native fishes decline (Walters et al. 2003a). It is reasonable to assume that urbanization will increase vulnerability of Southeastern streams and native *Cyprinella* to red shiner invasion, barring management actions to mitigate the detrimental effects of urbanization on stream ecosystems.

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Table 1. Environmental variables collected from mainstem tributaries in the upper Coosa River system.

| acronym              | variable description             | units               | transform           | range     | mean (1SD)    |
|----------------------|----------------------------------|---------------------|---------------------|-----------|---------------|
| <b>landscape</b>     |                                  |                     |                     |           |               |
| DTMS                 | distance to mainstem (m)         | m                   | log                 | 0.2-7.8   | 2.3 (1.6)     |
| DA                   | drainage area (km <sup>2</sup> ) | km <sup>2</sup>     | log                 | 4.6-456.3 | 46.7 (80.6)   |
| URB                  | basin urban area                 | %                   | arcsine square-root | 3.1-56.1  | 15.3 (13.2)   |
| FOR                  | basin forested                   | %                   | arcsine square-root | 18.7-86.7 | 55.8 (16.3)   |
| AG                   | basin agriculture                | %                   | arcsine square-root | 2.9-67    | 21.5 (12.9)   |
| IA                   | impervious area, 1-km radius     | %                   | arcsine square-root | 0.2-26.1  | 4.1 (6.5)     |
| <b>reach habitat</b> |                                  |                     |                     |           |               |
| GR                   | gradient                         | %                   | arcsine square-root | 0.01-1.4  | 0.3 (0.3)     |
| W                    | average width                    | m                   | log                 | 2.8-12.5  | 6.4 (2.5)     |
| V                    | maximum velocity                 | m sec <sup>-1</sup> | log                 | 0.03-1.12 | 0.57 (0.26)   |
| V-m                  | mean velocity                    | m sec <sup>-1</sup> | log                 | 0-0.3     | 0.11 (0.1)    |
| D                    | average depth                    | m                   | log                 | 0.2-1     | 0.4 (0.2)     |
| PS                   | average particle size            | phi                 | log <sub>2</sub>    | 1.6-8     | 4.1 (1.3)     |
| SAND                 | proportion sand                  | %                   | arcsine square-root | 0-59      | 12.8 (15.1)   |
| GRAV                 | proportion gravel                | %                   | arcsine square-root | 4.8-96.3  | 62.5 (22.8)   |
| COBB                 | proportion cobble                | %                   | arcsine square-root | 0-74      | 9.5 (18.2)    |
| BR                   | proportion bedrock               | %                   | arcsine square-root | 0-67.9    | 15.2 (18.5)   |
| LWD                  | proportion large woody debris    | %                   | arcsine square-root | 0-66      | 13.3 (13.7)   |
| EH                   | proportion of erosional habitat  | %                   | arcsine square-root | 6-56.2    | 28.1 (14.7)   |
| CV-D                 | CV <sup>a</sup> depth            | %                   | none                | 31-99.5   | 61.2 (18.2)   |
| CV-V                 | CV velocity                      | %                   | none                | 0-612.3   | 147.2 (117.1) |
| OC                   | % open canopy                    | %                   | arcsine square-root | 1.8-72.7  | 27.1 (19.5)   |
| <b>water quality</b> |                                  |                     |                     |           |               |
| CON                  | conductivity                     | μS cm <sup>-2</sup> | log                 | 88-573    | 232.8 (84.8)  |
| NTU                  | mean turbidity                   | NTU <sup>b</sup>    | log                 | 1.4-25.1  | 6.6 (5.3)     |
| DO                   | dissolved oxygen                 | mg l <sup>-1</sup>  | log                 | 2.4-9.7   | 7.1 (1.8)     |
| pH                   | pH                               | NA                  | none                | 3.8-8.7   | 7.8 (0.8)     |

<sup>a</sup> coefficient of variation

<sup>b</sup> nephelometric turbidity units

Table 2. Percent population contribution of red shiner (RS), hybrids exhibiting asymmetry favoring red shiner ( $H_r$ ),  $F_1$  hybrids, hybrids exhibiting asymmetry favoring blacktail shiner ( $H_{bt}$ ), and blacktail shiner (BTS) collected in the study area. Sample locales are shown in Figure 2.

| Site code     | Site name         | n          | RS         | $H_r$      | $F_1$      | $H_{bt}$    | BTS         |
|---------------|-------------------|------------|------------|------------|------------|-------------|-------------|
| 1             | Coahulla Cr.      | 73         | 0          | 9.6        | 0          | 12.3        | 78.1        |
| 2             | Little Cr.        | 2          | 0          | 0          | 0          | 50          | 50          |
| 3             | Drowning Bear Cr. | 1          | 0          | 0          | 0          | 0           | 100         |
| 4             | Jobs Cr.          | 70         | 0          | 2.9        | 4.3        | 28.6        | 64.3        |
| 5             | Swamp Cr.         | 61         | 0          | 11.5       | 4.9        | 23          | 60.7        |
| 6             | Polecat Br.       | 5          | 20         | 20         | 0          | 20          | 40          |
| 7             | Town Cr.          | 47         | 4.3        | 2.1        | 6.4        | 34          | 53.2        |
| 8             | Dry Cr.           | 35         | 2.9        | 5.7        | 5.7        | 25.7        | 60          |
| 9             | Oothkalooga Cr.   | 11         | 0          | 9.1        | 9.1        | 27.3        | 54.5        |
| 10            | Snake Cr.         | 26         | 0          | 3.8        | 3.8        | 19.2        | 73.1        |
| 11            | Bow Cr.           | 6          | 16.7       | 16.7       | 0          | 0           | 66.7        |
| 12            | Rocky Cr.         | 6          | 0          | 16.7       | 33.3       | 0           | 50          |
| 13            | Johns Cr.         | 29         | 0          | 0          | 3.4        | 20.7        | 75.9        |
| 14            | Lovejoy Cr.       | 6          | 0          | 0          | 33.3       | 66.7        | 0           |
| 15            | Woodward Cr.      | 16         | 0          | 0          | 6.3        | 25          | 68.8        |
| 16            | Silver Cr.        | 12         | 0          | 0          | 0          | 8.3         | 91.7        |
| 17            | Webb Cr.          | 2          | 0          | 0          | 0          | 0           | 100         |
| 18            | Kings Cr.         | 1          | 0          | 100        | 0          | 0           | 0           |
| <b>Totals</b> |                   | <b>409</b> | <b>1.2</b> | <b>6.1</b> | <b>4.6</b> | <b>22.7</b> | <b>65.3</b> |



Table 3. Predictive habitat models for presence of blacktail shiner (BTS), hybrids, and red shiner (RS) as well as relative abundance of hybrids presented in rank order. K is the number of estimable parameters in the model; AICc is Akaike's Information Criterion corrected for small sample size (smaller is better);  $\Delta_i$  is the difference in AICc from the best-fit model; and w is the model weight (models with higher weights are more parsimonious). Explanations of predictor variables are given in Table 1. Variable categories: reach = reach habitat; wq = water quality; land = landscape.

