LIFE HISTORIES AND LONG-TERM COMMUNITY TRENDS OF FISHES IN THE CONASAUGA RIVER

by

Andrew Joseph Nagy

(Under the Direction of Mary C. Freeman)

ABSTRACT

Freshwater ecosystems face numerous threats that contribute to decreases in biodiversity and ecosystem function loss. Southeastern United States rivers are among the most biodiverse freshwater systems in the temperate world and support numerous endemic, rare, and imperiled fishes. Long-term monitoring in the Conasauga River in northwest Georgia has revealed declines in some fish taxa, but causes are uncertain. This study (1) collected data on spawning timing and behavior for 16 species, enhancing our understanding of the life histories of fishes in the Conasauga River; (2) quantified changes in fish community composition and size over 24 years, and evaluated streamflow effects on community change; and (3) analyzed relationships between traits and inter-specific variation in abundance trends. We found weak flow effects on community change, but evidence that the traits of crevice-spawning, insectivory and small body size were associated with steeper population declines over the study period.

INDEX WORDS: Natural history, Fish ecology, Community ecology, Time-series, Population decline, Rivers, Life history

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CHAPTER 1

INTRODUCTION AND RESEARCH OBJECTIVES

Introduction:

Freshwater ecosystems are among the most impacted ecosystems by humans. Threats including climate change, harmful land-use practices, habitat fragmentation and destruction, and pollution have led to substantial loss of both freshwater biodiversity and ecosystem functioning (Tickner et al. 2020). Even though freshwater ecosystems contribute disproportionately to global biodiversity, actions to conserve them have been inadequate to stop or reverse harm (Harrison et al. 2018). Although this is in part due to a lack of political and social will to enact strong conservation policies, it is also recognized that there are many outstanding questions that must be answered to develop effective management plans (Harper et al. 2021). One of these pertinent research areas is better understanding of how organisms respond to changes in the environment.

Freshwater fishes are of particular conservation concern due to limited migration and dispersal potential and high rates of endemism, among other factors. Globally, nearly half of freshwater fishes are endemic to a single ecoregion (Abell et al. 2008). The southeastern U.S. has a particularly high number of narrowly distributed species, many of which are considered imperiled (Elkins et al. 2019). Human modifications to the landscape including deforestation, damming of rivers, urban development, and intensive agriculture have led to declines of aquatic taxa throughout the Southeast (Benz and Collins 1997). Researchers have observed declines in endemic taxa in disturbed watersheds, leading to decreases in biodiversity and increasing biotic homogenization among fish communities (Scott 2005).

Community composition shifts in response to human impacts may stem from differences in life histories among taxa (Green et al. 2022). For instance, researchers have found that life history traits mediate fish responses to flow stochasticity (Hitt et al. 2020), hydrologic alteration (Mims & Olden 2012), climate change (Troia et al. 2019), and deforestation (Leitao et al. 2018). Linking traits to fish population responses allows researchers to 1) identify stressors that elicit shared responses among taxa and 2) identify taxa susceptible to declines.

The Conasauga River in the headwaters of the Coosa River basin is renowned for its aquatic biodiversity (Freeman et al. 1996). The river is home to >75 native fishes (Walters 1997) including 4 taxa listed as Threatened or Endangered under the Endangered Species Act as well as 16 additional taxa identified as being of conservation concern by the states of Tennessee and Georgia (GNDR, TWRC 2018). However, long-term fish population monitoring efforts have revealed a decline in several fish taxa in recent years (Freeman et al. 2017, Stowe et al. 2020). Other studies in the Conasauga River have identified longitudinal declines in insects (Baker 2012) and aquatic macrophytes associated with habitat quality (Argentina et al. 2010). Although researchers have hypothesized causes of decline stemming from intensive agriculture, industry, urbanization, and flow effects, a mechanistic link between stressors and population changes has not been identified in the Conasauga River. Pinpointing causes of decline is challenging due to the likelihood of multiple confounding stressors influencing the riverine community (Heugens et al. 2001). However, identifying primary stressors is imperative to implement conservation measures to protect and recover fish populations. Sparse life history information for many narrowly-distributed fish species often makes it difficult to identify causes of community change.

Although ecologists have developed databases of species-specific trait values, including FishTraits (Frimpong and Angermeier 2009), trait data for many species are often drawn from one or a few studies. Some traits such as spawn timing and fecundity are particularly imprecisely known, yet these traits could strongly influence how taxa respond to environmental shifts.

Research Objectives

This research has two primary goals; 1) enhance our understanding of the life histories of fishes in the upper Coosa River watershed and 2) investigate shifts in the Conasauga River fish community using time-series population data. In chapter 1, we conducted snorkel surveys in several rivers in the upper Coosa watershed to study life history. We recorded occurrences of shoal-dwelling fish spawning events and noted timing, temperature ranges, habitat use, and behavior. In addition, we collected gravid female individuals of common shoal-dwelling taxa to estimate reproductive investment, including clutch sizes and egg mass. We compared our observations to previous studies to expand the range of life history information within taxa. Our work highlights previously unknown behaviors among understudied taxa as well as challenges, and future steps of life history research in fishes. In chapter 2, we used a 19-year, six-site time-series of Conasauga River fish abundances to investigate changes in community size and composition and relate them to river flow. Additionally, we assembled and used trait data for 32 taxa to analyze physiological and behavioral characteristics associated with differential abundance trends among taxa.

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CHAPTER 2

LIFE HISTORIES OF FISHES IN THE UPPER COOSA RIVER BASIN

Introduction:

The Coosa River system is among the most diverse freshwater ecosystems in the temperate world. The Georgia headwaters including the Conasauga, Etowah, and Coosawattee Rivers are home to >90 native extant species of fish, approximately 23 of which are endemic to the Alabama River system (Freeman et al. 2007), and several of which have been extirpated from other areas of the Coosa River system. The Coosa River headwaters contain 8 fish species listed by the U.S. Fish and Wildlife Service as Threatened or Endangered (GDNR) and many more listed as species of conservation concern by the states of Tennessee, Alabama, and Georgia (GDNR, TWRC 2018). Consequently, rivers and tributaries in the Upper Coosa have been identified as priority freshwater conservation targets (Elkins et al. 2019). Furthermore, recent studies using long term monitoring datasets in the Conasauga and Etowah rivers in the Upper Coosa basin have identified population declines in several shoal-dwelling fishes (Freeman et al. 2017, Stowe et al. 2020). Researchers have hypothesized that causes of freshwater fish declines are primarily human impacts from intensive agriculture and urbanization, but mechanistic links between environmental variables and fish population changes are difficult to identify (Wenger et al. 2008, Brown et al. 2009). One challenge to determining the causes of decline in upper Coosa fishes is the scarcity of life history information for much of the shoal-dwelling community.

Natural history information for riverine fishes can inform ecological research by providing biologically relevant information that may explain how taxa respond to environmental factors including flow alteration, temperature fluctuations, and anthropogenic stressors such as pollution and damming. Life history strategies have long been known to influence community organization in varied environments (Winemiller & Rose 1992), and trait-based ecology has become an increasingly popular approach to answer questions about how life history influences population and community response to global change (Green et al. 2022). There have been several studies in recent years investigating the links of fish life histories to population success in human impacted systems. For instance, one study showed that dispersal behavior is likely to influence the ability of taxa to utilize thermal refugia in response to warming climates (Troia et al. 2019). Other studies have shown that life history traits mediate population responses of riverine fish to flow stochasticity (Hitt et al. 2020, Tonkin et al. 2021, Malone et al. 2022) and human altered flow regimes (Mimms & Olden 2013). In addition, there are several studies attempting to link traits to variation in fish populations through space. Researchers in the Amazon River basin have used landscape scale variables such as deforestation and fragmentation to explain local fish community trait assemblages (Leitão et al. 2018, Brejão et al. 2018). In most of these studies, researchers use publicly available trait data or measure morphometric indexes of habitat utilization and behavior.

Natural history information can be an important tool to guide management decisions. Conservation managers often attempt to predict how fish populations will respond to human action and mitigate any potentially harmful effects. Life history components such as spawning habitat and timing may mediate a fish population’s response to a variety of human actions such as damming, flow regulation, and construction activities. This information can be leveraged in order to alter human actions in ways that support fish reproduction and survival. For instance, identification of critical spawning habitat for anadromous or other highly migratory fishes has led to alterations of dams including installations of fish passages (Keefer et al. 2021) and removal of dams altogether (Burdick & Hightower 2006, Johnston et al. 2018). Identification and enhancement of spawning substrate have led to increased reproduction and population recovery in fishes (Taylor et al. 2019). Conservation managers may also use information about when fishes spawn to plan construction activities, such as bridge construction and culverts, to minimize negative impacts on reproduction (Wenger et al. 2021). Furthermore, understanding the temperature ranges at which fish spawn can inform watershed land-use standards, such as the protection and restoration of riparian tree coverage and reduction of impervious surface to maintain stable and cool temperatures (Pool & Berman 2001). Life history information has even been used to establish metrics of extinction risk (Kopf et al. 2017). Although attempts have been made to incorporate life history information into fish conservation and management plans (van Treeck et al. 2020), it remains difficult for species with limited available data.

Trait-based research and targeted conservation action require accurate life history information. Although trait databases such as FishTraits (Frimpong and Angermeier 2009) provide data for freshwater fishes, information for many taxa is either absent or based on a single study of one population, which may not be generalizable to a taxon’s entire distribution (Hughey et al. 2012). In addition, trait analyses of rare fish often rely on information from sister taxa, and for many rare small-bodied fishes in the southeastern U.S., comprehensive life history is lacking altogether (Boschung & Mayden 2004). Consequently, targeted study may often be necessary to obtain the life history data to inform conservation and management of particular fishes and river systems.

Motivated by the scarcity of life history information for many shoal-dwelling fishes in the upper Coosa River system and the conservation concern of many taxa in the basin, we conducted snorkel surveys and fish collections at multiple sites in the Conasauga and Etowah River basins in northwest Georgia during the spring and summer spawning season in 2021. In this study, we had two primary goals; 1) to record occurrences of shoal-dwelling fish spawning events and note timing, temperature ranges, habitat use, and behavior; and 2) to collect gravid female individuals of common shoal-dwelling taxa to estimate reproductive investment, including clutch sizes and egg mass. We compare our observations to previous studies as available to expand the range of life history information within taxa. Our work highlights previously unknown behaviors among understudied taxa as well as challenges and future steps of life history research in fishes.

Methods:

Sites

We selected five sites (Figure 1) for snorkeling observations based on available access, water clarity, and known fish diversity. Sites with high biodiversity and low turbidity at base flows (<5 NTU) were prioritized to increase chances of seeing spawning activity across multiple taxa. Three sites were located on the Conasauga River in NW Georgia. Two of these sites were located within the Blue Ridge physiographic province and one was located in the Ridge and Valley physiographic province. Our fourth site was located on Holly Creek, a tributary to the Conasauga River in the Blue Ridge physiographic province. A fifth site was located on Shoal Creek, a tributary to the Etowah River in the Blue Ridge physiographic province. Snorkeling reaches varied from 40m – 100m covering shallow riffles, eddies, and pools within shoals. Sites had substantial overlap in fish communities, although there were differences in abundances of certain taxa. Shoal Creek contained two taxa endemic to the Etowah River system (*Etheostoma etowahae* and *Etheostoma scotti*). Likewise, Conasauga River and Holly Creek sites contained *Etheostoma jordani*, a congener to *Etheostoma etowahae*, and *Cyprinella caerulea*.

Spawning Observations

We initiated snorkel surveys in February with the intent to observe onset of spawning in shoal fishes. We rotated surveys among the five study sites depending on weather and water clarity, at approximately weekly intervals, ending after nuptial activity had not been observed for 4 surveys. Surveys began on 25 February and ended on 7 July. During each survey, two to five snorkelers moved systematically from downstream to upstream to cover all habitat within shoals. Observers looked for spawning behavior including spawning, nest construction, sparring between males, and males courting or following females. Observers recorded all taxa observed, regardless of spawning behavior. Survey durations ranged from one to two hours of snorkeling. We recorded the water temperature at the beginning of each survey.

When spawning was observed, we marked the location with a flag and noted the substrate in which spawning occurred as well as general behavior. Following surveys, we returned to flags and recorded depth and velocity measurements. Depth and velocity were measured using a velocity head rod constructed with a 0.10 m x 1.0 m plexiglass panel and 1 m measuring rods following Fonstad et al. 2005. The VHR was calibrated using 83 paired measurements with a magnetic flowmeter to convert the VHR reading to a velocity in meters per second. The calibrated equation ($R^2 = 0.72$) used to convert head readings to velocity was;

$$v = 0.675 * (2gh)^{0.5} – 0.14$$

where v is water velocity in meters per second, g is acceleration due to gravity (9.8 m/s^2) and h is the difference in water height (head depth) between the upstream and downstream ends of the head rod.

Fish Collections and Ova Measurements

We collected female individuals of 9 common taxa to investigate reproductive investment. Collections occurred over 21 surveys between 6 March and 24 June. Gravid fish were collected with a 8' x 6' x 1/8" seine, euthanized in a buffered solution of MS-222, and preserved in 10% formalin to be taken back to the lab. Fin clips were collected and stored in 98% ethanol for archival in the Georgia Museum of Natural History Tissue Collection. In the lab, we first measured standard length of each collected specimen, then incised the abdomen to determine the presence or absence of ova. Ovaries were stored in 10% formalin until ova could be counted. Ovaries often contained ripe ova, i.e., eggs that are ready to be ovulated, as well as a mix of ova in varying stages of development. To estimate clutch sizes, we used a dissecting microscope to count the number of maturing (ME) or mature ova (MA), rather than ripe ova (RE) following Heins and Baker 1993. Ripe ova were not used for clutch estimates due to the possibility of the fish having already deposited some of them in the stream prior to collection. Consequently, ripe ova often present an underestimate of clutch production. If ripe ova were present, a sub-sample of 5-20 was removed and placed in a drying oven at 40 °C for 12-24 hours until desiccated and then weighed. Egg mass estimates in Table 2 are the dry weight of a single egg.

Results:

Summary

We completed 30 snorkel surveys across our five sites in 2021, beginning on February 25 and ending on July 7. We noted 67 occurrences of spawning behavior in 15 taxa (Table 1), the first of which occurred on April 11 and the last occurring on June 14. Spawning temperatures for Upper Coosa shoal fish ranged from 14.5 °C (*Etheostoma coosae*) to 23.5 °C (*Cyprinella trichroistia*, *Etheostoma jordani, and Percina palmaris*). Fish spawned in a broad range of velocities, ranging from 0 m/s (*Cyprinella caerulea*) to 1.23 m/s (*Etheostoma coosae*). Depths ranged from a shallow 0.06 m (*Lepisosteus osseus*, observed spawning on the edge of a *Justicia americana* bed with much of their body out of water) to deeper shoal habitat (0.91 m, *Cyprinella callistia)*. It should be noted that because we conducted our surveys in shoal habitats, we were unlikely to observe spawning in deep (>2 m) pool habitats.

We sampled fish with nets 21 times in the same February to July period to collect individuals for ovarian analysis and note nuptial condition. We collected a total of 91 individual fish across 9 taxa, yielding 63 total estimates of clutch sizes (Table 2). Mean clutch sizes within taxa ranged from a minimum of 43.5 (*E. coosae*) to a maximum of 332.25 (*C. trichroistia*). Most ovaries contained eggs at varying stages of development, with the exception of *Cottus carolinae* all of which contained a single clutch of uniformly sized eggs. Aside from *C. carolinae*, it is likely that these taxa spawn multiple times over the course of a season. Mean dry egg mass ranged from 0.15 mg (*N. stilbius*) to 3.2 mg (*C. carolinae*). Mean egg sizes ranged from 0.95 mm (*N. stilbius*) to 2.6 mm (*C. carolinae*). Among the fish we collected, there seemed to be a trade-off between egg size and fecundity that was associated with family. Cottidae (1 species) was characterized by low fecundity and large egg size and Leuciscidae (3 species) was characterized by higher fecundity and smaller eggs. Percidae had intermediate values, with *Etheostoma* (3 species) tending to have smaller eggs and clutch sizes than *Percina* (3 species).

Species specific spawning information:

Etheostoma coosae

We noted 22 occurrences of *Etheostoma coosae* spawning across 8 snorkel surveys in 4 sites in 2021. The earliest spawning was observed on April 11 and the latest observation was June 14. This is similar to findings from a previous study from a stream in Alabama that noted *E. coosae* spawning from mid-March to late May (O'Neil 1981). Water temperatures during spawning varied substantially, ranging from 14.5 to 22 °C. This was the largest temperature and time range we noted in our surveys, indicating that *E. coosae* may have an extended spawning season compared to other darters.