| Model            | Predictor variables | K | AICc  | $\Delta$ AICc | w    | % Correctly predicted |         |         |
|------------------|---------------------|---|-------|---------------|------|-----------------------|---------|---------|
|                  |                     |   |       |               |      | Presence              | Absence | Overall |
| BTS presence=    |                     |   |       |               |      |                       |         |         |
| (reach+wq)       | V, W, NTU           | 4 | 29.04 | 0             | 0.19 | 81.3                  | 82.4    | 81.8    |
| (reach)          | V, V-m, COBB        | 4 | 29.37 | 0.33          | 0.19 | 75.0                  | 76.5    | 75.8    |
| (reach+land)     | W, V, DTMS          | 4 | 32.95 | 3.91          | 0.16 | 81.3                  | 82.4    | 81.8    |
| (reach+land+wq)  | W, NTU, DTMS        | 4 | 33.00 | 3.96          | 0.16 | 68.8                  | 76.5    | 72.7    |
| (land+wq)        | NTU, URB, CON       | 4 | 37.55 | 8.51          | 0.12 | 75.0                  | 82.4    | 78.8    |
| (wq)             | NTU, CON, DO        | 4 | 38.62 | 9.58          | 0.12 | 81.3                  | 70.6    | 75.8    |
| (land)           | AG, FOR, DTMS       | 4 | 50.47 | 21.43         | 0.07 | 43.8                  | 47.1    | 45.5    |
| Hybrid presence= |                     |   |       |               |      |                       |         |         |
| (land+wq)        | AG, NTU, DTMS       | 4 | 25.00 | 0             | 0.18 | 87.5                  | 88.2    | 87.9    |
| (reach+wq)       | D, NTU, CON         | 4 | 26.64 | 1.64          | 0.17 | 75.0                  | 70.6    | 72.7    |
| (reach)          | V, EH, GRAV         | 4 | 27.55 | 2.55          | 0.16 | 81.3                  | 82.4    | 81.8    |
| (reach+land+wq)  | W, NTU, DTMS        | 4 | 29.11 | 4.11          | 0.15 | 81.3                  | 88.2    | 84.8    |
| (wq)             | NTU, CON            | 3 | 32.54 | 7.54          | 0.13 | 81.3                  | 88.2    | 84.8    |
| (reach+land)     | D, W, DTMS          | 4 | 32.69 | 7.69          | 0.13 | 75.0                  | 70.6    | 72.7    |
| (land)           | URB, DTMS           | 3 | 41.06 | 16.06         | 0.08 | 75.0                  | 76.5    | 75.8    |
| RS presence=     |                     |   |       |               |      |                       |         |         |

|                  |                |   |       |       |      |      |      |      |
|------------------|----------------|---|-------|-------|------|------|------|------|
| (reach+wq)       | V-m, NTU, CV-D | 4 | 15.49 | 0     | 0.21 | 60.0 | 89.3 | 84.8 |
| (reach+land+wq)  | V-m, FOR, NTU  | 4 | 18.94 | 3.45  | 0.17 | 40.0 | 92.9 | 84.8 |
| (reach+land)     | V-m, FOR, W    | 4 | 22.67 | 7.18  | 0.14 | 60.0 | 92.9 | 87.9 |
| (reach)          | D, V-m         | 3 | 23.44 | 7.95  | 0.14 | 40.0 | 96.4 | 87.9 |
| (land+wq)        | DTMS, NTU      | 3 | 25.32 | 9.83  | 0.13 | 20.0 | 89.3 | 78.8 |
| (wq)             | NTU, DO        | 3 | 26.49 | 11.0  | 0.12 | 40.0 | 96.4 | 81.8 |
| (land)           | FOR, DTMS      | 3 | 32.27 | 16.78 | 0.09 | 0.0  | 92.9 | 78.8 |
| Hybrid abundance |                |   |       |       |      |      |      |      |
| (reach+land+wq)  | DO, AG, GR     | 4 | -5.9  | 0     | 0.17 |      |      |      |
| (reach+wq)       | GR, DO, EH,    | 4 | -4.4  | 1.5   | 0.15 |      |      |      |
| (land+wq)        | FOR, URB, DO   | 4 | -3.9  | 2.0   | 0.15 |      |      |      |
| (reach+land)     | GR, FOR, V-m   | 4 | -3.4  | 2.5   | 0.15 |      |      |      |
| (land)           | AG, FOR        | 3 | -2.4  | 3.5   | 0.14 |      |      |      |
| (reach)          | LWD, EH, GR    | 4 | -1.1  | 4.8   | 0.13 |      |      |      |
| (wq)             | DO, CON        | 3 | 1.4   | 7.3   | 0.12 |      |      |      |

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Table 4. Parameter estimates for best-fit models from Table 3. Logistic and linear regression models are based on occurrence and relative abundance, respectively.

| Logistic model | N  | Wald chi-square | P          | Parameter | Parameter estimate | Standard error | 95% Confidence Interval |       |
|----------------|----|-----------------|------------|-----------|--------------------|----------------|-------------------------|-------|
|                |    |                 |            |           |                    |                | Lower                   | Upper |
| BTS            | 33 | 8.1             | 0.0448     |           |                    |                |                         |       |
|                |    |                 |            | Intercept | -17.3              | 6.3            | -29.6                   | -5.0  |
|                |    |                 |            | NTU       | 6.6                | 3.2            | 0.3                     | 12.8  |
|                |    |                 |            | V         | 7.7                | 3.3            | 1.2                     | 14.2  |
|                |    |                 |            | W         | 10.7               | 5.1            | 0.7                     | 20.6  |
| Hybrid         | 33 | 6.9             | 0.0760     |           |                    |                |                         |       |
|                |    |                 |            | Intercept | 15.0               | 11.0           | -6.6                    | 36.5  |
|                |    |                 |            | DTMS      | -13.5              | 5.7            | -24.7                   | -2.2  |
|                |    |                 |            | AG        | 20.9               | 8.9            | 3.4                     | 38.4  |
|                |    |                 |            | NTU       | 13.7               | 5.3            | 3.3                     | 24.1  |
| RS             | 33 | 1.8             | 0.6176     |           |                    |                |                         |       |
|                |    |                 |            | Intercept | -22.3              | 19.5           | -60.7                   | 16.0  |
|                |    |                 |            | V-mean    | 140.1              | 120.7          | -96.4                   | 376.6 |
|                |    |                 |            | NTU       | 27.8               | 27.7           | -24.4                   | 82.0  |
|                |    |                 |            | CV-D      | -0.3               | 0.3            | -0.7                    | 0.3   |
| Linear model   |    | $r^2$           | F, Pr>F    |           |                    |                |                         |       |
| Hybrid         | 18 | 0.50            | 4.6 (0.02) |           |                    |                |                         |       |
|                |    |                 |            | Intercept | 0.9                | 0.5            | -0.3                    | 2.0   |
|                |    |                 |            | DO        | -1.5               | 0.5            | -2.6                    | -0.4  |
|                |    |                 |            | AG        | 0.9                | 0.4            | 0.1                     | 1.6   |
|                |    |                 |            | GR        | 0.4                | 2.9            | -5.9                    | 6.7   |

## List of Figures

Figure 1. A) Historical spread of red shiners and *C. lutrensis* x *C. venusta* hybrids in the upper Coosa River system. Key locales and dates illustrate the earliest known collections in the system and the rapid upstream migration of phenotypic red shiner and hybrids. For illustration purposes, 2001-2003 records for the Conasauga River only include the upstream-most site where red shiner or hybrids were collected. B) Extent of fish collections 1993-98 (when the hybrid swarm was first discovered) and 1998-2001 (corresponding with the period of rapid upstream dispersal). Blacktail shiners are widely distributed in 3<sup>rd</sup> order and larger streams, but red shiners and hybrids were collected at only three locales upstream of Lake Weiss prior to 1998 (see Figure 1A). No red shiners or hybrids were collected in the Coahulla Creek or Conasauga River systems from 1993 – 1998, suggesting that the red shiners and hybrids did not radiate southward from a northern introduction. Samples collected from 1998-2001 confirm that red shiners and hybrids upstream of Weiss Lake were limited to the mainstem Oostanaula and Conasauga rivers.

Figure 2. Collection locales in mainstem tributaries. Relative abundance (percent) of red shiner, blacktail shiner, and hybrids are provided in pie charts. Open circles indicate locales where both species and hybrids were absent. Site codes are in Table 2.