Spawning occurred in shallow shoals, often near margins in moderate flow over cobble, gravel, and sand mixtures. The average water velocity in spawning habitat was 0.44 m/s and average depth was 0.39 meters. Spawning behavior was typical of other members the *Etheostoma* genus (Winn 1958). Males followed females while females searched for a location suitable for egg deposition. Nearby males frequently competed for female attention, including nipping the competitor's fins and flexing their dorsal, caudal, and pelvic fins. Once a suitable location was determined and a mate chosen, males moved their pelvic region towards that of the female and vibrated, fertilizing the egg as it was deposited. Eggs were primarily deposited and attached to mid-sized cobble (5-15 cm). Upon release and fertilization of an egg, females typically moved a short distance away to find a new location to deposit an egg, and courtship behavior by nearby males resumed, often including the male that had already spawned with the female. This behavior is different than that reported in aquaria, where *E. coosae* was observed spawning in rock crevices and under ledges (O'Neil 1981).

Etheostoma stigmaeum

We noted 9 occurrences of *Etheostoma stigmaeum* spawning across 3 surveys in 3 of our sites. Seven of these spawning occurrences were during a single survey on April 15 at our most downstream Conasauga River site. The remaining two occurred on April 11 and May 2 in our middle Conasauga Site and Holly Creek respectively. In addition, we observed two large male individuals sparring in our Shoal Creek site on April 28, indicating that spawning was still occurring at this time. Our observations are congruent with notes from unpublished research by Patrick O'Neil that *E. stigmaeum* is likely to spawn in late March to early May in Barbaree Creek in Alabama (Boschung and Mayden 2004). We noted water temperatures during spawning ranging from 14.5-16.7 °C, also in agreement with O'Neil's suggestion of 14 to 17 °C. We observed spawning habitat similar to that of *E. coosae*; however, *E stigmaeum* tended to spawn closer to shoal margins rather than in the center of channels, often alongside gravel and cobble banks and islands. Depths averaged 0.49 m and water velocities averaged 0.35 m/s, however, the velocity varied substantially, ranging from 0.05 m/s to 0.58 m/s.

Fishes of Alabama (Boschung and Mayden 2004) notes that *Etheostoma stigmaeum* has not been observed spawning in nature. However, Winn (1958) describes *E. stigmaeum* spawning in aquaria, noting egg burying behavior in sand and gravel substrate. We observed the same behavior in the field. Males were seen chasing after nearby females, occasionally fending off potential competitors that approached the area. Although we did not see any spawning during our April 28 survey, we did note two males sparring for over 10 minutes, flexing their dorsal, caudal, and anal fins and nipping at the fins of the other male. This behavior occurred in similar habitat to that in which we saw spawning at other sites, corroborating O’Neil’s account of males defending and sparring for spawning territory (Boschung and Mayden 2004). As males fended off challengers, the female moved in the shoal to find a spot suitable for spawning. In all of our observations, this was coarse sand substrate, often interspersed with cobble. The male mounted the female, moved its pelvic region and caudal and they vibrated, releasing gametes into the substrate. We were unable to determine whether these events resulted in a single egg or multiple eggs deposited and fertilized. Winn (1958) notes that in aquaria, an average of 4-5 eggs were deposited at one time.

Percina palmaris

We noted 6 observations of *Percina palmaris* spawning across 4 snorkel surveys. All of the observations of *P. palmaris* spawning occurred in our furthest upstream site in the Conasauga River. The earliest spawning event occurred on May 1 and the last occurred on June 13. Previous research from the Tallapoosa River system in Alabama has noted that the spawning period of *P. palmaris* begins in mid-March and ends in mid-June (Wieland 1984). Although we did not note spawning prior to May, this may be explained by the cooler temperatures of the Upper Conasauga River in the Blue Ridge Province. Water temperatures during spawning ranged from 15 to 23.5 °C, corresponding with the peak in spawning at 18 to 20 °C found in the Tallapoosa River population (Wieland 1984).

We were unable to find published information about the spawning behavior of *Percina palmaris*. Wieland (1984) noted that attempts to spawn in aquaria were unsuccessful and occurrences in the field were not observed. We observed *P. palmaris* spawning in swift water (mean = 0.50 m/s) at moderate depths (0.40 m) near large boulders, bedrock, and mixtures of sand and gravel. We noted that males tended to be more stationary than other darters in this study, often staying near a particular boulder or bedrock cavity whilst defending the area against other males. When a nearby female was noticed by a male, the male engaged in courtship behavior, following her closely and continuing to defend from male competitors. Upon reaching a suitable spawning location, often the location that the male had been defending, the male moved its pelvic region towards the female's and vibrated vigorously, often sending sand and gravel up into the water column as eggs were fertilized and deposited and buried in the substrate. Burying eggs in sand and gravel is typical of darters in the *Percina* genus (Page 1983), however, *P. palmaris* appeared to have a specific affinity for cavities. We noted that all observed spawning occurred in substrate within spacious cavities formed by large boulders and bedrock slabs, possibly to provide additional protection from predation.

Etheostoma jordani

We noted 6 observations of *E. jordani* spawning across 4 snorkel surveys. Spawning was observed at 3 locations in the Conasauga River, with the earliest being May 25 and the latest being June 14. Previous research in Alabama has noted some differences in spawning periods between Piedmont *E. chuckwachatte* (formerly *E. jordani*) and Coastal Plains populations of *E. jordani* (Orr & Ramsey 1990), although peak spawning for both populations occurred from late April to late June. A study in Barbaree Creek in Alabama noted a similar spawning window, but identified a peak in early May (O'Neill 1980). We measured temperatures ranging from 20-23.5 °C during spawning in the Conasauga River, the warmest range among *Etheostoma spp*. observed in our surveys. Orr & Ramsey documented spawning in temperatures from 18-29.4 °C, far beyond the range that we observed, but noted a peak in spawning behavior from 21.1-23.9 °C.

We found *E. jordani* spawning in swift flows (~0.63 m/s), consistent with velocities reported in prior research (~0.55 m/s) in Opintlocco Creek in Alabama (Orr & Ramsey 1990). Spawning occurred in shallow areas with large cobble and boulder substrates. Like many darters in the *Nothonotus* subgenus (Page 1983, Near et al. 2011), *E. jordani* displayed egg burying behavior, depositing and fertilizing eggs in sand and gravel substrate. Males tended to stay in the same location in the shoal, often a patch of sand or gravel located in between or underneath large cobble or boulders. The male would fend off competing males that attempted to claim their territory. Once a female came within close proximity of the male, courtship would begin, with males closely following females until they reached substrate suitable for spawning. Although O'Neil (1980) noted females burying their body in the substrate as they were spawning, we did not observe this. Rather, the male moved its pelvic region towards that of the female positioned on top of sand and gravel and vibrated, depositing eggs within the substrate.

Cyprinella caerulea

We observed 3 instances of *C. caerulea* spawning across 3 snorkel surveys. We observed these on 11 May at our Holly Creek site, 28 May at our most downstream Conasauga River site, and June 4 at our most upstream Conasauga River site. Temperatures during spawning ranged from 16.4 - 23.3 °C. Our observations are consistent with a previous study using gonad inspection in the Conasauga River that suggested a peak spawning season of late May to early June (Krotzer 1990) as well as previous snorkel observations that noted spawning on 23 May and 1 June 1996 (Johnston & Shute 1997).

Spawning occurred in areas of the shoal with moderately low water velocity (mean = 0.19 m/s) at moderate depths (mean = 0.33 m). Substrate was characterized by sand, gravel, and silt bottoms, often with large woody debris. Like others in the *Cyprinella* genus, *C. caerulea* exhibits a crevice spawning strategy (Johnston and Page 1992). We observed male individuals (often the largest and most colorful in the area) guarding suitable crevices in logs, often chasing away other males that got close. Nearby females made passes over the crevice, turning sideways as they positioned their ovipositors facing the crevice depositing eggs. The male followed closely in a similar position, fertilizing the eggs along the crevice. Like Johnston and Shute (1997), we witnessed multiple females spawning in the same crevice but only one male. Our observations of *C. caerulea* differed from those of *C. trichroistia* and *C. callistia* in that *C caerulea* was only observed depositing eggs in log crevices, not large boulders or bedrock.

Cyprinella trichroistia

We observed 3 instances of *C. trichroistia* spawning across 2 surveys at a single site; our most upstream Conasauga River site. The first two of these occurred on 25 May and the latter occurred on 13 June. Water temperatures ranged from 20-23.5 °C. It was surprising that we only observed this 3 times over the course of our sampling, given that *C trichroistia* was an abundant minnow at most of our sites, barring the most downstream Conasauga River site. However, we did note the development of tubercles on males as early as 1 May, indicating that spawning may have begun earlier than our first observation. We noted similar breeding condition of males as late as June 29, again suggesting a spawning season beyond what we observed snorkeling. Nonetheless, our range fell within the late-April to late-July or August spawning period suggested by previous research based gonadal development in the Coosa River system in Alabama (Scott and Mayden 2008).

We observed C *trichroistia* spawning in crevices within large boulders and bedrock in deep (mean = 0.53 m) shoal habitats with swift water velocities (mean = 0.59 m/s, max = 0.76 m/s). Behavior was congruent with that described above for *C. caerulea*, with males sparring around crevices suitable for spawning. Females in the area would make passes along the crevice, with the dominant male following and fertilizing eggs as they were deposited. Although spawning in crevices in logs has been reported for *C. trichroistia*, we did not observe this. This may be due to the lack of large wood within the swift areas of the shoal where we observed spawning.

Cyprinella callistia

We observed two instances of *C. callistia* spawning, both of which occurred in mid to late May. One of these occurred on 19 May in our middle Conasauga River site and the other occurred on 25 May at our most upstream Conasauga River site. Water temperatures during these two samples were 16 and 18 °C, respectively. Although *C. callistia* was one of the most abundant minnows in all of our sites and was observed during >90% of surveys, we obtained limited observations of spawning. We did, however, note nuptial condition including tubercle development, spawning coloration, and/or presence of ova from late March to late June. This is consistent with previous research from Turkey Creek in Alabama that noted a spawning period from April through July (Ferguson 1990).

We observed *C. callistia* spawning in crevices in large boulders and bedrock at depths of 0.61 and 0.915 m (the deepest spawning observation in our study) and in moderate velocities (0.23-0.42 m/s). The behavior was similar to that of the other *Cyprinella sp.* observed in this study, with large males sticking close to a crevice and fending off other males. Nearby females made runs over the crevice to deposit their eggs, while the male followed closely to fertilize. During one of these spawning occurrences, we noted groups of *C. trichroistia* engaged in sparring around the crevice used by *C. callistia*. Although it is possible that interspecific competition for spawning habitat may occur between these two taxa, we did not see any direct evidence for competition or interference between the two groups.

Cyprinella venusta

We observed 2 instances of *C. venusta* spawning, occurring within 2 days in mid-June. These occurred in Holly Creek and the middle Conasauga River Site on 12 June and 14 June respectively. Water temperatures were 21 and 22 °C. Like the other *Cyprinella spp*. in our study, it is unlikely that we captured the range of the spawning season with our limited spawning observations. Unlike the other members of this genus, *C. venusta* tended to have lower abundances within the habitats that we surveyed, possibly due to their preference for deeper, slower moving water. Consequently, we have little record of the nuptial condition over the course of the spawning season. Previous research from Mississippi has suggested an extended spawning season for *C. venusta*, ranging from March to early-October (Heins and Dorsett 1986). However, *C venusta* has been shown to vary substantially in life history across its large range (Heins and Baker 2018).

We observed *C. venusta* spawning in moderate depths from 0.69-0.74 m and slow water velocities (mean = 0.12 m/s). Spawning occurred in crevices in logs positioned in deep eddies at the bottom of shoal areas over sandy substrates. *Cyprinella venusta* displayed similar behavior to the other *Cyprinella* species, but we did not note any sparring between males. In both instances, the male that was seen spawning was much larger than the other individuals in the area. Females and males made passes over the crevice, depositing and fertilizing eggs. We observed multiple females spawning in the crevice with a single male. Like *C. caerulea*, we only witnessed *C. venusta* spawning in crevices in wood, not rocky substrate.

Etheostoma brevirostrum

We observed two *E. brevirostrum* spawning events during a single survey on 1 May. These occurred at our most upstream Conasauga River site which was the only site in our study with apparent sizeable populations of *E. brevirostrum*. The water temperature during this survey was 15 °C. Our observations were similar to previous observations in 1996 from the same locality in the Conasauga River (Johnston and Shute 1997). This previous study noted spawning on 25 May while the water temperature was 19 °C. Our observations of habitat were similar to this previous study as well. Johnston and Shute (1997) note that *E. brevirostrum* spawns in shallow (0.22 - 0.34m) cobble and boulder substrates in swift velocities (0.54 - 81 m/s). We noted spawning in similar substrates at depths of approximately 0.5m. One of our observations was in fast flowing water (0.70 m/s), however, the other instance occurred in slower moving water than expected (0.16 m/s). A study from the Etowah River system (Anderson 2009) showed spawning from late-April to mid-May, consistent with our observations. However, they noted that *E. brevirostrum* consistently spawned in velocities lower than 0.23 m/s, indicating that habitat use can be quite variable. Our observations of behavior were consistent to the previous studies as well, with *E. brevirostrum* using an egg attaching strategy similar to that of *E. coosae* and other snubnose darters (*Ulocentr*a) (Page 1985).

Percina nigrofasciata

We observed two instances of *P. nigrofasciata* spawning during two surveys in early-May in our Holly Creek site. Water temperatures during spawning ranged from 16-16.4 °C. Both spawning events occurred in shallow areas (~0.3m) in moderately fast current (0.53 – 0.65 m/s) in areas with cobble, gravel, and sand substrates. *P. nigrofasciata* exhibited egg burying behavior like others in the *Percina* genus, with males sticking close to females until a spot suitable for spawning was chosen. A female would lie on the substrate as the male positioned itself over top, at which point both fish vibrated vigorously, disturbing the sand and gravel as eggs were deposited and fertilized, then buried by the moving substrate.

Due to our limited observations, it is difficult to infer the range of habitat and environmental conditions in which Upper Coosa populations of *P. nigrofasciata* spawn. Like *C. venusta*, *P. nigrofasciata* has a broad geographic range with significant phylogenetic variation (Hayes and Piller 2018). Even within small geographic areas, studies have showed divergence in life history among populations (Hughey et al. 2012). Consequently, natural history studies from other localities may not yield information that is generalizable to the Conasauga. This is likely true for many taxa, but is especially apparent in *P. nigrofasciata*. For instance, studies from the Choctawhatchee River drainage in Florida suggested a spawning period spanning from December to September (Hughey et al. 2012). In contrast, studies from Alabama and Louisiana have suggest spawning periods as short as two months (Mathurs 1973, Boschung & Mayden 2004).

Percina kusha

We observed two pairs of *P. kusha* spawning during a single survey on 4 June at our most upstream site in the Conasauga River. The water temperature on this day was 19 °C. Although our observations were limited to a single day, these observations are the second recorded observation of *P. kusha* spawning in-situ and confirm the behavior noted by Anderson (2009) and extending the known spawning season. These observations help confirm presumptions that *P. kusha* spawns a similar manner to related species in the *Percina* genus. Anderson (2009) observed spawning in *P. freemanorum* (formerly *P. kusha*) from mid-April to mid-May, earlier than our observations. In addition, previous research on *P. smithvanizi*, a congener to *P. kusha*, suggests that spawning occurs from late March to July and ovulation occurs when water temperatures range from 12 to 15.5 °C (Boschung & Mayden 2004). Our observations fall within this time period, although the low abundances of *P. kusha* in our sites may have prevented us from observing spawning or nuptial characteristics over a longer period.

We observed both pairs of *P. kusha* spawning in similar habitats in a run above a shoal. The water was approximately 0.5 m deep and the velocity was slow (0.07-0.09 m/s). Substrate consisted of a mixture of sand and gravel interspersed with cobble. We did not observe any competition between males, and pairs were isolated from other *P. kusha* individuals. Males followed females closely, as the female moved around looking for a spot appropriate for spawning. Like others in the *Percina* genus, spawning was characterized by egg burying. After a site was deemed appropriate for spawning, the male mounted the female, moving its pelvic region towards the ovipositor of the female. Both fish vibrated vigorously as the female sunk into sand and gravel substrate, depositing eggs and the male fertilized them. After spawning, the individuals appear to stay near their mate, but we did not see additional spawning events by the same pairs.

Cottus carolinae

We did not directly observe *Cottus carolinae* spawning. However, we were able to estimate the approximate time of spawning due to collections of females before and after spawning, determined by the presence or absence of eggs. In our Shoal Creek site, we collected gravid individuals on March 10 and were unable to find any gravid individuals on March 21, indicating that it is likely that the Shoal Creek population spawned in mid-March, during which time the water temperatures warmed from 11 °C to 17 °C. This is substantially later than the December and January spawning timing observed in the Roaring River watershed. However, that study noted that there is likely substantial geographic variation in spawning initiation, driven by photoperiod and temperature regimes among streams (Gebbhard and Perkin 2017).

Discussion:

Our study of the life history of fishes in the Upper Coosa yielded several key insights including 2 previously unpublished accounts of in-situ spawning (*E. stigmaeum* and *P. palmaris*). Furthermore, we observed spawning of 13 additional taxa and compared them to previous studies, expanding understanding of their life histories. A community approach to studying life history highlights the diversity of strategies among taxa with substantial variation in spawning timing, behavior, and habitat use. Recognizing the diversity of life histories within riverine communities can lead to more comprehensive conservation actions that protect all essential habitats (Williams et al. 2011). Despite the importance of life history research, scientists have observed a decline in natural histories studies in recent decades (Tewksbury et al. 2014, Able 2016). In particular, life history research for small-bodied stream fishes remains sparse. As we observed in our comparisons to previously published studies, information for many taxa is limited to a single study with limited spatial or temporal extent or aquarium observations of spawning behavior.

Obtaining comprehensive life history information for fishes presents several challenges. Firstly, freshwater fish life histories can vary substantially between populations across geographic gradients due to environmental differences (Blanck & Lamouroux 2007). We would also expect timing of spawning to vary among streams with different temperature regimes (Hubbs 1985). In addition, genetic divergence may lead to life history differences between populations within small geographic areas (Hughey et al. 2018). This creates a particular challenge for fish with broad geographic ranges and high intraspecific genetic diversity, as it is unlikely that life history traits such as spawning timing and reproductive investment are uniform across the range. Consequently, researchers should use caution when using life history information based on single studies. Although it is a labor-intensive undertaking to study multiple populations of fishes, it can yield important information about the breadth of life histories within taxa.

Rare fishes present their own challenges to life history research. The southeastern U.S. supports numerous endemic fish species with limited ranges, many of which have small population sizes and are listed under the Endangered Species Act as Threatened or Endangered (Elkins et al. 2019). Small population size limits opportunities for in-situ observation, and protected status largely precludes specimen collection for genetic or physiological analysis. For example, the Amber Darter (*Percina antesella*), a Federally Endangered darter endemic to the upper Coosa River system, has rarely been observed spawning in-situ and there are no published accounts of its spawning behavior and habitat. Furthermore, remaining *P. antesella* populations in the Etowah and Conasauga Rivers are in decline (Freeman et al. 2017, Stowe 2020) and the species has not been observed in monitoring efforts in the Conasauga River in recent years. Understanding the spawning behavior of this fish may yield insights important to its conservation. However, observing *P. antesella* spawning is a difficult task due to low abundances, high turbidity in its limited range, and its seemingly short spawning window. Furthermore, collection-based life histories studies like ovarian analyses are unfeasible due to its low abundances and protected status. There are many imperiled freshwater fish taxa like *P. antesella* that are either difficult or impractical to study in detail. Consequently, it may be a priority to conduct life history research on rare fishes before they decline to the point that the study becomes unfeasible.

Despite the challenges, we encourage research on the life histories of understudied fishes. We have identified 3 key areas of life history research which we believe would greatly benefit ecology and conservation alike:

1) *Intraspecific variation in life histories*. Understanding how life histories of fishes vary through space and time yields important information about the breadth of strategies across populations. Studies or combinations of studies across a broad geographic range of a species can provide more accurate information to researchers and conservationists using life histories in analyses and conservation management. Studies that show large amounts of variation across a taxon's range point to the need for system-specific information. Likewise, studies that show little variation allow one to have greater confidence that data are representative. Studies across gradients of human impacts may indicate the degree to which life histories are able to adapt to stressors such as hydrologic alteration and land-use effects. Fishes with high plasticity may be more likely to persist in altered systems (Hamel 2020). Likewise, multi-year studies may elucidate the degree of plasticity that taxa have in response to variation in stream temperature and flow regimes. This plasticity is likely to influence a taxon's resiliency in the face of climate change, which could create mismatches in phenology between photoperiod and spawning temperature ranges (Woods 2022).

2) *Behavioral observations*. Identifying seemingly subtle behavioral idiosyncrasies between related taxa may be key to understanding varying habitat affinities as well as differential success in changing environments. For instance, in our study we noted that *Percina palmaris* buried eggs in the sand and gravel much like other *Percina* species. However, it also seemed to prefer cavities in between and underneath large boulders and bedrock slabs, a behavior not commonly reported for its relatives. This behavior may explain its apparent affinity for

shoals with large rocky substrate, such as the streams in the Blue Ridge physiographic province. Furthermore, it may indicate that it is especially susceptible to population declines as a result of the loss of those cavity habitats via sedimentation. It is likely that there are other understudied species with conservation relevant behaviors that differ from their close relatives.

3) *Reproductive investment*. Quantifying reproductive investment in small-bodied shoal fishes is difficult, yet traits such as egg size and fecundity are an integral component of life history strategies (Winemiller & Rose 1992) and widely used in functional trait ecology (Laureto et al. 2015). Challenges in quantifying variables such as fecundity and total reproductive investment arise due to the uncertainty of the number of spawning events for many taxa. Although some families are known to deposit single clutches at once (e.g., *Cottidae*), others like *Leuciscidae* and *Percidae* spawn multiple times over the course of a season. In the past, researchers have used counts of all mature or ripe ova as well as smaller developing ova present to estimate fecundity (Page 1983), however, this fails to account for eggs already deposited as well as eggs that are not deposited and then reabsorbed by the female. Although clutch size is relatively easy to determine, identifying the number of spawning bouts for small-bodied shoal fishes is difficult but necessary to determine fecundity (Heins & Baker 1993, Heins & Brown-Peterson 2022). Consequently, researchers should use caution when using fecundity estimates as reported in datasets for species with unknown numbers of spawning bouts.

It is as important as ever to pursue natural history research of fishes. Fishes and other freshwater taxa are facing unprecedented declines (Tickner et al. 2020) and an understanding of life histories plays an important role in identifying threats to fish populations and creating effective conservation action. However, this work is resource intensive. For example, we mostly observed spawning only on a few occasions even for commonly occurring taxa, and despite visiting multiple sites over a period of 5 months. To comprehensively capture spawning periods could require multiple years of intensive surveys; extending such work over larger regions would likely be prohibitively costly. However, we are in a unique position today where collaboration and community engagement are more feasible than ever. Crowdsourced information has become an invaluable tool for natural history research. For instance, data from social media platforms such as iNaturalist have supported hundreds of natural history studies across a broad range of taxa. Examples include studies of seasonal distributions of ticks (Cull 2022), monitoring rare butterflies in the tropics (Mesaglio et al. 2021), and enhancing understanding of fish species richness in coral reefs (Roberts et al. 2022). Although deriving fish spawning information such as timing and behavior from a platform like iNaturalist is difficult, studies of freshwater fish life history may be able to leverage passionate communities of fish enthusiasts such as the North American Native Fishes Association (NANFA). The NANFA Facebook page contains many images and descriptions of spawning across a broad geographic range. Encouraging hobbyists to record observations with date and temperature data and aggregating these observations in a database such as the Tennessee Aquarium Conservation Institute's "Freshwater Information Network" (TNACI) could substantially expand information on freshwater fish spawning phenology and plasticity. Creative and collaborative efforts such as this may yield great advancements in our understanding of fish life history responses to environmental cues as well as variation across populations.

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Tables:

Table 2.1**:** Spawning Information from 67 observations across 30 snorkel surveys in 2021. Data are range of dates when spawning was observed, temperatures, depths, and water velocities. Entries show the mean ± standard deviation and ranges of observed values in parentheses.

Species	**Date Range**	**Temperature (°C)**	**Depth (m)**	**Velocity (m/s)**	**No. of Observations**
Cyprinella caerulea	11 May – 4 June	19.6 ± 3.5 (16.4 – 23.3)	0.33 ± 0.08 (0.26 – 0.42)	0.20 ± 0.34 (0 – 0.59)	3
Cyprinella callistia	19 May – 25 May	18.0 ± 2.8 (16 – 20)	0.76 ± 0.22 (0.61 – 0.92)	0.32 ± 0.14 (0.23 – 0.42)	2
Cyprinella trichroistia	25 May – 13 June	21.2 ± 2.0 (20 – 23.5)	0.53 ± 0.24 (0.30 – 0.77)	0.59 ± 0.23 (0.33 – 0.76)	3
Cyprinella venusta	12 June – 14 June	21.5 ± 0.7 (21 – 22)	0.72 ± 0.04 (0.69 – 0.74)	0.12 ± 0.14 (0.02 – 0.23)	2
Etheostoma brevirostrum	1 May	15	0.5 ± -.08 (0.44 – 0.56)	0.43 ± 0.39 (0.16 – 0.71)	2
Etheostoma coosae	11 April – 14 June	17.1 ± 2.3 (14.5 – 22)	0.39 ± 0.14 (0.22 – 0.61)	0.45 ± 0.24 (0.16 – 1.23)	22
Etheostoma jordani	25 May – 14 June	22.6 ± 1.4 (20 – 23.5)	0.22 ± 0.10 (0.11 – 0.37)	0.63 ± 0.17 (0.36 – 0.87)	6
Etheostoma scotti	18 April	16	0.12	0.22	1
Etheostoma stigmaeum	11 April – 2 May	16.4 ± 0.7 (14.5 – 16.7)	0.49 ± 0.09 (0.32 – 0.59)	0.35 ± 0.15 (0.05 – 0.58)	9
Lepistosteus osseus	19 May	16	0.10 ± 0.06 (0.06 – 0.16)	0.62 ± 0.15 (0.46 – 0.76)	3
Micropterus henshalli	14 June	22			1
Percina kusha	4 June	19	0.50 ± 0.01 (0.50 – 0.51)	0.08 ± 0.01 (0.07 – 0.09)	2
Percina nigrofasciata	2 May – 11 May	16.2 ± 0.28 (16 – 16.4)	0.30 ± 0.01 (0.30 – 0.31)	0.59 ± 0.08 (0.53 – 0.65)	2
Percina palmaris	1 May – 13 June	17.8 ± 3.89 (15 – 23.5)	0.40 ± 0.19 (0.10 – 0.61)	0.50 ± 0.17 (0.28 – 0.81)	7

Table 2.2: Egg size and clutch size information from 63 individuals across 9 taxa collected in 2021**.** Data are the clutch sizes, single egg masses in milligrams, and egg diameter in millimeters. Entries show the mean ± the standard deviation, ranges of observed values in parentheses, and number of individuals counted.

Species	**Clutch Size**	**Egg Mass (mg)**	**Egg Diameter (mm)**
Cottus carolinae	56 ± 13.2 (41-73) N=5	3.21 ± 1.0 (1.52-4.13) N=5	2.63 ± 0.35 (2.23-3.08) N=6
Cyprinella callistia	226.3 ± 31.7 (207-263) N=3	0.39 ± 0.05 (0.36-0.43) N=2	1.43 N=1
Cyprinella trichroistia	332.3 ± 135.0 (112-569) N=8	0.54 ± 0.39 (0.26-1.14) N=5	1.28 ± 0.18 (1.05-1.43) N=4
Etheostoma coosae	43.5 ± 10.0 (20-59) N=13	0.34 ± 0.15 (0.1-0.775) N=13	1.41 ± 0.11 (1.24-1.53) N=9
Etheostoma jordani	46.1 ± 23.2 (18-92) N=11	0.62 ± 0.18 (0.36-0.84) N=9	1.71 ± 0.14 (1.48-1.81) N=5
Etheostoma stigmaeum	53.5 ± 27.0 (26-98) N=6	0.29 ± 0.07 (0.20-0.38) N=6	1.43 ± 0.26 (1.22-1.81) N=4
Notropis stilbius	297 ± 159.8 (184-410) N=2	0.15 N=1	0.95 N=1
Percina nigrofasciata	100.3 ± 37.6 (21-132) N=8	0.54 ± 0.14 (0.34-0.69) N=8	1.57 ± 0.46 (1.18-2.33) N=5
Percina palmaris	96.6 ± 52.0 (21-148) N=7	0.85 ± 0.23 (0.52-1.13) N=8	1.79 ± 0.13 (1.65- 2.00) N=6

Figures:

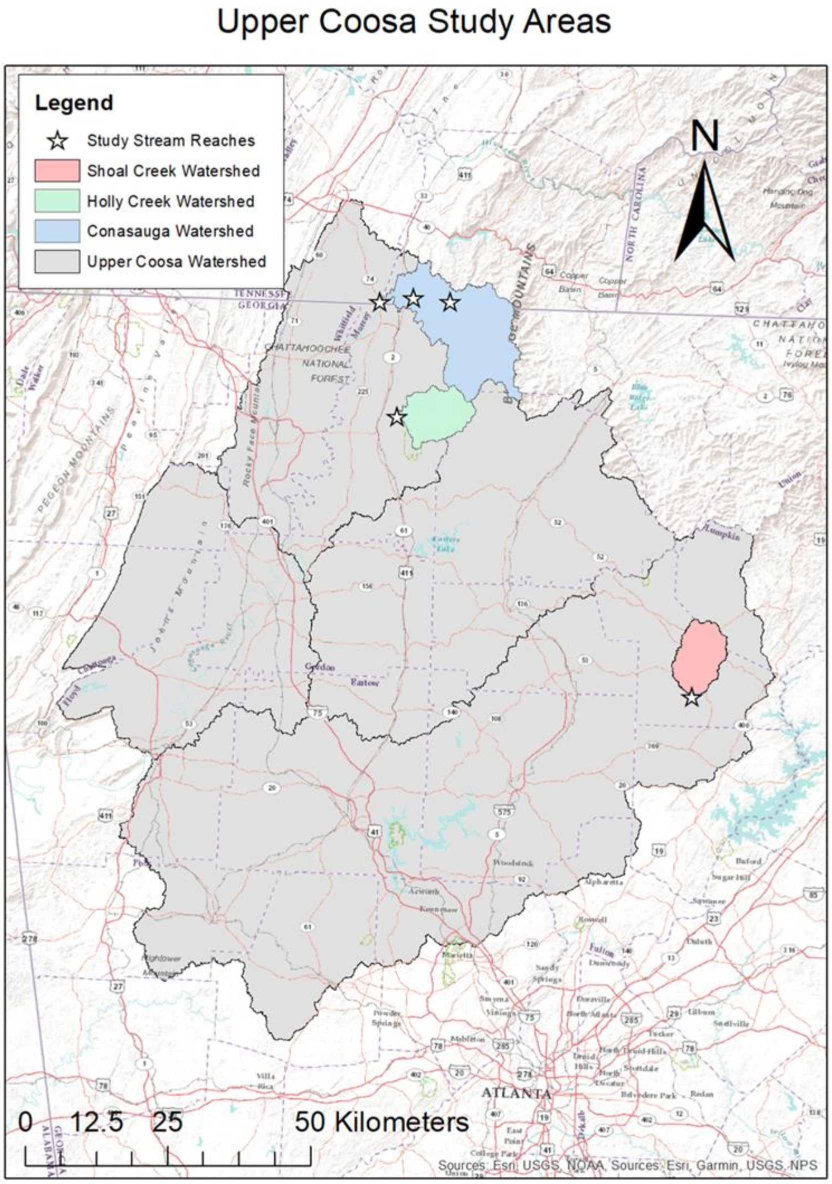

Figure 2.1: Map of 5 snorkel survey sites in the upper Coosa River watershed. Sites shown with stars. HUC 8 watersheds for the Conasauga, Coosawattee, Oostanaula, and Etowah Rivers (together making the Upper Coosa) shown in grey. Upstream watersheds of each site are shown in red, green, and blue for Shoal Creek, Holly Creek, and Conasauga River respectively.