Figure 3. Box and whisker plots of caudal spot intensity, lateral line scales, and length-to-depth ratio of for red shiners (RS), hybrids exhibiting asymmetry favoring red shiner (H<sub>r</sub>), F<sub>1</sub> hybrids, hybrids exhibiting asymmetry favoring blacktail shiner (H<sub>bt</sub>), and

blacktail shiner (BTS) collected in the study area. Top and bottom boundaries of the box indicate 75<sup>th</sup> and 25<sup>th</sup> percentiles, respectively, and the line within the box indicates the median. Whiskers above and below the box indicate the 90<sup>th</sup> and 10<sup>th</sup> percentiles. Points outside the boxes are outliers (observations beyond the 10<sup>th</sup> and 90<sup>th</sup> percentiles).



Figure 2.

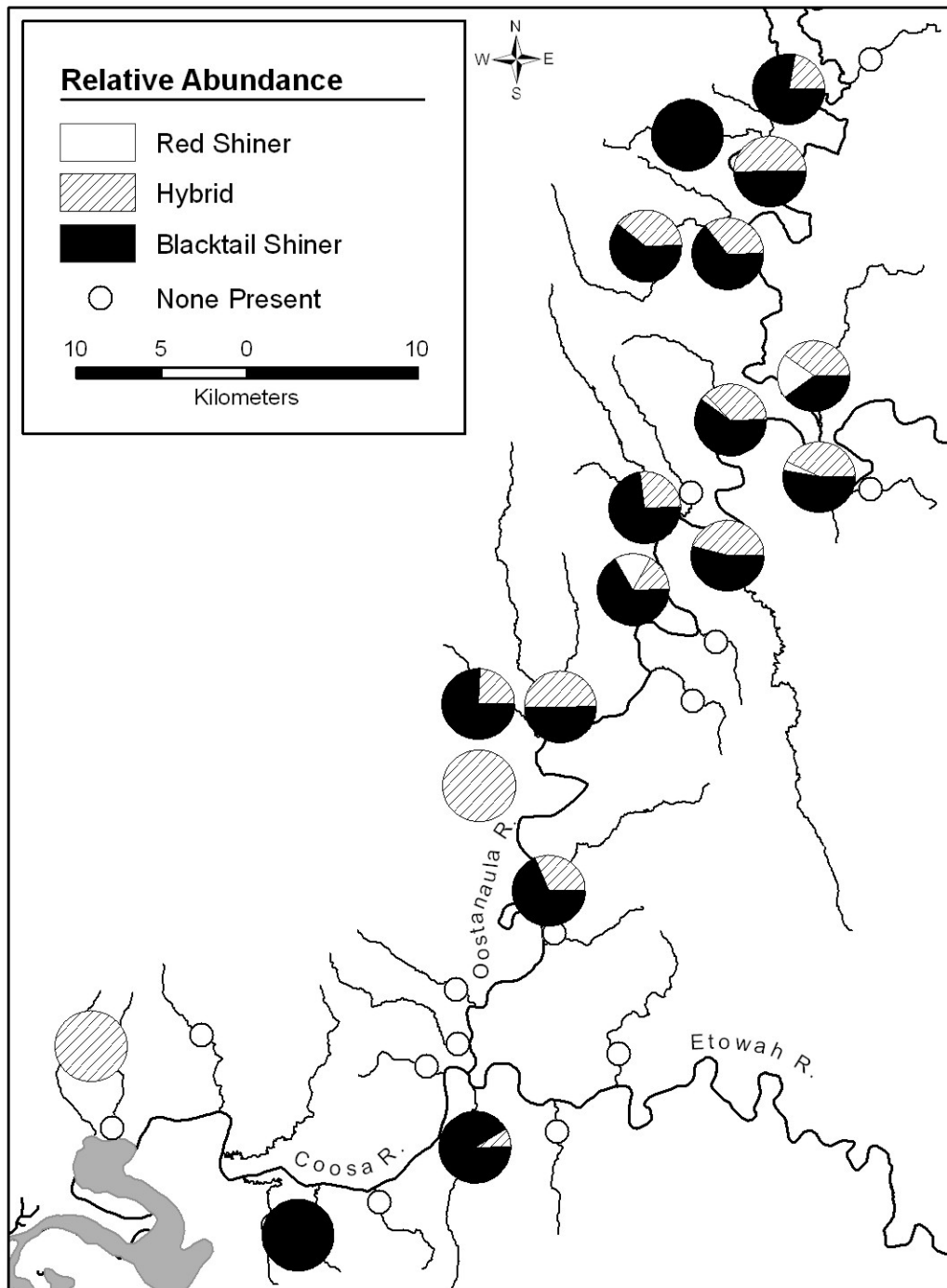
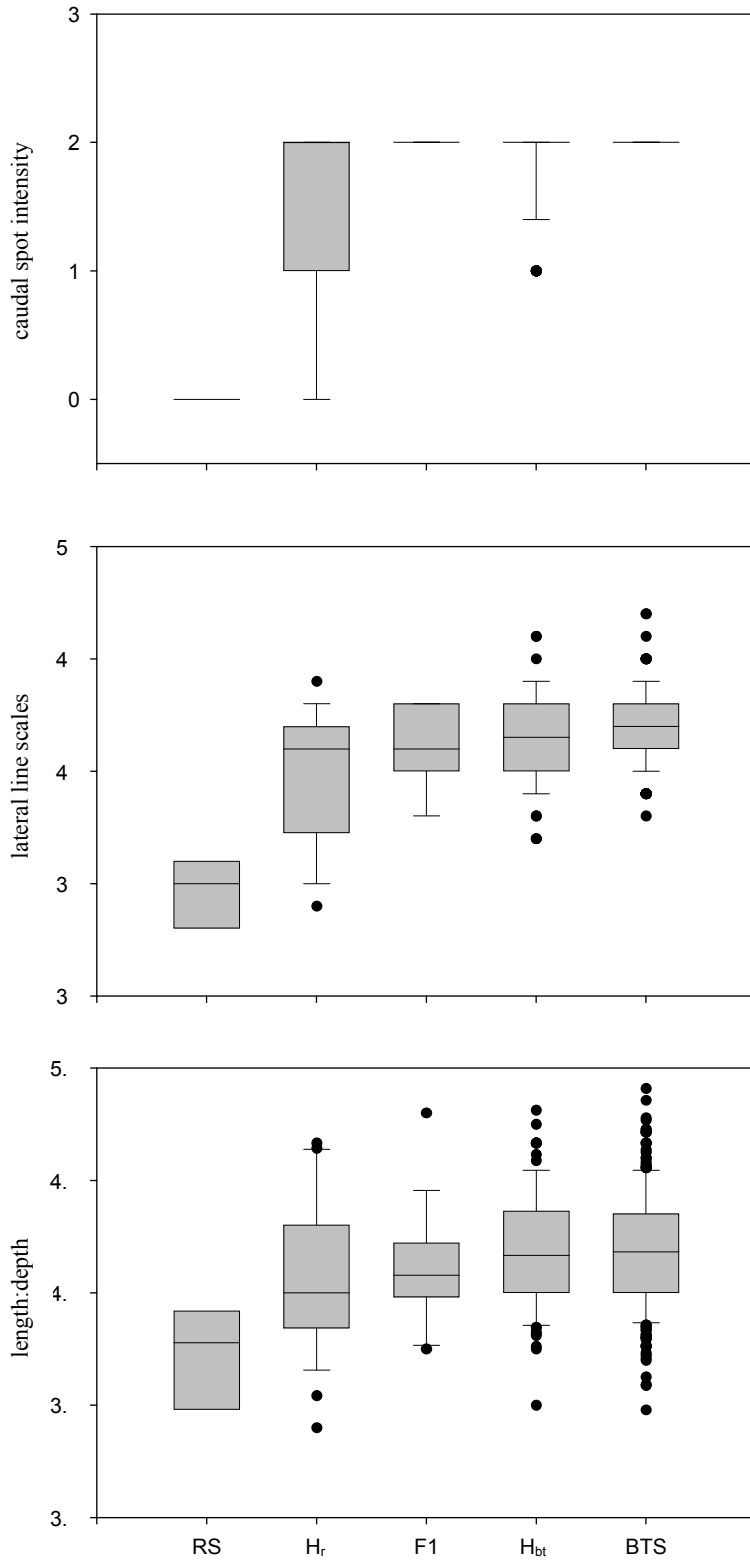


Figure 3.





## CHAPTER 2.

Preventing loss of native species following hybridization with an invasive species;  
Lessons from prezygotic and postzygotic reproductive isolation between introduced red  
shiner and native blacktail shiner (Cyprinidae: *Cyprinella*)

Michael J. Blum<sup>1,2\*</sup>, David M. Walters<sup>1</sup>, Noel M. Burkhead<sup>3</sup>,  
Byron J. Freeman<sup>4</sup>, Brady A. Porter<sup>5</sup>

<sup>1</sup> National Exposure Research Laboratory, US Environmental Protection Agency,  
Cincinnati, OH 45268

<sup>2</sup> Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA  
70118

<sup>3</sup> Florida Integrated Science Center, US Geological Survey, Gainesville, FL 32653

<sup>4</sup> Georgia Museum of Natural History and Odum School of Ecology, University of  
Georgia, Athens, GA 30602

<sup>5</sup> Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282

\*corresponding author

**Contact information:** Department of Ecology and Evolutionary Biology, Tulane  
University, New Orleans, LA 70118

Phone: 504-862-8295

Fax: 504-862-8076

Email: [mjblum@tulane.edu](mailto:mjblum@tulane.edu)

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## ***ABSTRACT***

Biological invasions involving hybridization can lead to rapid displacement and loss of native species. Timely management that aids or reinforces pre- and postzygotic isolation could be effective for preventing hybridization and curtailing the loss of native species. Such strategies should account for how isolating mechanisms vary across species interactions and environments. Here we present a study of prezygotic and postzygotic reproductive isolation between non-native red shiner (*Cyprinella lutrensis*) and blacktail shiner (*C. venusta stigmatura*) from the Coosa River basin where a hybrid swarm is rapidly expanding. We conducted conservative no-choice spawning trials to measure mating preferences and raised broods from intra- and interspecific crosses to assay hybrid viability through early juvenile development. Females of both species were significantly more responsive to conspecific versus heterospecific mates, although blacktail shiner females responded more often to heterospecific mates than did red shiner females. Heterospecific crosses resulted in lower fertilization and egg hatching rates, but we found no other evidence of inviability. Rather, cumulative post-fertilization and larval mortality of hybrids were significantly lower than parent species, which is suggestive of heterosis. Considering prior studies that have linked high water turbidity to the formation of *C. lutrensis* x *C. venusta* hybrid swarms, a water quality improvement plan focused on turbidity reduction could potentially reinstate sexual isolation and prevent further hybridization between introduced red shiner and endemic blacktail shiner in the Coosa River basin.

## ***INTRODUCTION***

Finding evidence of hybridization between an invasive species and a native congener has become tantamount to tolling a death knell for the native species. Hybridization can provide a foothold for the establishment of non-native species, and can initiate widespread invasions (Ellstrand and Schierenbeck, 2000; Sakai et al., 2001; Lee, 2002). Biological invasions resulting from hybridization can lead to rapid displacement and loss of native species via genetic dilution or assimilation (Rhymer & Simberloff, 1996). Wolf et al. (2001) estimated that the genetic extinction of an endemic species can occur as quickly as five generations following an initial hybridization event. Under such potentially dire circumstances, can anything be done to prevent the loss of native species? Is it possible to avoid genetic extinction by preventing or even eliminating hybridization between non-native species and native congeners?

Timely management that aids or reinforces pre- and postzygotic isolation could be effective for preventing hybridization and curtailing the loss of native species. Such strategies must account for how isolating mechanisms vary across species interactions and environments. In some sexually dimorphic freshwater fishes, for example, prezygotic isolation can be sufficient to prevent hybridization between congeners (Mendelson, 2003). However, elevated turbidity due to eutrophication or excessive sedimentation can weaken sexual selection and result in hybridization (Hubbs & Strawn, 1956; Seehausen et al., 1997; Järvenpää & Lindström, 2004). Similarly, the spread of hybrids is often constrained by environmental conditions that favor parental species, but disturbance can create marginal habitats where selection against hybrids is comparably weak (Anderson, 1949).

Developing management approaches intended to prevent hybridization requires identifying causal factors of pre- and postzygotic isolation, particularly when it is unclear how environment influences species interactions or the success of hybrids. Hubbs et al. (1953) observed that episodes of hybridization between sexually dimorphic red shiner (*Cyprinella lutrensis*) and blacktail shiner (*C. venusta*) in the Guadalupe River coincided with increased turbidity resulting from excessive sedimentation. Jurgens (1951) discovered a second *C. lutrensis* x *C. venusta* hybrid swarm in a turbid reach of the San Marcos River adjacent to active oil fields. Hybridization in the San Marcos River abated as water quality improved, leading Hubbs & Strawn (1956) to conclude that turbidity disrupts species recognition during spawning, and by extension, that hybridization abates because prezygotic isolation is reinstated as turbidity declines. Although this is consistent with other studies that show increased turbidity weakens or alters sexual selection (Järvenpää & Lindström, 2004; Candolin et al., 2006; Engström-Öst & Candolin, 2007) some evidence suggests that *C. lutrensis* x *C. venusta* F<sub>1</sub> and F<sub>2</sub> hybrids may be inviable or have comparably low fitness (Hubbs & Strawn, 1956). Identifying causal factors of pre- and postzygotic isolation between red shiner and congeners would help clarify whether mitigating environmental disturbance improves species recognition or eliminates conditions favoring the persistence of hybrids, thereby laying the groundwork for management strategies to curtail hybridization.

The spread of red shiner and hybridization with endemic blacktail shiner (*C. venusta stigmatura*) in the Coosa River basin presents an opportunity to implement novel management practices to control a nuisance species if more were known about the nature of reproductive isolation among *Cyprinella* species. Recent surveys of the upper Coosa

River basin have documented extensive hybridization between introduced red shiner and endemic blacktail shiner. The hybrid swarm formed around 1998, following at least 6 years of intermittent hybridization (Walters et al., unpublished data). Subsequent surveys have tracked the rapid upstream expansion of the swarm (Walters et al., unpublished data). The hybrid swarm now overlaps with the largest remaining population of federally Threatened blue shiner (*C. caerulea*) in the Conasauga River. Continued expansion of red shiners is a viable threat to this population, since they electively hybridize with blue shiner under laboratory conditions (Burkhead et al., 2006). Considering that prior studies (Jurgens, 1951; Hubbs & Strawn, 1956; Page & Smith, 1970) of red shiner and blacktail shiner have linked hybridization with deteriorated water quality, it might be possible to modify existing water quality management practices to prevent hybridization. Greater understanding of isolation mechanisms among red shiner and blacktail shiner would help guide development and application of such modifications.

Here we present a study of prezygotic and postzygotic reproductive isolation between non-native red shiner and blacktail shiner from the Coosa River basin. Prior field and laboratory studies (Hubbs & Strawn, 1956) found that red shiners electively hybridize with blacktail shiners, but no studies have documented the strength or symmetry of prezygotic isolation between the two species. Similarly, it has been shown that *C. lutrensis* x *C. venusta* hybrids can be fertile (Hubbs & Strawn, 1956), but hybrid fertility and inviability have not been quantified. We conducted conservative no-choice laboratory trials with wild-caught fish to measure mating preferences, and raised broods generated from intra- and interspecific crosses in common garden conditions to assay hybrid viability from post-fertilization to early juvenile development. Comparison of pre-

and postzygotic isolation enabled us to infer what range of management practices could potentially prevent further hybridization between introduced red shiner and native congeners.