CHAPTER 3

CHANGES IN CONASAUGA RIVER FISH COMMUNITY AND RELATIONSHIPS BETWEEN TRAITS AND VARIABLE TEMPORAL TRENDS

Introduction:

Ecologists have long been interested in understanding how environmental conditions shape species assemblages. In recent years, there has been a growing focus on how natural and anthropogenic stressors can change community structure (Sirami et al. 2017). For instance, systems have shown shifts in functional assemblages through time in response to stressors, which in turn can alter ecosystem functions such as metabolic processes (Handa et al. 2014), trophic web stability (Kaur & Dutta 2019), and nutrient cycling (Lü et al. 2020). Changing community structure also can alter biotic interactions and lead to decreased species richness and a decline in taxonomic diversity (Sandor et al. 2021). Furthermore, community ecology analyses have proven useful in determining which species are resilient to change and which species may be particularly susceptible to declines (Kopf et al. 2017). Community analyses have benefits over single-species models because they can identify shared responses (Chapman and Purse 2011), shifts in functional diversity (Laureto et al. 2015), and directly compare trends among taxa (Cazalis 2022).

Time-series records of species identities and abundances at a consistent set of sites are an invaluable tool to investigate how communities are changing in response to environmental conditions. For instance, decades of data from a French monitoring program have allowed scientists to assess changing distributions of fishes in response to climate change (Grenouilet & Comte 2014). Although resource intensive, time series of community data allow researchers to quantify temporal trends and relate variation between samples to environmental variables (Wenger et al. 2022). Furthermore, time series datasets are more suitable than synoptic surveys across gradients when investigating the role of environmental variables on changes in community composition (Damgaard 2019). Samples taken at one or two points in time may be adequate to answer questions about species presence and variation across multiple sites but do not inform understanding of temporal stochasticity of community composition and abundances. Consequently, time series data are useful in resolving whether differences reflect directional change or expected variation in a steady-state system (Matthews & Marsh-Matthews 2016).

Rivers support dynamic communities of fishes, invertebrates, plants, and microbes that are constantly shifting in response to natural environmental variation such as flow and temperature. However, riverine communities are susceptible to human activity including dams, land-use changes, and pollution which can influence long-term community trends in abundance and composition. Fish, in particular, are susceptible to stressors that influence their feeding habits, reproductive success, and survival. Fish communities have been shown to shift at large time scales in response to climate change (Comte et al. 2021), deforestation (Brejao et al. 2018), and river regulation (Pereira et al. 2021) as well as to vary at smaller time scales in response to disturbances such as drought (Malone et al. 2021) and resource pulses (Bailey & Moore 2020). Traits influence how fish populations respond to disturbances (McLean 2019). For instance, Troia et al. (2019) show that traits such as body size and dispersal ability may mediate a species' ability to seek refuge in low order streams under climate change.

Here, we use a long-term fish monitoring dataset from six sites within the Conasauga River to test hypotheses related to community changes through time. The Conasauga River in the headwaters of the Coosa River Basin is renowned for its aquatic biodiversity (Freeman et al. 1996). It is home to over 75 fishes (Walters 1997) and 25 mussel species, many of which are imperiled. Studies have shown a decline in several fish taxa in recent years (Hagler et al. 2011, Freeman et al. 2017, Stowe et al. 2020) as well as longitudinal declines in insects (Baker 2012) and an aquatic macrophyte associated with habitat quality (Argentina et al. 2010). Consequently, the Conasauga River has been identified as a priority target for conservation measures (Elkins 2019). Several hypotheses have been proposed to explain the decline in fishes, although none have been identified as the primary driver. These include habitat degradation stemming from sedimentation and eutrophication caused by agricultural practices in the watershed, reduced recruitment due to anomalous flows, and increased mortality due to pollution. Previous analyses of fish in this system have focused on single species models, with less investigation at the whole-community scale. A community analysis may provide novel insight into mechanisms of decline in stressed systems by identifying community response to environmental variables such as flow. It may also be possible to identify species traits associated with differential temporal trends in abundance that underlie changes in community composition.

First, we investigate trends in fish community size and composition as well as responses to variable flow conditions over a 24-year time frame using a community turnover metric proposed by Shimadzu et al. (2015). Specifically, we hypothesized that anomalously low and high flows during the spawning and rearing season, two critical periods for fish recruitment (Craven et al. 2010), would impact the total and relative abundances of taxa (Table 3.1). In addition, we used community turnover to investigate directional change in community composition and size over the course of the time-series. Secondly, we investigated the influence of fish traits on trends in abundances through time. We hypothesized that fish with certain traits associated with postulated stressors would be more likely show declines through time (Table 3.2).

Methods:

Study Area

The Conasauga River is located in northwestern Georgia and southeastern Tennessee where it flows from the steep landscape of the Southern Blue Ridge geographic province into the lower-gradient Ridge and Valley province. Along with the Etowah and Coosawattee Rivers, the Conasauga River forms the headwaters of the Coosa River in the Mobile River basin, a major basin renowned for its high aquatic biodiversity (Abell et al. 2008). The river is home to >75 native fishes (Walters 1997), including 4 taxa listed as Threatened or Endangered under the Endangered Species Act, as well as 16 additional taxa identified as being of conservation concern by the states of Tennessee and Georgia (GDNR, TWRC 2018). The lower reaches of the Conasauga River within the Ridge and Valley province is heavily impacted by human activity including intensive agriculture, industry, and urban development which may negatively impact freshwater biota (Wenger et al. 2007).

Fish Data

Fish count data were obtained from a long-term monitoring program initiated in the mid-1990s to track potential faunal changes in the Conasauga River in Murray and Whitfield counties in northwest Georgia. The fish community in a series of shoals (shallow areas of the river with swift currents) was sampled in the autumn of each year when flows were sufficiently low to effectively seine most of the available habitat. Fish were captured using an 8’ by 6’ seine using kick sets, in which two people held the net on the riverbed while others upstream used their feet to displace substrate while walking downstream, causing fish to swim into the net (Stowe 2020). In areas with inadequate flow for this method, two people moved the net through the water column to capture fish. Captured fish were identified, counted, and released. We attempted to use the same number of seine sets for each site across all years to limit variability in sampling effort. To limit the influence of spatial variability in yearly sampling locations, a subset of 6 sites, representing the core of the sampling reach, was selected for this analysis due to the completeness of annual samples over the period of monitoring (1996-2019). No surveys were completed in 2009 nor 2011 due to high flows. In addition, only five sites were sampled in 2013, 2016, and 2017, and only two sites were sampled in 2018. A total of 113 surveys in 19 years were used in this analysis. Thirty-two taxa were included in this analysis, accounting for >90% of the individual fish caught during the monitoring program.

Flow Data

Daily discharge data for the Conasauga River at GA Highway 286 (USGS Stream Gauge 02384500) were obtained using the R package “waterData” (Ryberg & Vecchia 2012). Following Sabo and Post (2008), daily discharge values were Fourier transformed using the R package ‘discharge’ to obtain a normalized seasonal discharge signal (i.e. the expected discharge for each day in a year; Figure 2). Daily anomalies were defined as the residual between the normalized discharge of any given day and the seasonal discharge signal. To calculate flow variables used in regressions, we summed the number of days with anomaly values above (high flows) or below (low flows) the 90th percentile and 10th percentile of all anomaly values across all years. We summed high- and low-flow anomalies within the spawning (March-June) and rearing (June-September) seasons for each year, 1996-2019. All flow variables (spawning high flows, spawning low flows, rearing high flows and rearing low flows) were scaled to a mean of 0 and SD of 1. In addition, we calculated lagged flow variables (i.e. flows from the year prior to annual sampling) to test for delayed responses in community change.

Trait Data

To investigate the effects of species-specific traits on temporal trends in abundance, we assigned values for 10 traits for each species representing the variation in behavior and physiology including spawning strategies, lifespans, sizes, and feeding behavior (Table 3.3). Fish trait data were obtained from Fishes of Alabama (Boschung and Mayden 2004), Fishes of Tennessee (Etnier and Starnes 1993), the FishTraits database (Frimpong & Angermeier 2009), and additional observational study (Chapter 1). When literature sources reported multiple values for a continuous trait of a particular taxon, we used the median value for the analysis. Trait variables were a mix of categorical (e.g. spawning method), binary (e.g. insectivorous versus not), and continuous (e.g. maximum length). All continuous variables were scaled to a mean of 0 and SD of 1. We also defined a trait for relative tributary abundance in the Conasauga River watershed using records from a collection database maintained by the Georgia Museum of Natural History. This was defined as the proportion of total abundance for each taxon that was caught in tributaries to the Conasauga River (i.e. values near 1 indicate most individuals of a taxon were caught in a tributary, whereas a value of 0 means all of the individuals of a taxon were caught in the mainstem).

Community turnover and flow

We calculated temporal turnover values, indices of community change, following Shimadzu et al. (2015). This framework decomposes the total turnover (“D”) into the change in composition (i.e. taxa-specific proportional abundances, “D1”) and the change in total community size (i.e., number of individual fish caught, “D2”). Change in community size (D2) from time t to time u is defined as:

$\log(lambda(u)/\ lambda(t))$, where

$lambda$ = sum of expected abundances across all taxa (Shimadzu et al., 2015).

Change in community composition (D1) is defined as:

$-\sum((\log(p_i(t)/\ p_i(u))*p_i(t))$, where

p_i = the proportional abundance of taxon i (Shimadzu et al., 2015).

We calculated a mean community composition in each year by averaging abundances of taxa across 6 sites and then calculated annual turnover values (D, D1, D2). These turnover values are calculated relative to the first year in the time series (Shimdazu et al., 2015), and so in our case represent shifts in community composition and community size in each year relative to 1996. Then, we calculated the interannual change in turnover, or ΔD, to correlate community change with year-specific flow variables.

We used these turnover values to ask (1) whether there was a temporal trend in community turnover, and (2) whether year to year changes in community composition or size were related to flow variables. To answer the first question, we used linear regression with year as a predictor variable to measure temporal trends in D, D1, and D2.

$$D_t \sim \beta_0 + \beta_1 \mathrm{Year}_t \qquad (1)$$

Where D_t is the turnover value in year t, β_0 is the intercept parameter, and β_1 is the slope associated with year t.

To investigate the impact of flow on the year-to- year change in community turnover, we used a linear model as follows:

$$\Delta D_t \sim \beta_0 + \beta_1 F_t + \beta_2 S_t \qquad (2)$$

Where ΔD_t is the change in turnover value between year t and the previous year t -1, β_0 is the intercept parameter, β_1 is the parameter describing the effect of flow variable F, and β_2 is the parameter describing the effect of discharge S during sampling in year t. Flow variables (F) are year-specific (e.g. magnitude of high flows during the rearing season). S_t is the mean discharge across days sampled in each year and was included to account for variable capture efficiency under different flow conditions.

Trait regressions

We investigated taxa-specific trends in abundance through time as well as the influence of traits on these trends using a generalized linear mixed model adapted from Miller et al. (2019). We used counts as a response variable and included a fixed effect for year to investigate the overall trend in abundances through time. We included a random intercept for site to account for differences among shoals in fish abundance. We included a random intercept for taxon to allow different species to have different overall abundances. To investigate the influence of traits on trends through time, we fit a model with a fixed effect of trait as well as an interaction between trait and year. This interaction effect is the target of this analysis and indicates the degree to which a taxon’s trait value influences its predicted abundance through time. The distributional assumption for the count model is as follows:

$$Y_{ijkl} \sim NB(\mu_{ijkl}, \kappa) \qquad (3)$$

where Y_{ijkl} is the observed count during year i, for trait status j, at site k, and for taxon l that follows a negative binomial distribution with an expected value of μ_{ijkl} and an overdispersion parameter (κ). In the model, a log link was used so as to represent the log of the expected value, which is η_{ijkl}, in a linear equation:

$$\eta_{ijkl}=\beta_0+\beta_1 C_i+\beta_{2.}T_j+\beta_{3.}(C_i T_j)+a_k+b_l, \qquad (4)$$

where β_0 is an intercept parameter, β_1 is the fixed slope for the effect of i^{th} sampling year (C), β_2. is the adjustment for j^{th} trait level (T), β_3. is the slope change for the trait-year interaction term, a_k is the random intercept adjustment for each site k, and b_l is the random intercept adjustment for each taxonomic group l. Random effects for site and taxon were assumed to be normally distributed (not shown).

We tested multiple distributions common to regressions with count data, including Poisson, negative binomial, zero-inflated Poisson, and zero-inflated negative binomial. We selected zero-inflated negative binomial as the top performing model based on AIC comparison. We used this distribution for all regressions in this analysis. Trait models were fit using the R package “glmmTMB” (Brooks et al. 2017). In addition, we ran this same model using subsets of taxa in two families (Percidae and Leuciscidae) to investigate whether the effects of trait were consistent among different taxonomic groupings.

We originally included a random slope for each taxon; however, when a year-trait interaction was included, our models would not converge. Consequently, we removed the random slope, resulting in fitted models that treat species sharing trait values as having the same slope. However, we do report random slopes from the model not containing a trait-year interaction to compare trends through time among taxa irrespective of trait values.

Results:

Fish abundances, variation in traits, and streamflow variability.

Taxa-specific abundances within a sample ranged from 0 to 743, with a mean of 16.02 and a median of 3. Of 3840 total counts of taxa within a sample, 1321 were 0 (i.e. we did not catch that species in the survey). Mean counts within a sample for individual taxa ranged from 0.06 (*Notropis xaenocephalus*) to 131.1 (*Cyprinella venusta*). Of the 32 taxa, 4 were present in every survey and 4 were caught in less than 10% of surveys. Trait values encompassed a wide range of life history strategies, including 6 different spawning methods, spawning timing ranging from January to July, fecundities from 29.5 to 24050, maximum ages from 2 to 10 years, and maximum lengths from 5 to 64 cm (Table 3.3). More than 90% of the included taxa were insectivorous, with herbivory and piscivory exhibited by 18.8% and 12.5% of taxa, respectively.

Normalized discharge values exhibited a seasonal signal with a peak in late winter and spring and a trough in late summer and early fall (Figure 3.2). Flow anomaly values (the day-specific departure from the seasonal signal) ranged from -0.53 to 0.87. Across the entire time series of flow anomaly data, there were 6178 days with positive flow anomalies (i.e. the flow was higher than expected for that day) and 7692 days with negative flow anomalies (i.e. the flow was lower than expected for that day).

Community turnover and flow

We found a significant negative trend in community turnover (D) through time ($p < 0.05$). Independently, both composition (D1) and abundance (D2) displayed negative trends, although the D1 slope was more strongly negative (Figure 3.3). Most of the change through time in D was accounted for by the change in D1.

We found little evidence that flow during the spawning and rearing seasons affected year-to-year changes in turnover values. Most parameter values for the effects of high and low flow had substantial overlap with 0 (Figures 3.4 and 3.5) except for two effects of high flow anomalies during the rearing seasons (Figure 3.6). We found weak evidence for a negative effect of high flows during the rearing season (June - September) on change in abundance (ΔD2) ($\beta_{1} =$ -0.224, 95% confidence interval = -0.503 – 0.054). Increased number of high flow days was associated with a decline in total community size in the corresponding year. Conversely, increased number of high flow days was also associated with an increase in community size (D2) in the subsequent year (β_1 = 0.191, 0.007 – 0.376).

Influence of Traits on Variable Trends in Abundance

Our mixed-effects regression of species-specific counts explained little of the variance in counts (R^2 ~ 0.10 in each model). However, we found evidence supporting an overall decline in counts through time. The parameter estimate for year (-0.0206, $p<0.05$) corresponds to approximately 2% decline in total counts each year, indicating an approximate 40% decline in total abundances from 1996 - 2019. However, we found that trends in abundance through time varied among species. Random slope parameters for species trends through time varied from -0.23 to 0.07 (Figure 3.7). Taxon-specific random slopes and random intercepts were weakly correlated (r = 0.50), indicating that rarer taxa were more likely to be declining through time.

We found that some traits were associated with differing declines through time, as indicated by β_3 whose 95% confidence intervals differed from zero (Figures 3.8 and 3.9). Fish that spawn in crevices showed greater declines than taxa with other spawning strategies (β_3 = -0.015, 95% confidence interval = -0.03 – 0.00; Table 3.4, Figure 3.8). Fish with diets that included insects were likely to be declining (β_3 = -0.028, -0.049 – -0.007) whereas we found no evidence of differential decline in piscivorous or herbivore/detritivorous fish (Table 3.5). We found a positive effect of body size, maximum age, and fecundity on trends through time (Figure 3.9), indicating that large bodied, long-lived fish with high reproductive output were less likely to be declining than other taxa. We also found a positive effect of spawn timing, indicating that fish that spawn later in the year were less likely to be declining (β_3 = 0.007, 0.001 – 0.013). Finally, we found that fish with greater presence in tributaries were more likely to be declining (β_3 = -0.005, -0.011 – 0.00).

We found some evidence that traits are associated with differential decline within the families Leuciscidae and Percidae, although fewer traits showed an effect than when analyzed across all taxonomic groups. Within Leuciscidae, we found a positive effect of fecundity on trends through time (β_3 = 0.010, 0.047 – 0.152). Within Percidae, we found a positive effect of body length (β_3 = 0.078, 0.030 – 0.127) and spawn timing (β_3 = 0.018, 0.010 – 0.026). These effects were consistent with those found at the community level (Table 3.5).

Discussion:

Our analysis of riverine fish communities across 6 sites and 19 years revealed substantial change in the total count of fish as well as the relative abundances of individual taxa. Contrary to our predictions, flow did not explain much variation in year-to-year patterns in community turnover. Generalized linear mixed models of fish counts revealed a decline (~2% a year) in total fish numbers at the study sites. However, this decline was not shared evenly across taxa. Some taxa showed a much steeper rate of decline than the average whereas 8 species increased in abundance over the sampling period. Including traits of each taxon in the model explained some of the variation in abundance trends. Several of these relationships were consistent with our hypotheses, but not all of our hypotheses were supported.

The application of Shimadzu et al's approach to community indices proved useful in separating components of the community (size and composition) that are intertwined in traditional indices such as Shannon-Weiner or Simpson. Importantly, we found that most of the turnover in the community was due to a change in composition. This supports our prediction that not all taxa are responding similarly to apparent stressors in the Conasauga River. In addition, separating community size and composition allowed us to test mechanistic hypotheses of fish community responses to high and low flow events (Table 3.1). For instance, ecologists have shown that abnormally high flows during the rearing season can decrease young of year survival due to physical displacement (Harvey 1987) and decreased feeding efficiency and growth (Haworth & Bestgen 2016). We hypothesized that all taxa would be negatively impacted by high rearing season flows because of shared rearing habitat and, consequently, the community size (D2) would decrease. Our results showed that these high flows reduced D2 in the corresponding year. However, we found that in the following year, D2 was likely to increase in response to the prior year's high flows. This may be explained by a "rebound effect" where fish populations were likely to increase following a decline. Potential causes for this include relaxed density dependence (Velez-Espino & Koops 2012) where decreases in populations free up resources, subsequently increasing survival and recruitment. In addition, it is possible that the decrease in community size following high rearing season flows is a result of flow-driven dispersal effect that changes local community abundance (Hedden and Gido 2020). During extreme high flow events, fishes may move from shallow shoal areas to seek shelter from extreme flow conditions. Re-aggregation of fishes within shoal habitats after long periods of high flow may occur over extended time periods. Consequently, fall shoal samples following high flows during the summer may yield fewer fish. Although we found evidence of high flow effects on fish community sizes, we found no effect of low flows on community size or composition during the spawning and rearing seasons. A review of previous flow studies shows that responses to spawning and rearing-season low flows can vary among taxa (Freeman et al. 2022). Consequently, it may be difficult to detect an effect in a whole-community analysis. Although we were unable to explain much variation in the fish community through time with flow, our analysis did reveal that the community is changing in composition and decreasing in size through time.

Freshwater fish declines in long-term monitoring datasets have been noted in many river systems across the world. For example, studies comparing extant with historical species occurrence data reveal shrinking distributions of native fishes in European (Mueller et al. 2018), Asian (Liu et al. 2019) and North American (Olden and Poff 2005) river basins. Causes of fish declines are not always obvious. Several studies have noted decline in fish populations following damming (Pringle et al. 2000, Pereira et al. 2021), but identifying the specific causes of decline in unregulated systems has proven difficult. Rivers often experience multiple stressors from human activity including urbanization, intensive agriculture, and industrial pollution (Birk et al. 2020, Tickner et al. 2020). Increases in urbanization (Wenger et al. 2008, Brown et al. 2009) and agricultural development (Diamond et al. 2002, Waite & Carpenter 2000) within a watershed can alter fish communities, decreasing species richness and favoring more tolerant taxa (Chen and Olden 2020). Furthermore, stressors from watershed-scale land-use impacts can compound with flow effects from climate change to alter fish communities (Comte et al. 2021, Walker 2019). Disentangling these effects can require large amounts of time-series abundance and environmental data. Despite these challenges, determining primary drivers of declines of fishes is an upmost priority and a pre-requisite for implementing effective conservation action.

Including traits in community analyses can be useful for identifying mechanisms by which fish communities are structured and changed (Poveda-Cuellar et al. 2021) and identifying which taxa are most susceptible to declines (Kopf et al. 2017). The effect of environmental stressors may depend on differences in biology among taxa. Winemiller & Rose (1992) describe a continuum between three life history strategies: opportunistic (small, short lived, early maturing), periodic (large, long lived, high fecundity), and equilibrium (variable size, low fecundity, high parental investment) and suggest that they are likely to respond differently to various disturbances. Life-history traits have been shown to influence variable trends through time among taxa in a community. For instance, researchers have shown that late maturing, larger fish are declining at faster rates in rivers in France and postulate that declines of periodic species stem from the slow recovery after perturbations in human-altered streams (Santos et al. 2021). Studies have demonstrated that traits influence fish community and population response to a wide range of human actions, including damming (Pereira et al. 2021), stochastic flow events (Malone et al. 2022, Hitt et al. 2020), land-use change (Zhang et al. 2019), and stream warming (Stefani et al. 2020).

Our analysis of the influence of traits on variable trends of fish abundances through time showed that shifts in the fish community may reflect differences in life history and physiology among taxa. We found that large-bodied, more fecund, and longer-living fishes associated with periodic strategies were less likely to show declines than smaller, short-lived taxa associated with equilibrium and opportunistic strategies. Researchers have postulated that small short-lived taxa with low dispersal ability along with small home ranges may be vulnerable to increasing degradation and fragmentation within rivers (Kopf et al. 2017). Small-bodied fish may be less suited to seeking out refuge in response to environmental events such as drought, flooding, or high temperatures. Furthermore, we found support for our hypothesis that sedimentation may be reducing the quality of spawning habitat for taxa that rely on specialized benthic substrate such as crevices and cavities. In contrast, fish that spawn broadly on benthic surfaces in shoals or that construct and clean nests are less likely to be declining.

We were surprised to find a negative relationship between relative tributary abundance and mainstem Conasauga River population trends. Our hypothesis was that tributary populations of fish could subsidize mainstem populations, preventing or masking declines. Source-sink and subsidy dynamics have been documented in fishes (Tsuboi et al. 2021) and macroinvertebrates (Milner et al. 2019) in flow-regulated rivers as well as fish in urbanized rivers (Waits 2008), but there is less research in stream systems heavily influenced by agriculture. Contrary to our prediction, we found that taxa with high tributary abundances were more likely to be declining in the mainstem. It is possible that within the mainstem, individuals of taxa that have high tributary abundances are a result of a spillover effect, where tributary populations are large enough that small numbers of individuals emigrate to mainstems. Spillover effects have been documented in fish in lakes (Zolderdo et al. 2018) and marine areas (da Silva et al. 2015) and it is likely to occur in stream populations as well. If tributary populations are declining and emigration to mainstems is declining as well, we expect that abundances in our mainstem Conasauga River surveys would decline.

Fish community analyses using fish time series presents numerous challenges. Even within extensive, multi-year datasets, it can be difficult to identify temporal trends due to large amounts of variation typically present in community data. Research has suggested that stream fish community changes may experience naturally occurring cyclic variation (Matthews & Matthews 2016). In this paradigm, communities have an “average” structure, but diverge from this from time to time before returning. Consequently, apparent directional trends at smaller time scales may represent natural departures or returns to a baseline state. Furthermore, it can be difficult to know the timeframe and frequency with which sampling must occur to be able to detect a reliable trend. Factors such as environmental stability (Death & Winterbourn et al. 1994) and generation time (Arkilanian et al. 2020) influence the amount of temporal variation present in community data. Consequently, efforts to collect time-series data should consider the context of the community to ensure appropriate timeframe and temporal resolution sampling. Nonetheless, time-series data are an invaluable tool for ecologists to detect trends in communities and disentangle environmental influences.

Our analysis using time-series data from Conasauga River shoal fishes has demonstrated methods that are useful in quantifying community change and identifying trends in trait assemblages. However, as mentioned above, a lack of additional data has created challenges for linking these community data to specific mechanisms. We recommend that researchers engaged in long-term monitoring of declining fish populations collect supplemental data to test hypothesized mechanisms of decline. For instance, monitoring of insect biomass in shoals would allow us to strengthen evidence for a decline in insectivores resulting from decreased food supply. Monitoring algal growth and sedimentation during spawning seasons can allow researchers to test the link between spawning habitat quality and recruitment. Furthermore, additional natural history study may be useful to identifying behavioral and physiological differences among taxa that influence their resilience to stressors.

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Tables:

Table 3.1: Hypotheses for the effects of three flow components of community turnover on freshwater fishes. Community response variables are changes in composition (D1, relative abundances among species) and size (D2, total abundance summed over all species; Shimadzu et al. 2015).

Flow Variable	**Response Variable**	**Predicted Relationship**	**Mechanism**
High Rearing Season Flows	Community Size (D2)	Negative	High flow in the rearing season months (June-September) reduces the amount of shallow low-flow marginal habitat needed for juvenile survival, leading to larval displacement (Harvey 1987) and lowered feeding efficiency and growth (Haworth & Bestgen 2016).
Low Spawning Season Flows	Community Size (D2)	Negative	Low flows in the spawning season months (March – June) reduces the amount of spawning habitat available for shoal fishes and can lead to dewatering of eggs spawned in shallow areas (Gabrowski & Isely 2007).
High Spawning Season Flows	Community Composition (D1)	Negative or Positive	High flows during spawning seasons differentially affect fishes with different spawning strategies, leading to larger shifts in community composition. Fishes relying on drift dispersal may benefit (Rodger et al. 2016) whereas other taxa may experience egg displacement and mortality (Harvey 1987).

Table 3.2: Five hypotheses for fish trait influences on variable temporal trends in abundance

Trait	Predicted Relationship	Mechanism
Crevice and Cavity Spawning	Negative	Sedimentation from land-use disturbance reduces the amount and quality of spawning habitat (Burkhead & Jelks 2011).
Insectivory	Negative	Decreases in insect abundances within sampled reaches (Baker 2012) decrease food resources available to insectivorous taxa leading to decreased growth and reproduction (Wallace & Webster 1996).
Age and Fecundity	Positive	Fish with longer lifespans with higher fecundity will show less decline if conditions that are unfavorable to larval and juvenile survival (e.g. algal blooms, extreme flow events, etc) are common because taxa have more opportunities for successful recruitment. In addition, longer lived species may show a temporal lag in population responses to environmental stressors (Figueiredo et al. 2019).
Relative Tributary Abundance	Positive	Fish with larger tributary populations will show less decline due to subsidy opportunities (Waits 2008).
Spawning Timing	Negative	Fish that spawn later in the year will be more likely to decline due to algal overgrowth in shoals and shoal margins decreasing spawning and rearing habitat quality later in the season (Khanaev et al. 2016).

Table 3.3: Traits tested for influence on temporal trends in 32 fishes in the Conasauga River. Entries list the trait, data type, text description of the trait, and the mean and range of traits among the 32 taxa included in the analysis.

Trait	Data Type	Description	Mean	Range
Spawning Method	Categorical	Behavioral trait describing how and where a species lays eggs	NA	NA
Length	Continuous	Maximum adult length in millimeters reported in literature	150.6	49 - 640
Fecundity	Continuous	The total number of eggs produced in a spawning season	1924	29.5 - 24050
Max Age	Continuous	Maximum age reported in literature	4.27	2 - 10
Reproductive Longevity	Continuous	Number of reproductive years (i.e. the maximum age minus the age at maturity)	2.77	0.5 – 8.75
Spawn Timing	Continuous	Ordinal month of the onset of spawning	3.91 (late April)	1 – 7 (January – July)
Tributary Abundance	Continuous	Index of relative abundance in tributaries in the Conasauga River	0.235	0 – 0.617
Trophic Level	Continuous	Numeric approximation of trophic position in food web	3.03	2 – 4.5
Insectivory	Binary	Indication of whether insects are present in diet	0.906	NA
Herbivory	Binary	Indication of whether live plant matter or detritus is present in diet	0.188	NA
Piscivory	Binary	Indication of whether fish are present in diet	0.125	NA

Table 3.4: Results of linear regressions of counts for 32 species at 6 sites in the Conasauga River from 1996 – 2019 testing for effects of spawning methods on temporal trends. Entries show the parameter estimate for the year effect (β_1) and the year * spawning method interaction effect (β_3). 95% confidence intervals are shown in parentheses.

Parameter	**Estimate**
Year	-0.016 (-0.028 – -0.004
Cavity	-0.006 (-0.025 – 0.013)
Crevice	-0.015 (-0.030 – 0.000)
Gravel mound	-0.003 (-0.020 – 0.014)
Nest excavating	0.003 (-0.019 – 0.026)
Pelagic dispersal	0.013 (-0.002 – 0.029)
Sand and gravel burying	-0.006 (-0.027 – -0.014)

Table 3.5: Results of linear regressions of counts for 32 species at 6 sites in the Conasauga River from 1996 – 2019 testing for effects of traits on temporal trends. Entries show the parameter estimate for the year effect (β_1) and the year * trait interaction effect (β_3). Results are shown for all taxa, Percidae only, Leuciscidae only. 95% confidence intervals are shown in parentheses.

	All Taxa		**Percidae**		**Leuciscidae**	
Trait	**Year**	**Year * Trait**	**Year**	**Year * Trait**	**Year**	**Year * Trait**
Length	-0.020 (-0.025 – -0.016)	0.007 (0.001 – 0.013)	0.030 (0.000 – 0.060)	0.078 (0.030 – 0.127)	-0.019 (-0.026 – -0.012)	0.009 (-0.005 – 0.023)
Fecundity	-0.020 (-0.025 – -0.016)	0.007 (0.001 – 0.012	-0.012 (-0.041 – 0.018)	0.011 (-0.067 – 0.090)	0.000 (-0.012 – 0.013)	0.010 (0.047 – 0.152)
Max Age	-0.020 (-0.025 – -0.015)	0.005 (0.000 – 0.010)	-0.012 (-0.030 – 0.005)	0.010 (-0.029 – 0.048)	-0.019 (-0.032 – -0.006)	0.002 (-0.020 – 0.024)
Reproductive Longevity	-0.020 (-0.025 – -0.016)	0.002 (-0.002 – 0.008)	-0.012 (-0.023 – -0.000)	0.011 (-0.012 – 0.035)	-0.022 (-0.031 – -0.013)	-0.006 (-0.021 – 0.009)
Spawn Timing	-0.019 (-0.024 – -0.014)	0.007 (0.001 – 0.013)	-0.011 (-0.019 – -0.004)	0.018 (0.010 – 0.026)	-0.025 (-0.033 – -0.017)	-0.015 (-0.029 – -0.001)
Tributary Abundance	-0.021 (-0.025 – -0.016)	-0.005 (-0.011 – 0.000)	-0.017 (-0.026 – -0.008)	-0.002 (-0.013 – 0.008)	-0.020 (-0.028 – -0.013)	-0.004 (-0.017 – 0.009)
Trophic Level	-0.021 (-0.026 – -0.016)	-0.002 (-0.007 – 0.002)	NA	NA	-0.022 (-0.029 – -0.015)	-0.006 (-0.012 – 0.001)
Insectivory	-0.006 (-0.014 – 0.027)	-0.028 (-0.049 – -0.007	NA	NA	NA	NA
Herbivory	-0.022 (-0.027 – -0.017)	0.007 (-0.005 – 0.018)	NA	NA	-0.019 (-0.027 – -0.011)	-0.005 (-0.021 – 0.011)
Piscivory	-0.020 (-0.025 – -0.015)	-0.009 (-0.025 – 0.007)	NA	NA	NA	NA

Figures:

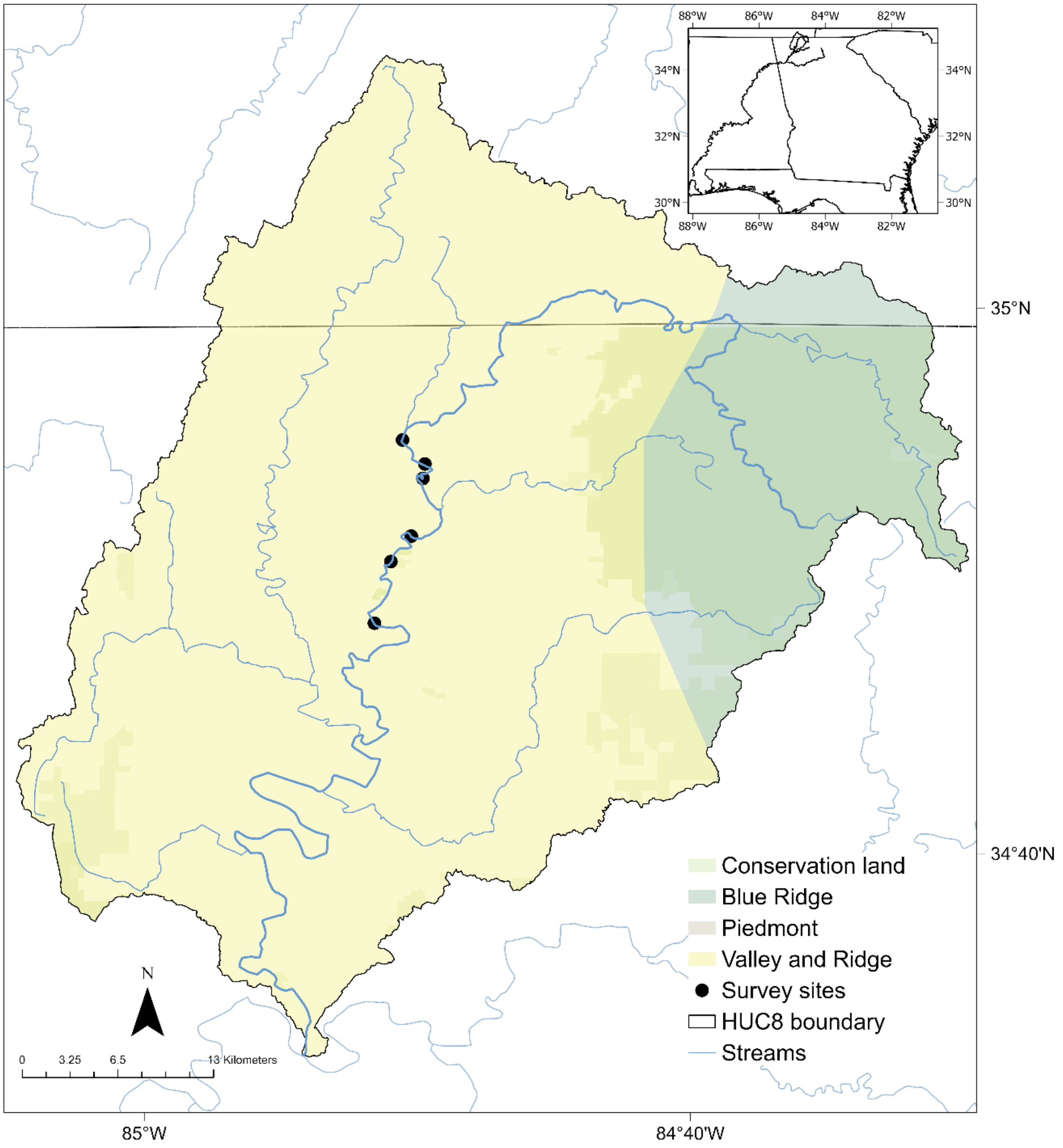

Figure 3.1: Site map of Conasauga River. Black points indicate long-term monitoring sites. Colors indicate physiographic provinces and presence of conservation lands including National Forests and state wildlife management areas.

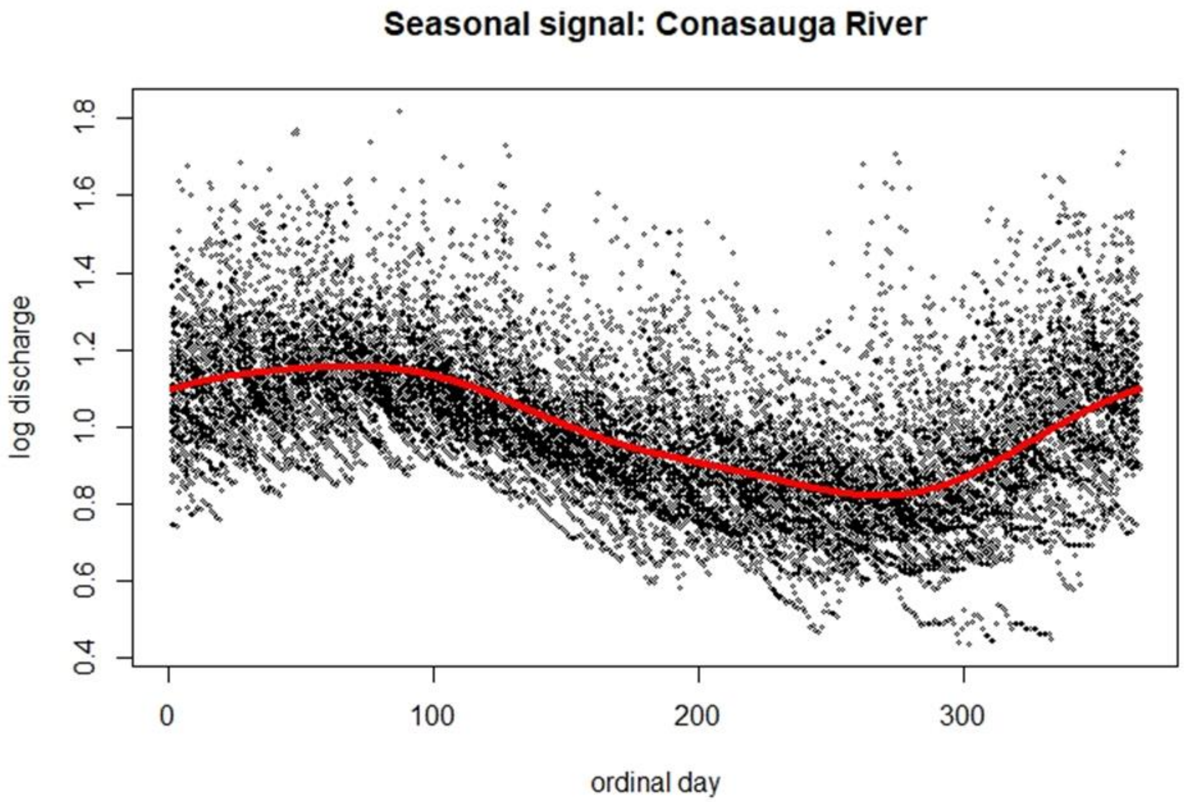

Figure 3.2: Daily flows in the Conasauga River (1981-2021) plotted against day of the year. Data points indicate day-specific log discharge. Red line shows the average seasonal signal across all years.

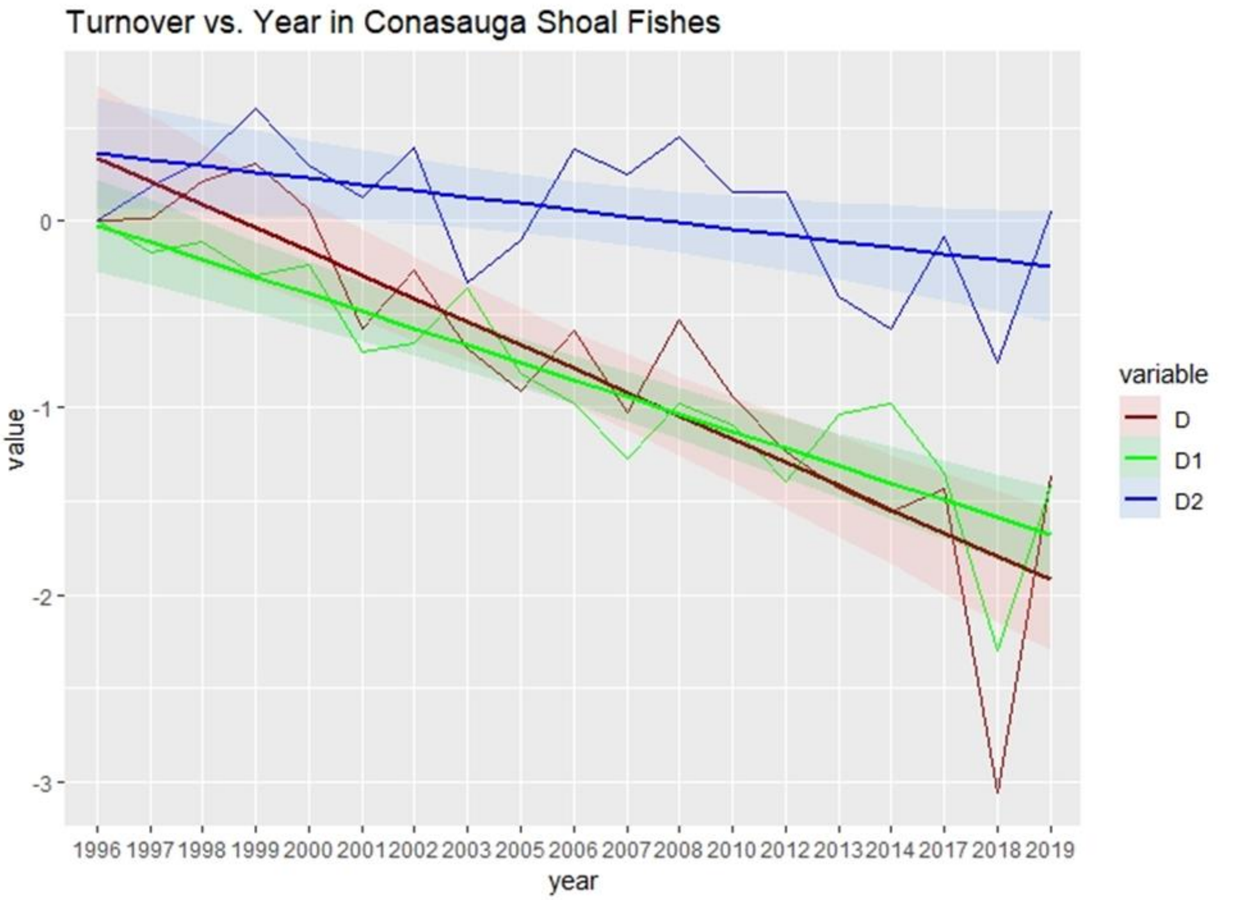

Figure 3.3: Community turnover for fishes in the Conasauga River through from 1996 to 2019 showing decline in community composition (D1) and community size (D2). Total turnover (D) is shown in red, size turnover (D2) is shown in blue, and composition turnover (D1) shown in green. Linear regressions of turnover value vs year are shown with 95% confidence interval shaded.

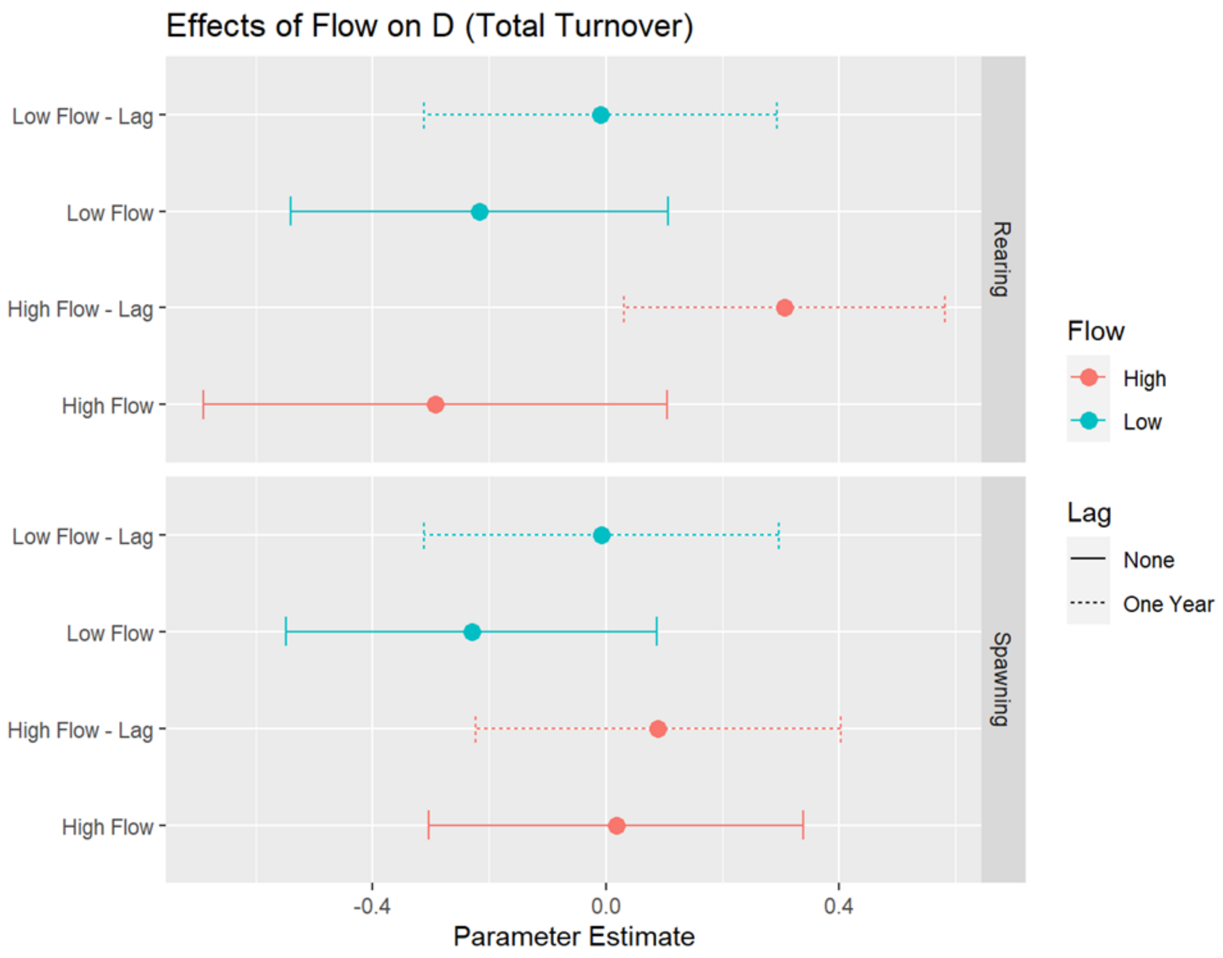

Figure 3.4: Parameter estimates for the effect of low and high flows (β_1) during the spawning and rearing seasons on year to year change in total community turnover (ΔD). Top and bottom panels show the effect of flows during the rearing and spawning season, respectively. Blue points indicate the effect of low flow anomalies, red points indicate the effect of high flow anomalies. Dashed lines indicate the effect of flow with a one-year lag.