## ***METHODS***

### *Field collection, transport and care*

Individuals used in trials were collected between July 2004 and October 2005. Blacktail shiners were collected from the upper Conasauga River in Whitfield County (GA), and Bradley County (TN) more than 28 river km above the upstream front of the *C. lutrensis* x *C. venusta* hybrid swarm in the Coosa River system (Walters et al., unpublished data). Blacktail shiners did not exhibit any intermediate or incongruent morphological or molecular genetic attributes indicative of hybridization (Blum et al., unpublished data), but it remains possible that all blacktail shiners in the system are nominally affected by introgression due to the extent of hybridization within the upper Coosa River basin. The *C. lutrensis* x *C. venusta* hybrid swarm encompasses all sites where red shiners are known to occur in the upper Coosa River basin. To avoid complications arising from introgression, we collected non-native red shiners from Proctor Creek (DeKalb Co., GA) in the adjacent Chattahoochee River basin. Red shiners are abundant and widespread in Atlanta metropolitan area streams, like Proctor Creek (Couch et al., 1995; DeVivo, 1996). No evidence of persistent hybridization between red shiner and blacktail shiner (*C. venusta cercostigma*) has been documented in this region, and blacktail shiners are rare in these highly urbanized streams (Couch et al., 1995; DeVivo, 1996; Herrington, 2004).

Fishes were collected by seine and transferred to live wells containing local, temperature-equilibrated water prepared with 2mg/L metomidate, 0.13ml/L Ammo Lock (Aquarium Pharmaceuticals), and 0.26ml/L Stress Coat (Aquarium Pharmaceuticals) to reduce stress during transport. In the laboratory, fishes were transferred to 38L tanks on a flow-through, well-water system. Density per tank was limited to 2.5 cm of fish per 3.8L of water. Individuals were segregated to prevent all contact between species and prophylactically treated using oxytetracyclin medicated flake food. Following treatment, individuals were transferred to 120L circular, flow-through tanks and maintained on a diet of 43% protein pellet food.

*Prezygotic reproductive isolation: Mating experiments*

Upon onset of male nuptial coloration (e.g. red and yellow fin coloration in male red and blacktail shiners, respectively) in May 2006, individuals of both species were sexed and segregated. Photoperiod was adjusted to a 12:12 day-night cycle to promote spawning behavior.

When females of both species appeared gravid, we transferred nuptial pairs to individual spawning tubs. Nuptial pairs consisted of one male and one female in four cross configurations: red shiner male  $\times$  red shiner female, red shiner male  $\times$  blacktail shiner female, blacktail shiner male  $\times$  red shiner female, blacktail shiner male  $\times$  blacktail shiner female. Twenty unique pairs of each configuration were established. No individual was used twice. This no-choice trial design (i.e., where a single male and female are allowed to interact) provides a conservative estimate of mating preferences (Coyne, 1993; Hatfield & Schluter, 1996). Each nuptial pair was kept for an initial period of seven days in a 66.5L black plastic, circular tub equipped with an Aquaclear powerhead (Model 402)

to create circulation, a 4cm air diffuser (0.5cm slip, medium pore) to maintain aeration, and a single spawning tower. Towers consisted of stacked large (6cm x 6cm) alternating with small (5cm x 5cm) black squares of 3mm thick plexi-glass arrayed onto a 0.6cm stainless steel rod attached to a clear plexi-glass base. Spawning tubs were inspected daily for eggs in and around the spawning tower. After seven days, nuptial pairs were transferred to separate 38L flow-through tanks containing a spawning tower and monitored for another 120 days.

Males of both species maintain spawning territories. Females inspect territories, and choose whether to deposit eggs within crevices in a male's territory. Thus, we measured female preference as whether or not a female deposited eggs in crevices. We did not measure male preference because it was impossible to distinguish between milting runs and visually similar behaviors such as inspection of spawning crevices.

*Post-mating prezygotic reproductive isolation and postzygotic reproductive isolation:*

*Fertilization, hatching success and larval mortality*

Spawning towers containing eggs were transferred to tubs containing temperature-equilibrated well water. Eggs were dislodged by gently disassembling the spawning towers. Eggs adhering to plates were removed using an extra-fine paint brush. Eggs were counted and checked for fertilization as a measure of postmating prezygotic isolation (Mendelson et al., 2006). Eggs from each cross were placed in separate rearing cones, and towers were returned to their original spawning tank. Eggs were held in rearing cones (#4 Brew Rite permanent gold-tone coffee filters set in Styrofoam platforms) until hatching. The platforms floated in 38L tanks with air stones placed under each cone to oxygenate the water around the eggs and to increase circulation enough to gently roll the



eggs. Eggs were inspected daily to determine development times to the eyed stage and to first hatch, as well as time between the first and last hatch. Eggs were checked daily for fungal infections and mortalities. Dead and fungal-infected eggs were counted and discarded to protect the health of adjacent eggs.

We recorded hatch mortalities (when a larva is unable to completely exit its egg) as hatching commenced. Surviving hatchlings were transferred into cross-specific 20L white tubs filled to a depth of 10cm with well water slightly aerated with an air stone. After yolksacs were absorbed, larvae were fed on a mixed diet of concentrated brine shrimp nauplii and rotifers. Flake food supplements were added when larvae began surface feeding. Each tub was checked daily and all mortalities were recorded as occurring at one of the following stages: early yolksac, late yolksac, post-yolksac, or young. Larvae were transferred to separate 38L tanks upon attaining lengths of approximately 1cm and were fed flake food for a period of 180 days, after which the study was terminated. All mortalities during this time were removed and recorded as having occurred at the young stage.

#### *Statistical analyses*

Mating preference was assessed as the number of crosses where females deposited eggs and as the number of eggs laid by females in conspecific and heterospecific pairings since maternal investment can vary according to mate quality (Mousseau & Fox, 1998). We used chi-squared tests to determine if, overall, females deposited eggs more often with conspecific versus heterospecific mates. Chi-squared tests were also used to determine if red shiner and blacktail shiner females differed in response to conspecific males and heterospecific males (Prohl et al., 2006). Two-tailed t-tests were used to test whether

females deposited more eggs with conspecific versus heterospecific mates. We used analysis of variance (ANOVA) with a post-hoc Tukey test to determine if the number of eggs deposited varied among all cross configuration (Bolnick & Near, 2005; Mendelson et al., 2006).

The proportion of fertilized eggs in conspecific versus heterospecific crosses served as our measure of postmating prezygotic isolation (Mendelson et al., 2006). Postzygotic isolation was assessed by comparing development rates, hatching success and larval mortality at each growth stage in broods resulting from conspecific versus heterospecific crosses. We used two-tailed t-tests to determine whether fertilization rates, development rates and measures of larval mortality differed between conspecific and heterospecific crosses. We used ANOVA routines with post-hoc Tukey tests to assess differences across the four cross configurations. All statistical tests were performed using Systat v.10 (SPSS, Inc.).