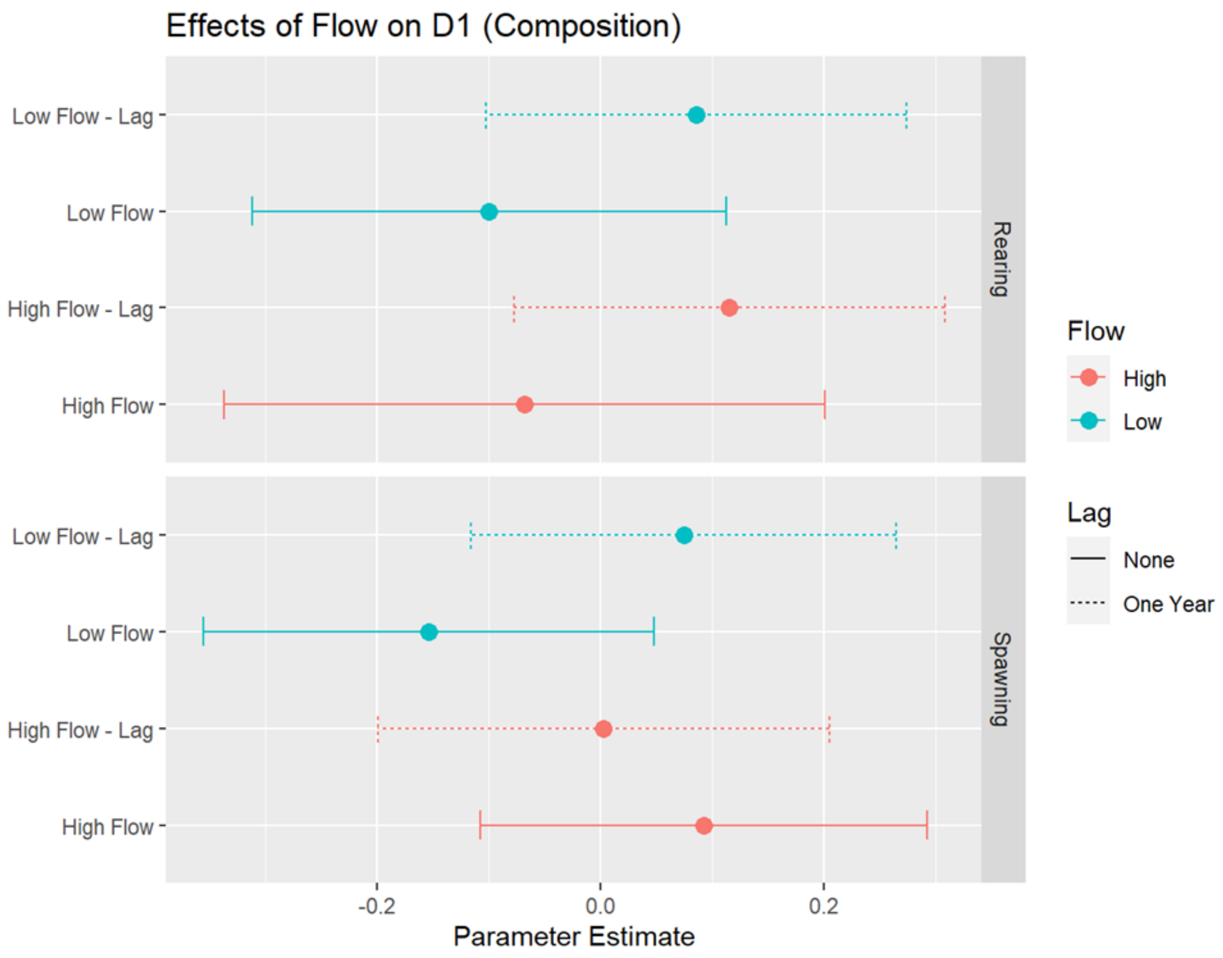

Figure 3.5: Parameter estimates for the effect of low and high flows ($\beta 1$) during the spawning and rearing seasons on year to year change in community composition turnover (ΔD1). Top and bottom panels show the effect of flows during the rearing and spawning season, respectively. Blue points indicate the effect of low flow anomalies, red points indicate the effect of high flow anomalies. Dashed lines indicate the effect of flow with a one-year lag.

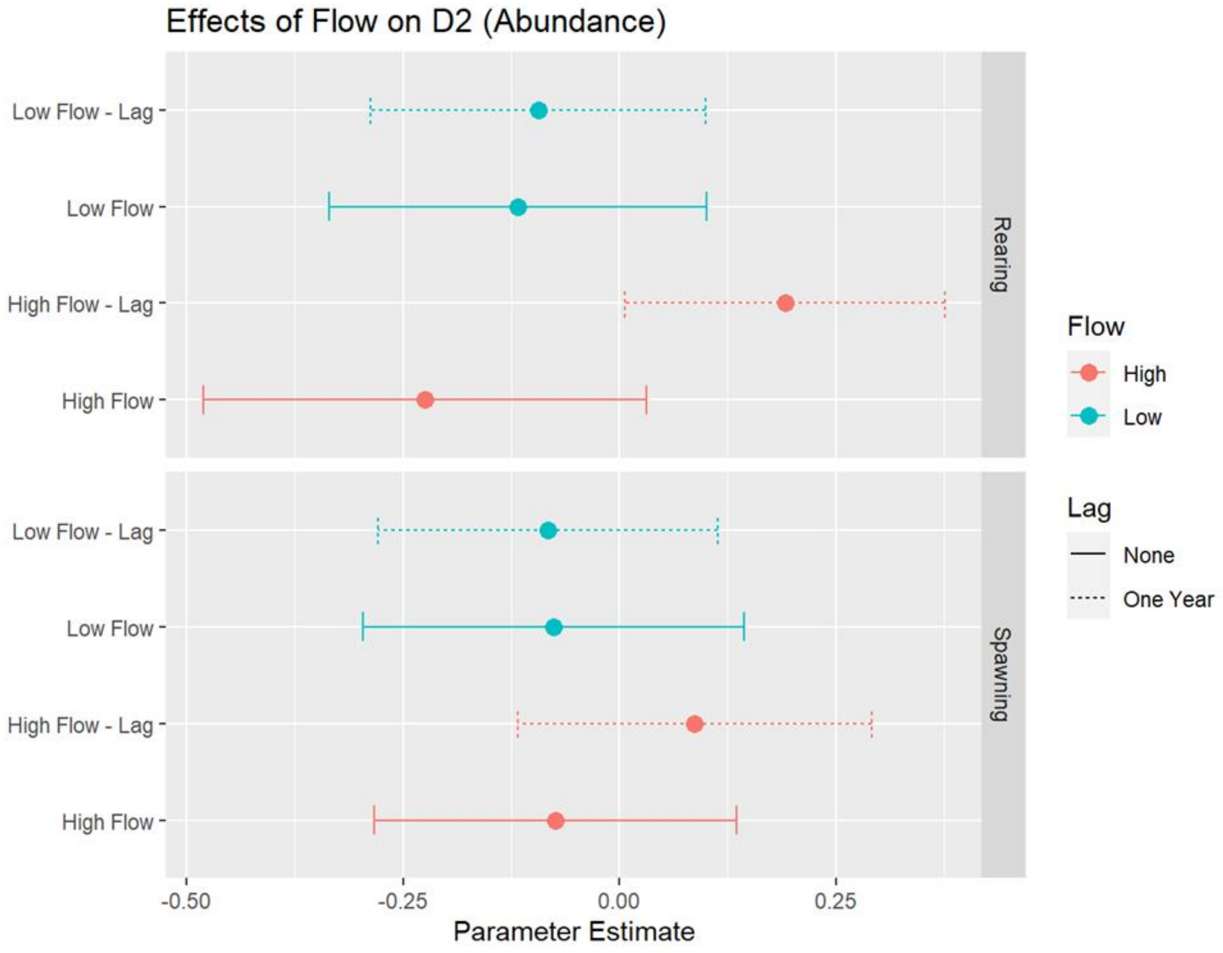

Figure 3.6: Parameter estimates for the effect of low and high flows ($\beta 1$) during the spawning and rearing seasons on year to year change in community abundance turnover (ΔD2). Top and bottom panels show the effect of flows during the rearing and spawning season, respectively. Blue points indicate the effect of low flow anomalies, red points indicate the effect of high flow anomalies. Dashed lines indicate the effect of flow with a one-year lag.

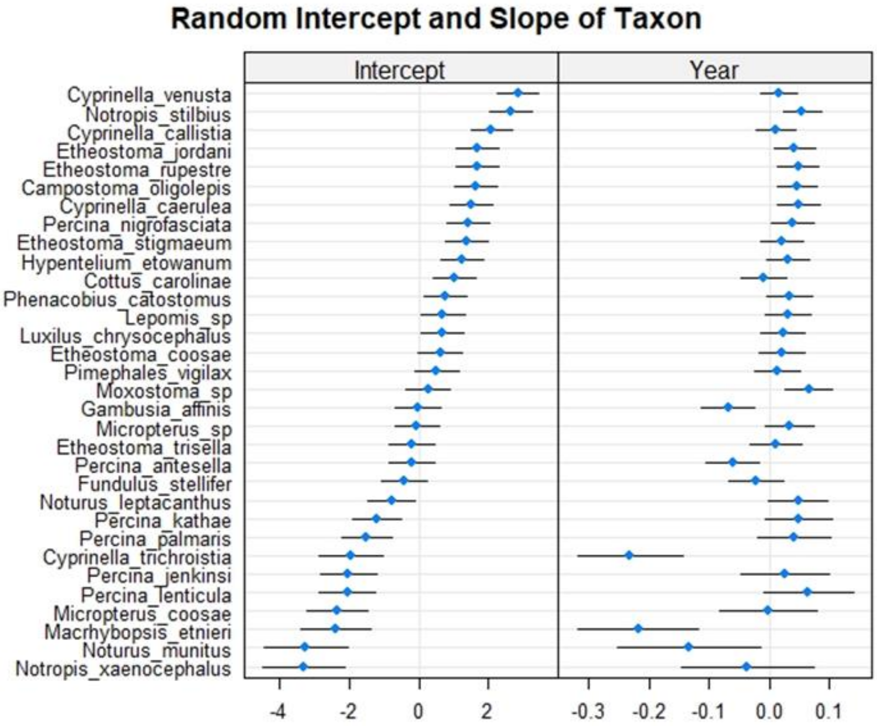

Figure 3.7: Random effect estimates for slope and intercept of abundance trends of 32 taxa in 6 sites in the Conasauga River from 1996 to 2019. Estimates are from a mixed-effect regression model of species and site specific counts through time where each taxon could vary in their intercept and slope. Bars indicate 95% confidence intervals.

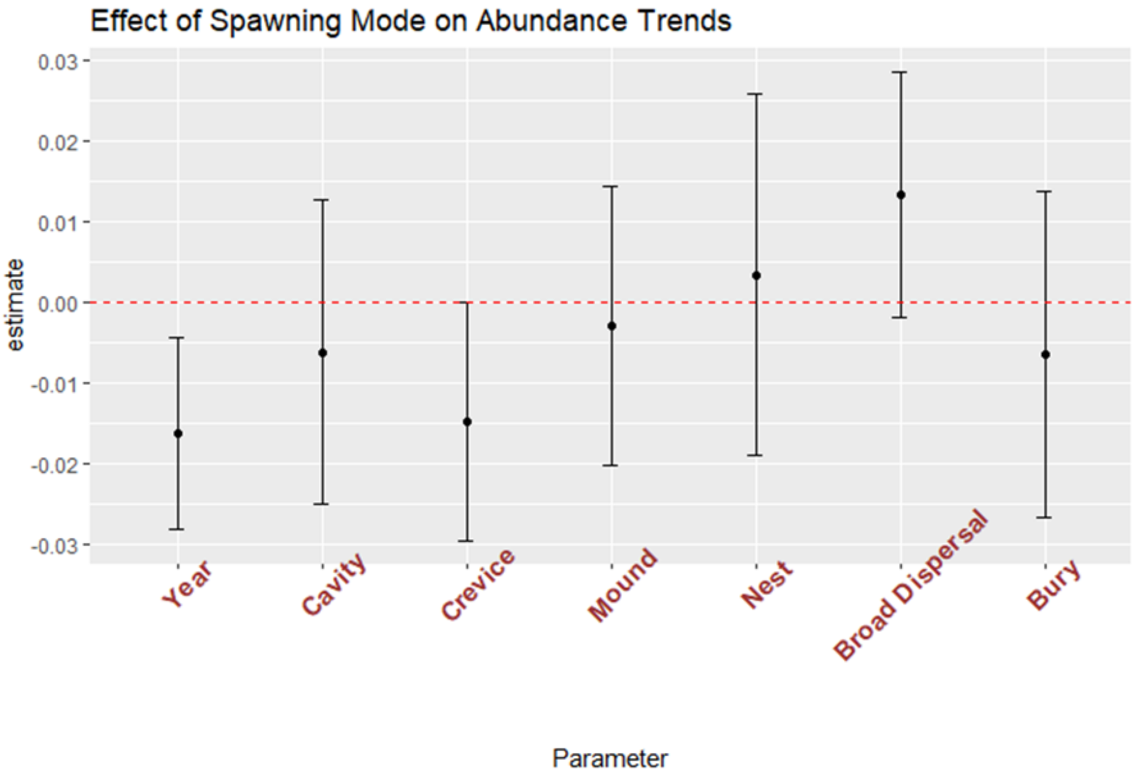

Figure 3.8: Parameter estimates from a mixed-effects regression model determining the influence of spawning method on temporal trends of 32 taxa in the Conasauga River from 1996-2019. Data show the effect of year ($\beta 1$) and spawning method * year interaction ($\beta 3$) on fish abundance trends. Error bars indicate 95% confidence interval. Dashed red line is positioned at 0 (no effect).

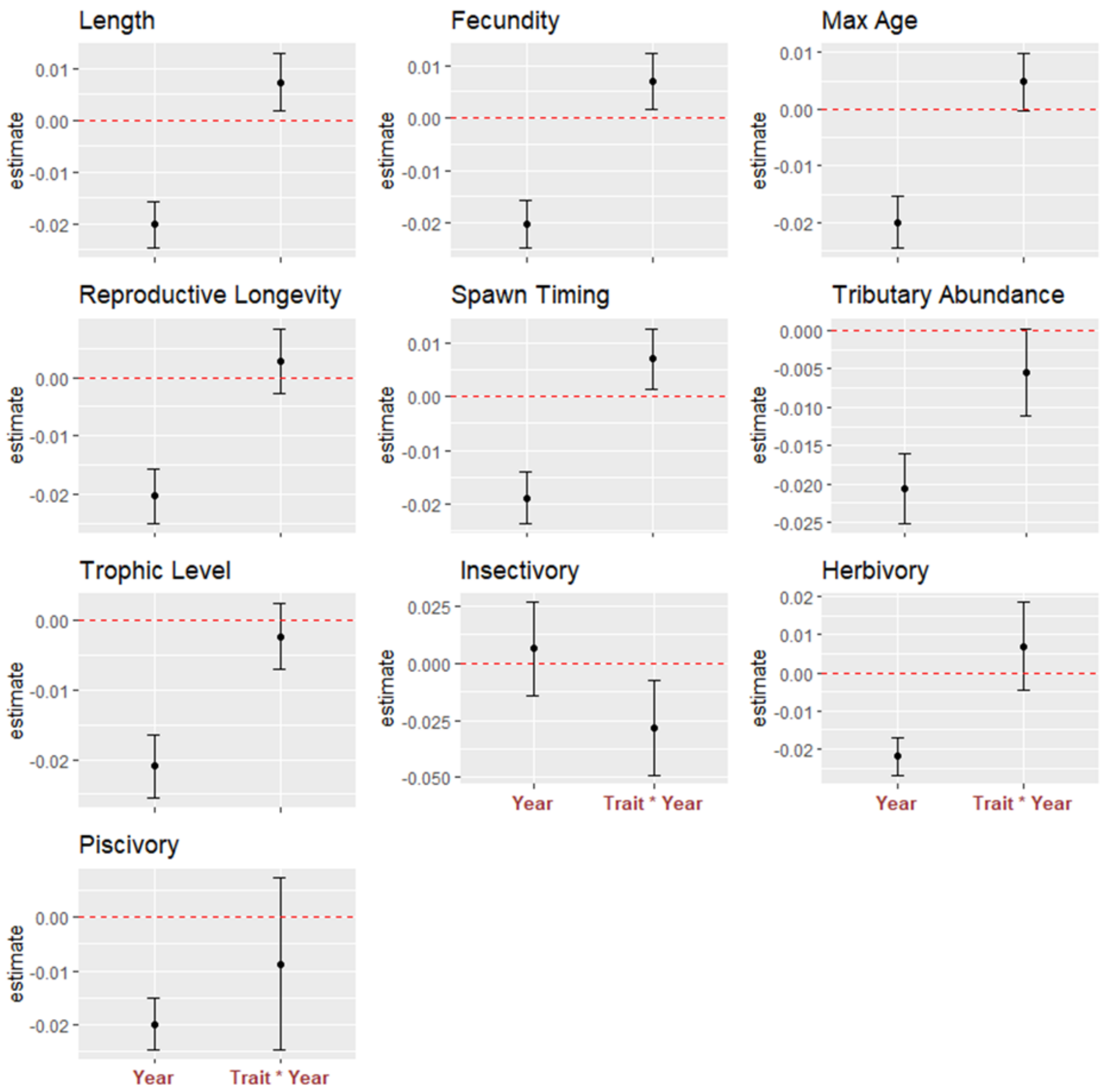

Figure 3.9: Parameter estimates from 10 mixed-effects regression models determining the influence of traits on temporal trends of 32 taxa in the Conasauga River from 1996-2019. Data show the effect of year ($\beta 1$) and trait*year interactions ($\beta 3$) on fish abundance trends. Error bars indicate 95% confidence interval. Within each panel, the left parameter is year and right parameter is the trait*year interaction. Dashed red line is positioned at 0.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Summary:

Our study of life histories of fishes in the upper Coosa River system yielded valuable information on the spawning timing, habitat, behavior, and reproductive investment of several taxa. We observed 67 spawning occurrences among 15 taxa from April to June of 2021. We observed a wide range of spawning behaviors, temperatures, and habitat characteristics such as depth, velocity, and substrates. In addition, we compared our observations with published records for 12 taxa. We also collected egg size and clutch data for 9 taxa. Publishing these data will add to the understanding of the life histories of understudied shoal fishes and inform future management decisions and ecological analyses.