## ***RESULTS***

### *Prezygotic reproductive isolation and fecundity*

Nineteen conspecific red shiner and 14 conspecific blacktail shiner crosses resulted in females depositing eggs. Females deposited eggs in five red shiner male  $\times$  blacktail shiner female crosses and only one of the blacktail shiner male  $\times$  red shiner female crosses. Females were significantly more likely to deposit eggs when paired with a conspecific male ( $\chi^2 = 36.5, p < 0.0001$ ). Within conspecific crosses, red shiner females were more likely to deposit eggs than were blacktail shiner females ( $\chi^2 = 4.32, p < 0.038$ ). Blacktail shiner females deposited eggs with heterospecific males more often than red shiner females, but the difference was not significant ( $\chi^2 = 3.13, p = 0.077$ ). Females

deposited more eggs when paired with conspecific males than with heterospecific males (Table 1), but the difference was not statistically significant ( $t=1.52$ ,  $p=0.13$ ). Within conspecific crosses, red shiner females deposited more eggs than did blacktail shiner females (*pairwise mean difference* = 262.3,  $p=0.018$ ; Figure 1A). Red shiner conspecific crosses had significantly higher spawning frequency than other configurations and pairwise differences among these other configurations were not significant (Figure 1B)

#### *Post-mating prezygotic and postzygotic reproductive isolation*

Comparisons of outcomes from conspecific versus heterospecific crosses demonstrated that hybrid progeny were equally or more viable than controls with two exceptions (Table 1). Eggs from heterospecific crosses were less likely to be fertilized ( $t=-3.38$ ,  $p=0.002$ ; Figure 2A) and fertilized eggs were less likely to hatch ( $t=-2.57$ ,  $p=0.014$ ; Figure 2B). However, the proportion of eggs that hatched from blacktail shiner conspecific crosses was comparable to the outcome of heterospecific crosses, whereas red shiner conspecific crosses exceeded other categories (Figure 2C). Days to eyed stage ( $t=1.28$ ,  $p=0.21$ ) and first hatch ( $t=0.82$ ,  $p=0.41$ ) did not differ between conspecific and heterospecific crosses (Table 1), although eggs from red shiner conspecific crosses reached the eyed stage faster than eggs from other cross configuration (Figure 3A). Eggs from red shiner conspecific crosses hatched faster compared with blacktail shiner conspecific crosses (*pairwise mean difference*=2.75,  $p=0.015$ ; Figure 3B) but not compared to heterospecific crosses. Larval mortality across developmental stages was similar between all cross configurations except during the post-yolksac stage (Figure 4A-E). Post-yolksac larvae from red shiner conspecific crosses suffered greater mortality than larvae from blacktail shiner conspecific crosses and red shiner female  $\times$  blacktail

shiner male crosses (Figure 4D). Cumulative larval mortality of hybrid progeny was significantly lower than larval mortality of progeny from control crosses ( $t = -2.60$ ,  $p = 0.014$ ). Total post-fertilization mortality, which includes hatching mortality, was also significantly lower for hybrid progeny ( $t = -4.08$ ,  $p < 0.0001$ ; Figure 4F).

## ***DISCUSSION***

### *Patterns of reproductive isolation*

Variation in sexual dimorphism, phylogenetic relationships and genetic distance between congeners provide some basis for inferring general patterns of reproductive isolation among *Cyprinella* species (Coyne & Orr, 1989; Sasa et al., 1998; Mendelson, 2003; Mendelson et al., 2004; Moyle et al., 2004; Bolnick & Near, 2005). Hybrid viability generally decreases with genetic distance and time since divergence (Coyne & Orr, 1989; Bolnick & Near, 2005). For example, Mendelson (2003) showed that sexual isolation evolves to completion faster than hybrid inviability between species pairs of *Etheostoma* darters. *Cyprinella* are similar to *Etheostoma* in that they commonly exhibit striking sexual dimorphism, suggesting that sexual isolation could be a more potent isolating mechanism than hybrid inviability among members of the genus (Mendelson, 2003). Evidence from Centrarchid fishes (Bolnick & Near, 2005) also suggests that hybrid inviability is a weak isolating mechanism among *Cyprinella*. Bolnick & Near (2005) found an initial lag time before the onset of hybrid inviability among Centrarchids, after which a hatching success declined 3.13% per million years of divergence. Thus, complete hybrid inviability has yet to evolve between the most divergent Centrarchid species (Bolnick & Near, 2005). Hybrid inviability among *Cyprinella* may evolve over a comparably slow rate. Fossil-calibrated divergence times have not been accurately

estimated for *Cyprinella*, but the viability of hybrids from crosses between red shiner and blue shiner (Burkhead et al., 2006) suggests that complete hybrid inviability has not evolved among distantly related congeners (Broughton & Gold, 2000).

Our study supports the hypothesis of strong sexual isolation among species of *Cyprinella*. We found that egg fertilization rates were lower among heterospecific crosses than conspecific crosses, and the no-choice trials we conducted indicate females are significantly more responsive to conspecific versus heterospecific mates. More research into why fertilization rates differ is warranted, especially to determine whether sperm precedence is a contributing factor (Mendelson, 2003). More extensive mate preference trials also will be necessary to assay what cues females use when choosing mates since females can refer to single or multiple cues (Hankison & Morris, 2002; Rosenfield & Kodric-Brown, 2003). However, it is likely that mate choice by *Cyprinella* females depends on several sexually selected courtship cues, including male aggression (Rosenfield & Kodric-Brown, 2003; Burkhead, unpublished data). If aggression is a courtship cue in both species, our finding that blacktail shiner females are more prone to choose heterospecific mates than are red shiner females could be a result of red shiner males being more aggressive than blacktail shiner males (Burkhead, unpublished data). By extension, this would suggest that factors impeding species recognition but not courtship cues could promote hybridization (Hankison & Morris, 2002).

Postzygotic reproductive isolation appears to be weak between red shiner and blacktail shiner. Lower egg hatching rates from heterospecific crosses is indicative of postzygotic reproductive isolation, but we found no other evidence of inviability. Rather, the comparatively low larval mortality of *C. lutrensis* x *C. venusta* hybrids is suggestive

of heterosis, where hybrids exhibit greater viability than controls. This finding is consistent with other studies of recently diverged taxa that have found evidence of hybrid vigor (Rosenfield et al., 2004; Bolnick & Near, 2005). However, we cannot exclude the possibility that some aspects of hybrid inviability are dependent upon ecological context and therefore are not measurable under laboratory conditions (Mendelson, 2003). We also cannot exclude the possibility that *C. lutrensis* x *C. venusta* F<sub>1</sub> hybrids are partially or completely infertile because we concluded our study prior to progeny from the crosses reaching sexual maturity. Additional work will be necessary to address this and other possibilities, such as the likelihood that F<sub>2</sub> offspring are inviable.

Evidence of sexual isolation suggests that heterospecific mating between introduced red shiner and native blacktail shiner in the Coosa River basin should be infrequent, but the frequency of interactions probably varies because the strength of sexual isolation can change according to environmental conditions (Seehausen et al., 1997; Järvenpää & Lindström, 2004; Candolin et al., 2006; Engström-Öst & Candolin, 2007). Prior studies suggest that elevated turbidity increases the frequency of interactions and the propensity of *Cyprinella* to hybridize (Jurgens, 1951; Hubbs & Strawn, 1956; Page & Smith, 1971). High turbidity obscures and potentially eliminates color differences among freshwater fishes (Seehausen et al., 1997; Seehausen & van Alphen, 1998). Since species of *Cyprinella* likely discriminate by color, elevated turbidity might reduce the efficacy of species recognition cues (Hubbs & Strawn, 1956), whereby hybridization proceeds because females favor sexually selected traits expressed in both conspecific and heterospecific mates. Similarly, hybridization could be a consequence of increased turbidity favoring heterospecific mates that expend greater energy (Engström-Öst &

Candolin, 2007) or compensatory plasticity in display tactics during courtship (Luyten & Liley, 1991). Carrying out additional experiments will be necessary to determine if one or more of these mechanisms promotes hybridization between red shiner and blacktail shiner in the Coosa River basin.

#### *Preventing hybridization and loss of native species*

Introductions of red shiner have put many congeners at risk of genetic extinction (Mayden, 1989; Fuller et al., 1999). No where is this more of a concern than in speciose southeastern US river basins that harbor the greatest number of *Cyprinella* species in North America. Red shiner have hybridized with at least nine of the 22 *Cyprinella* taxa found in southeastern drainages (Hubbs & Strawn, 1956; Page & Smith, 1970; Wallace & Ramsey, 1982; Johnson, 1999; Warren et al., 2000; W.C. Starnes, personal communication), and hybridize with federally Threatened blue shiner under laboratory conditions (Burkhead et al., 2006). Can measures be taken to limit the breadth or intensity of impact introduced red shiner are having on native congeners? Observational studies suggest that hybridization between red shiner and other congeners is mediated by environment (Jurgens, 1951; Hubbs & Strawn, 1956; Page & Smith, 1970). If hybrid swarms form and dissipate as environmental conditions decline and improve (Hubbs & Strawn, 1956), it could be possible to implement management practices to eliminate conditions favoring hybridization.

Hybridization does not necessarily cease following eradication or decline of a non-native species. The introduction of non-native smooth cordgrass (*Spartina alterniflora*) to San Francisco Bay (California, USA) led to the formation of a hybrid swarm with the endemic California cordgrass, *S. foliosa*. Smooth cordgrass has since

become exceedingly rare in San Francisco Bay while *S. alterniflora* x *S. foliosa* hybrids are spreading rapidly and overtaking marshes once occupied by *S. foliosa* (Ayres et al., 2004). Similarly, a single introduction of a very small number of non-native sheepshead minnow (*Cyprinodon variegatus*) into the Pecos River drainage led to hybridization with the endemic Pecos pupfish (*C. pecosensis*). Hybrids replaced Pecos pupfish throughout more than half of the species' range in less than five years (Echelle & Conner, 1989), and a *C. pecosensis* x *C. variegatus* hybrid swarm now exceeds the historic range of Pecos pupfish in the Pecos River (Wilde & Echelle, 1992; Childs et al., 1996).

Management involving steps beyond eradication has prevented the loss of some native species following hybridization. In two cases where hybridization has occurred between a second endemic pupfish (*C. bovinus*) and sheepshead minnow in the Pecos River drainage, a combination of trapping, application of piscicides, and reintroductions prevented the loss of the endemic pupfish (Echelle & Echelle, 1997; Rosenfield et al., 2004). The success of these strategies likely hinged on the comparatively small geographic extent of the *C. bovinus* x *C. variegatus* hybrid swarms (Rosenfield et al., 2004), which reinforces the idea that early detection and rapid response can help prevent loss of native congeners (Hobbs & Humphries, 1995). However, the success of the control strategies also depended on there being some degree of reproductive isolation between *C. bovinus* and *C. variegatus*. Rosenfield et al. (2004) argue that similar management strategies would be unlikely to prevent the loss of Pecos pupfish because female Pecos pupfish preferably breed with male sheepshead minnows (Rosenfield & Kodric-Brown, 2003) and because *C. pecosensis* x *C. variegatus* hybrids can exhibit greater vigor than either parental species (Rosenfield et al., 2004).



Management that strengthens prezygotic isolation between red shiner and blacktail shiner could help prevent the loss of blacktail shiner in the Coosa River basin. If additional work on mate choice confirms observations linking hybridization to elevated turbidity, then a prescription of eradication and water quality improvement with a focus on reducing turbidity would be in order. In the Coosa River basin, turbid conditions generally correspond to high levels of suspended sediments (Burkhead & Jelks, 2001; Walters et al., 2003; Roy et al., 2005). Improving capture of urban runoff or reducing soil loss in agricultural landscapes are just two among many potential strategies that could reduce sediment loads. Although reducing excessive sedimentation is a well recognized management priority for sustaining salmonids fisheries, it is under-appreciated for conserving non-game fishes (Burkhead & Jelks, 2001; Walters et al., 2003). Besides potentially leading to hybridization with invasive species, excessive sedimentation contributes to biotic homogenization of fish assemblages by creating habitat conditions favored by cosmopolitan species like red shiner (Matthews & Hill, 1979; Walters et al., 2003). Burkhead & Jelks (2001) also demonstrated that elevated suspended sediments can reduce the fecundity of native *Cyprinella* in southeastern US streams. Competition, hybridization and reduced fecundity could all be contributing to the decline of native *Cyprinella* in turbid southeastern US streams. Thus, improving water quality by reducing sedimentation could potentially eliminate one or more major invasion pathways and broadly benefit at-risk aquatic biodiversity.

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Table 1: Measures of prezygotic, post-mating prezygotic, and postzygotic reproductive isolation between non-native red shiner and native blacktail shiner. Comparisons are provided for conspecific versus heterospecific crosses, and among each of the four separate cross configurations. Values in the first column for each measure = range, values in the second column = mean (standard deviation).

| Cross          | Prezygotic |         |           |           |           | Post-mating Prezygotic |             |
|----------------|------------|---------|-----------|-----------|-----------|------------------------|-------------|
|                | N          | #spawns |           | Eggs Laid |           | % Fertilization        |             |
| Conspecific    | 33         | 1-6     | 2.4 (1.6) | 7-1059    | 252 (280) | 0.09-1                 | 0.67 (0.26) |
| Heterospecific | 6          | 1       | 1 (0)     | 19-330    | 151 (113) | 0.03-0.75              | 0.27 (0.31) |
| R x R          | 19         | 1-6     | 3.2 (1.6) | 53-1059   | 364 (314) | 0.28-0.95              | 0.74 (0.17) |
| B x B          | 14         | 1-3     | 1.3 (0.6) | 7-327     | 101 (119) | 0.09-1                 | 0.58 (0.33) |
| R♀ x B♂        | 1          | 1       |           | 19        |           | 0.58                   |             |
| R♂ x B♀        | 5          | 1       | 1 (0)     | 55-330    | 176 (104) | 0.03-0.75              | 0.21 (0.31) |

| Cross          | Postzygotic (prehatch development) |           |                     |           |                      |           |
|----------------|------------------------------------|-----------|---------------------|-----------|----------------------|-----------|
|                | Days to eyed                       |           | Days to first hatch |           | >Days to final hatch |           |
| Conspecific    | 1-8                                | 3.7 (1.7) | 2.8-14              | 7.1 (2.7) | 2-10                 | 4.8 (1.9) |
| Heterospecific | 2-7                                | 4.8 (1.9) | 7-9                 | 8 (0.6)   | 0-6                  | 3.4 (2.3) |
| R x R          | 1-5                                | 3 (1)     | 2.8-12.4            | 6 (2.1)   | 2-10                 | 4.8 (2.2) |
| B x B          | 3-8                                | 5.4 (1.8) | 5-14                | 8.8 (2.8) | 3-7                  | 4.9 (1.3) |
| R♀ x B♂        | 2                                  |           | 8                   |           | 6                    |           |
| R♂ x B♀        | 4-7                                | 5.5 (1.3) | 7-9                 | 8 (0.71)  | 0-5                  | 2.8 (2.1) |

| Cross          | Postzygotic (posthatch development) |             |              |             |                      |             |                     |              |
|----------------|-------------------------------------|-------------|--------------|-------------|----------------------|-------------|---------------------|--------------|
|                | % Egg hatch                         |             | % Hatch mort |             | % early yolksac mort |             | % late yolksac mort |              |
| Conspecific    | 0-1                                 | 0.61 (0.30) | 0-1          | 0.16 (0.28) | 0-1                  | 0.04 (0.18) | 0-0.17              | 0.007 (0.03) |
| Heterospecific | 0.03-0.75                           | 0.27 (0.31) | 0-0.12       | 0.02 (0.06) | 0                    | 0           | 0                   | 0            |
| R x R          | 0.28-0.95                           | 0.75 (0.17) | 0-0.74       | 0.13 (0.19) | 0-0.11               | 0.02 (0.04) | 0-0.17              | 0.01 (0.04)  |
| B x B          | 0-1                                 | 0.43 (0.34) | 0-1          | 0.21 (0.33) | 0-1                  | 0.09 (0.3)  | 0                   | 0            |
| R♀ x B♂        | 0.58                                |             | 0            |             | 0                    |             | 0                   |              |
| R♂ x B♀        | 0.03-0.75                           | 0.21 (0.31) | 0-0.12       | 0.02 (0.06) | 0                    | 0           | 0                   | 0            |

| Cross          | Postzygotic (posthatch development) |             |              |             |                     |             |              |             |
|----------------|-------------------------------------|-------------|--------------|-------------|---------------------|-------------|--------------|-------------|
|                | % post yolksac mort                 |             | % young mort |             | % cumul larval mort |             | % total mort |             |
| Conspecific    | 0-0.76                              | 0.24 (0.24) | 0-0.83       | 0.22 (0.24) | 0-1                 | 0.51 (0.27) | 0-1          | 0.67 (0.25) |
| Heterospecific | 0-0.37                              | 0.09 (0.15) | 0.02-0.2     | 0.13 (0.07) | 0.07-0.44           | 0.21 (0.14) | 0.13-0.44    | 0.23 (0.12) |
| R x R          | 0.01-0.76                           | 0.35 (0.24) | 0-0.52       | 0.17 (0.17) | 0.25-0.99           | 0.55 (0.2)  | 0.26-1       | 0.67 (0.21) |
| B x B          | 0-0.18                              | 0.05 (0.08) | 0-0.83       | 0.29 (0.33) | 0-1                 | 0.43 (0.36) | 0-1          | 0.64 (0.33) |
| R♀ x B♂        | 0                                   |             | 0.18         |             | 0.18                |             | 0.18         |             |
| R♂ x B♀        | 0-0.37                              | 0.1 (0.16)  | 0.02-0.2     | 0.11 (0.07) | 0.07-0.44           | 0.21 (0.15) | 0.13-0.44    | 0.24 (0.13) |

### **FIGURE LEGENDS**

Figure 1: The number of eggs laid per spawn (A) and the number of spawns (B) for each cross configuration. A bold asterisk indicates a significant pairwise difference (Tukey,  $p < 0.05$ ) to red shiner conspecific crosses. Axis legend: B = blacktail shiner; R = red shiner.

Figure 2: Fertilization rates (A) egg hatching rates per spawn for conspecific and heterospecific crosses (B) and for each cross configuration (C). A bold asterisk indicates a significant pairwise difference (Tukey,  $p < 0.05$ ) to red shiner conspecific crosses. Axis legend: B = blacktail shiner; R = red shiner.

Figure 3: The number of days for eggs to reach eyed stage (A) and to first hatch (B) for each cross configuration. A bold asterisk indicates a significant pairwise difference (Tukey,  $p < 0.05$ ) to red shiner conspecific crosses. Axis legend: B = blacktail shiner; R = red shiner.

Figure 4: Mortality rates at five progressive stages of development among all cross configurations (A-E) and total larval mortality of conspecific versus heterospecific crosses (F). A bold asterisk indicates significant pairwise difference ( $p < 0.05$ ) to red shiner conspecific crosses. Axis legend: B = blacktail shiner; R = red shiner.

Figure 1.

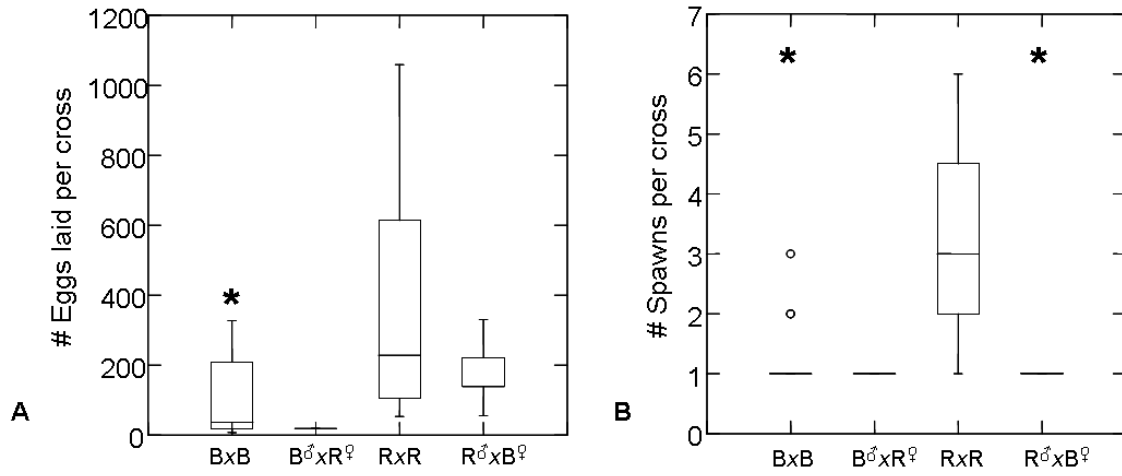




Figure 2.

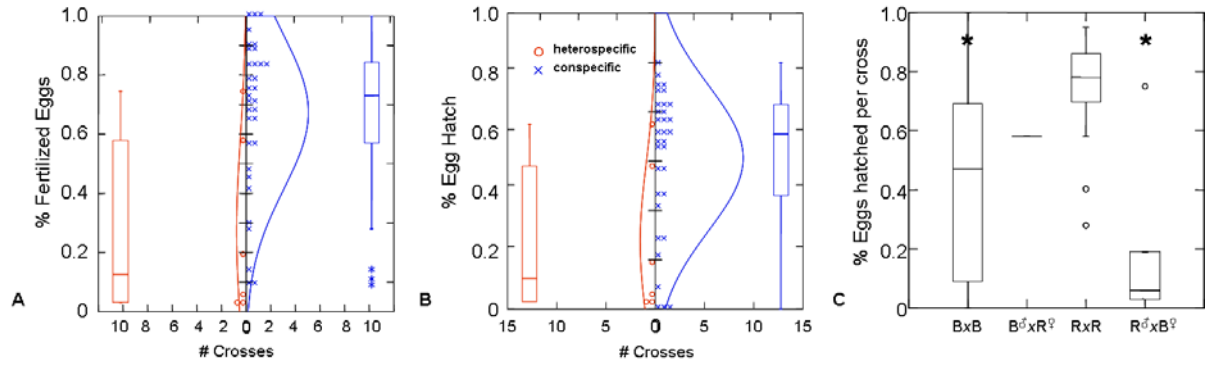


Figure 3.

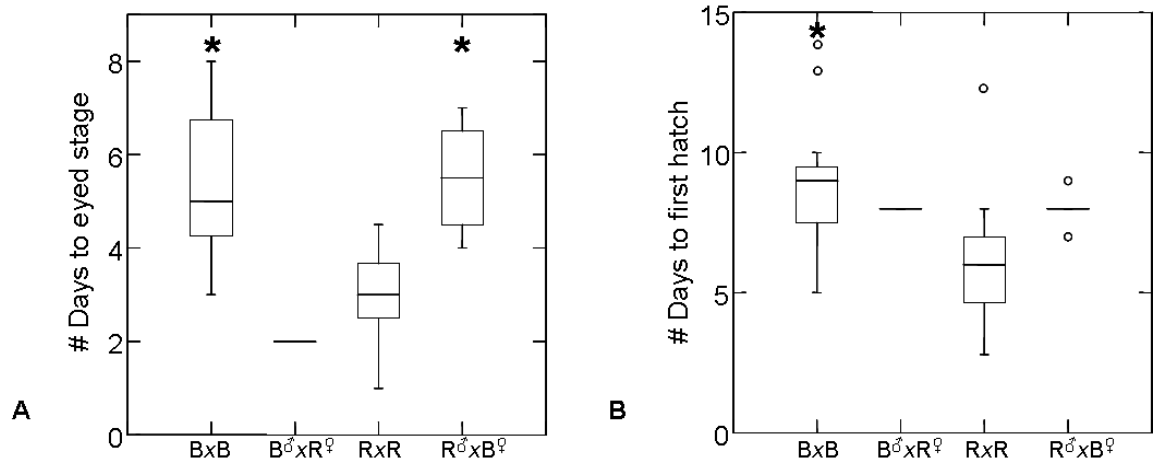


Figure 4.