Our analyses of fish community change in the Conasauga River identified temporal trends in both total fish abundances as well as the composition, or relative abundances, among taxa. Using a community turnover approach, we revealed that a large portion of the change in the community index is attributable to shifts in composition, indicating that taxa vary in their population trends. Although some taxa showed increasing populations through time, most species showed declines, amounting to a loss of total fish abundance of ~2% each year, or ~40% over the study period, within the six sites we analyzed. This raises concern for the health of a fish community that has already shown declines of individual taxa, including Federally Threatened or Endangered species (Freeman et al. 2017, Stowe et al. 2020). We were unable to find much evidence to support the role of flow in changes in community composition and size, although we did find that high flows during the rearing season led to decreases in the community size. This is consistent with research that shows that high flows can cause early-life stage mortality due to displacement and decreases in juvenile habitat availability (Harvey 1987, Haworth & Bestgen 2016). We did not find any effects of low flow or any effects of flow on community compositions. This may be due to substantial variability in the direction and magnitude of flow effects among taxa with varying life history strategies (Freeman et al. 2022).

We found evidence that some of the variation among taxa in abundance trends was explained by intraspecific variation in traits. Previous research has shown that sedimentation decreases reproductive success in crevice spawning *Cyprinella trichroistia* (Burkhead & Jelks 1999). We found that crevice spawners (fishes that deposit their eggs inside cracks in large rocks or logs) were more likely to be declining compared to those with other spawning strategies, indicating that sedimentation from, e.g., agricultural activity in the Conasauga River watershed may have variable influence on population success among taxa. In addition, we found that fish with characteristics corresponding to periodic life history strategies including large body size, high fecundity, and long lifespan (Winemiller and Rose 1992) were less likely to be declining compared to other taxa. Research from other systems have shown that some periodic species are more susceptible to declines in human modified streams (Santos et al. 2021), however, others have shown that small-bodied fishes are more susceptible to extinction and extirpation due to low dispersal abilities and high ecological specialization (Kopf et al. 2017). Although it is difficult to know the driver of these trends, it appears that larger-bodied fish populations are not experiencing the detrimental impacts of stressors in the Conasauga River to the same extent as other taxa. To our surprise, we found that fish with larger tributary populations were more likely to be declining within the mainstem Conasauga River. Although we expected that tributary populations of fishes would be able to subsidize declining mainstem populations, it is possible that tributary populations are in decline as well. Finally, we found that insectivorous fish were more likely to be declining than non-insectivores. A previous study of insect abundances and biomass revealed a longitudinal decline in insects along the Conasauga River, with low insect abundances and biomass in the reaches corresponding to the sites we analyzed in this study (Baker 2012). Food limitation may be negatively impacting the growth, mortality, and reproductive success of small-bodied taxa such as Percidae and Leuciscidae in the downstream reaches in the Conasauga River.

Future research:

Continued research of life history of fishes in the upper Coosa River system is necessary to predict and mitigate negative impacts of increasing anthropogenic activity in the watershed. Gaps in data for spawning timing, behavior, and habitat use still exist for many taxa, particularly rare, endemic, and/or threatened or endangered fishes. Discovering subtle behavioral idiosyncrasies among taxa may explain why some taxa appear to be declining while others are not. Furthermore, continued study of variation in spawning timing and habitat among taxa and populations will strengthen efforts to minimize disturbance during spawning periods. In addition, detailed life history information will allow researchers to test more specific mechanisms of how flow influences fish populations.

Future research in the Conasauga River should seek to collect supplemental environmental data to relate fish declines to specific mechanisms. Although time-series abundance data of fishes are an invaluable asset to monitoring and describing fish population patterns, additional data may elucidate important biotic and abiotic drivers of change. Determining drivers of declines of fish populations will aid managers in implementing effective protection and restoration efforts. We recommend additional studies of 1) insect communities, fish diets, and fish body-condition to determine foraging success; 2) spawning habitat quality assessments along a gradient of sedimentation influence; and 3) continued investigation of the effects of flow on fish populations, particularly among taxa with different life history traits such as spawning habitat and timing.

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APPENDICES

APPENDIX A: SPAWNING OBSERVATION AND EGG DATA FOR CHAPTER 2

Table 1. List of spawning occurrences for 12 taxa in the Upper Coosa River system. Entires include date, location, species, and habitat variables (temperature, depth, water velocity, and substrate).

Date	**Site**	**Species**	**Temperature (deg C)**	**Depth (m)**	**Velocity (m/s)**	**Substrate**
5/11/2021	Holly	*Cyprinella caerulea*	16.4	0.26	0.59	cobble, sand, wood
6/4/2021	Conasauga - upstream	*Cyprinella caerulea*	19.0	0.42	0.00	sand, wood
5/28/2021	Conasauga - downstream	*Cyprinella caerulea*	23.3	0.30	0.00	sand, wood
5/19/2021	Conasauga - middle	*Cyprinella callistia*	16.0	0.92	0.23	boulder
5/25/2021	Conasauga - upstream	*Cyprinella callistia*	20.0	0.61	0.42	bedrock/boulder
5/25/2021	Conasauga - upstream	*Cyprinella trichroistia*	20.0	0.30	0.69	bedrock/boulder, podostemum
5/25/2021	Conasauga - upstream	*Cyprinella trichroistia*	20.0	0.77	0.33	bedrock/boulder
6/13/2021	Conasauga - upstream	*Cyprinella trichroistia*	23.5	0.52	0.76	bedrock/boulder, podostemum
6/14/2021	Conasauga - middle	*Cyprinella venusta*	22.0	0.74	0.02	sand, wood, cobble
6/12/2021	Holly	*Cyprinella venusta*	21.0	0.69	0.23	sand, wood
5/1/2021	Conasauga - upstream	*Etheostoma brevirostrum*	15.0	0.56	0.16	boulder/bedrock, cobble, sand, podostemum
5/1/2021	Conasauga - upstream	*Etheostoma brevirostrum*	15.0	0.44	0.71	boulder/bedrock, cobble, sand
4/11/2021	Conasauga - middle	*Etheostoma coosae*	14.5			cobble, sand
4/11/2021	Conasauga - middle	*Etheostoma coosae*	14.5			cobble, sand
4/11/2021	Conasauga - middle	*Etheostoma coosae*	14.5			cobble, sand
5/19/2021	Conasauga - middle	*Etheostoma coosae*	16.0	0.48	0.16	cobble, bedrock/boulder

5/19/2021	Conasauga - middle	*Etheostoma coosae*	16.0	0.48	0.16	cobble, bedrock/boulder
5/19/2021	Conasauga - middle	*Etheostoma coosae*	16.0	0.58	0.46	cobble
5/19/2021	Conasauga - middle	*Etheostoma coosae*	16.0	0.28	0.28	cobble
6/14/2021	Conasauga - middle	*Etheostoma coosae*	22.0	0.56	0.49	clay, bedrock
6/14/2021	Conasauga - middle	*Etheostoma coosae*	22.0	0.25	0.65	cobble, sand, gravel
6/14/2021	Conasauga - middle	*Etheostoma coosae*	22.0			cobble, gravel
5/2/2021	Holly	*Etheostoma coosae*	16.0	0.27	0.35	cobble, justicia
5/11/2021	Holly	*Etheostoma coosae*	16.4	0.26	0.38	cobble
5/11/2021	Holly	*Etheostoma coosae*	16.4	0.26	0.56	cobble, justicia
5/30/2021	Holly	*Etheostoma coosae*	18.0	0.24	0.25	cobble, sand
5/16/2021	Conasauga - upstream	*Etheostoma coosae*	16.0	0.55	1.23	cobble, sand
5/25/2021	Conasauga - upstream	*Etheostoma coosae*	20.0	0.22	0.49	cobble, podostemum
4/15/2021	Conasauga - downstream	*Etheostoma coosae*	16.7	0.61	0.54	cobble, sand, podostemum
4/15/2021	Conasauga - downstream	*Etheostoma coosae*	16.7	0.61	0.44	cobble, sand
4/15/2021	Conasauga - downstream	*Etheostoma coosae*	16.7	0.30	0.37	cobble, justicia
4/15/2021	Conasauga - downstream	*Etheostoma coosae*	16.7	0.40	0.30	cobble, justicia
4/15/2021	Conasauga - downstream	*Etheostoma coosae*	16.7	0.35	0.32	cobble, justicia
4/15/2021	Conasauga - downstream	*Etheostoma coosae*	16.7	0.40	0.63	cobble, sand, justicia
6/14/2021	Conasauga - middle	*Etheostoma jordani*	22.0	0.24	0.59	cobble
5/25/2021	Conasauga - upstream	*Etheostoma jordani*	20.0	0.12	0.36	cobble, podostemum
6/13/2021	Conasauga - upstream	*Etheostoma jordani*	23.5	0.27	0.68	cobble
5/28/2021	Conasauga - downstream	*Etheostoma jordani*	23.3	0.21	0.87	cobble, sand
5/28/2021	Conasauga - downstream	*Etheostoma jordani*	23.3	0.11	0.58	cobble, sand
5/28/2021	Conasauga - downstream	*Etheostoma jordani*	23.3	0.37	0.71	cobble, sand
4/18/2021	Shoal	*Etheostoma scotti*	16.0	0.12	0.22	cobble, gravel, sand
4/11/2021	Conasauga - middle	*Etheostoma stigmaeum*	14.5			cobble, sand
5/2/2021	Holly	*Etheostoma stigmaeum*	16.0	0.32	0.05	cobble, gravel, sand

4/15/2021	Conasauga - downstream	*Etheostoma stigmaeum*	16.7	0.46	0.35	cobble, sand
4/15/2021	Conasauga - downstream	*Etheostoma stigmaeum*	16.7	0.49	0.33	cobble
4/15/2021	Conasauga - downstream	*Etheostoma stigmaeum*	16.7	0.58	0.35	cobble, sand
4/15/2021	Conasauga - downstream	*Etheostoma stigmaeum*	16.7	0.59	0.43	cobble, sand
4/15/2021	Conasauga - downstream	*Etheostoma stigmaeum*	16.7	0.52	0.58	cobble, sand
4/15/2021	Conasauga - downstream	*Etheostoma stigmaeum*	16.7	0.46	0.39	cobble, sand
4/15/2021	Conasauga - downstream	*Etheostoma stigmaeum*	16.7	0.49	0.29	sand, gravel
6/14/2021	Conasauga - middle	*Lampetra sp*	22.0	0.60	0.15	gravel
5/19/2021	Conasauga - middle	*Lepisosteus osseus*	16.0	0.16	0.65	cobble, justicia
5/19/2021	Conasauga - middle	*Lepisosteus osseus*	16.0	0.09	0.46	cobble, justicia
5/19/2021	Conasauga - middle	*Lepisosteus osseus*	16.0	0.06	0.76	cobble, justicia
6/14/2021	Conasauga - middle	*Micropterus henshalli*	22.0			silt, sand, cobble
6/4/2021	Conasauga - upstream	*Percina kusha*	19.0	0.50	0.09	sand, gravel
6/4/2021	Conasauga - upstream	*Percina kusha*	19.0	0.51	0.07	sand, gravel
5/2/2021	Holly	*Percina nigrofasciata*	16.0	0.31	0.65	cobble, gravel, wood
5/11/2021	Holly	*Percina nigrofasciata*	16.4	0.30	0.53	cobble, sand
5/1/2021	Conasauga - upstream	*Percina palmaris*	15.0	0.48	0.65	boulder/bedrock, sand
5/1/2021	Conasauga - upstream	*Percina palmaris*	15.0	0.61	0.28	boulder/bedrock, cobble, sand, podostemum
5/1/2021	Conasauga - upstream	*Percina palmaris*	15.0	0.50	0.81	boulder/bedrock, cobble, sand, podostemum
5/16/2021	Conasauga - upstream	*Percina palmaris*	16.0	0.56	0.42	bedrock/boulder, sand, podostemum
5/25/2021	Conasauga - upstream	*Percina palmaris*	20.0	0.10	0.49	cobble, justicia
5/25/2021	Conasauga - upstream	*Percina palmaris*	20.0	0.25	0.42	cobble, justicia
6/13/2021	Conasauga - upstream	*Percina palmaris*	23.5	0.29	0.46	sand, gravel, cobble

Table 2. List of egg data for 9 taxa. Entries show the date collected, species, length of fish, clutch size, and egg size including diameter and mass.

Date	**Species**	**Standard length**	**Clutch size**	**Egg diameter (mm)**	**Egg mass (mg)**
3/10/2021	*Cottus carolinae*	42	73	2.23	1.52
3/14/2021	*Cottus carolinae*	42	65	3.08	4.13
3/14/2021	*Cottus carolinae*	41	53	2.31	3.23
3/14/2021	*Cottus carolinae*	41	46	2.94	3.60
3/14/2021	*Cottus carolinae*	41	41	2.77	3.59
5/2/2021	*Cyprinella callistia*	80	209		
5/2/2021	*Cyprinella callistia*	70	207	1.43	0.36
5/28/2021	*Cyprinella callistia*	74	263		0.43
5/2/2021	*Cyprinella trichroistia*	70	569		
5/2/2021	*Cyprinella trichroistia*		112	1.43	1.14
5/2/2021	*Cyprinella trichroistia*	57	314		0.27
5/2/2021	*Cyprinella trichroistia*	60	366	1.05	0.26
5/28/2021	*Cyprinella trichroistia*	58	392		
5/28/2021	*Cyprinella trichroistia*	52	246	1.41	0.32
5/28/2021	*Cyprinella trichroistia*	71	402	1.24	0.73
5/28/2021	*Cyprinella trichroistia*	58	257		
3/13/2021	*Etheostoma coosae*	39	59	1.53	0.32
3/13/2021	*Etheostoma coosae*	36	38		0.29
4/5/2021	*Etheostoma coosae*	38	43		0.10
4/5/2021	*Etheostoma coosae*	41	57		0.37
4/5/2021	*Etheostoma coosae*	32	47	1.53	0.25
4/5/2021	*Etheostoma coosae*	30	20	1.24	0.35
4/5/2021	*Etheostoma*	34	38	1.43	0.34

	coosae				
4/5/2021	*Etheostoma coosae*	34	33	1.53	0.34
4/5/2021	*Etheostoma coosae*	38	47	1.43	0.78
5/2/2021	*Etheostoma coosae*	45	48	1.29	0.30
5/2/2021	*Etheostoma coosae*	43	46	1.43	0.27
5/2/2021	*Etheostoma coosae*	41	43		0.41
5/2/2021	*Etheostoma coosae*	43	47	1.29	0.31
3/13/2021	*Etheostoma jordani*	35	30		
5/1/2021	*Etheostoma jordani*	38	32	1.68	0.71
5/1/2021	*Etheostoma jordani*	39	37		0.36
5/1/2021	*Etheostoma jordani*	38	92	1.48	0.48
5/1/2021	*Etheostoma jordani*	38	43	1.76	0.65
5/2/2021	*Etheostoma jordani*	34	52		
5/2/2021	*Etheostoma jordani*	39	35	1.81	0.84
5/2/2021	*Etheostoma jordani*	38	32		0.36
5/2/2021	*Etheostoma jordani*	37	18	1.81	0.75
5/2/2021	*Etheostoma jordani*	43	50		0.80
5/2/2021	*Etheostoma jordani*	43	86		0.64
3/13/2021	*Etheostoma stigmaeum*	36	42	1.22	0.33
3/13/2021	*Etheostoma stigmaeum*	36	26		0.20
3/13/2021	*Etheostoma stigmaeum*	34	33	1.29	0.35
3/13/2021	*Etheostoma stigmaeum*	37	50	1.41	0.38
3/24/2021	*Etheostoma stigmaeum*	39	72		0.27
3/24/2021	*Etheostoma stigmaeum*	47	98	1.81	0.24
4/5/2021	*Notropis stilbius*	61	410		
5/28/2021	*Notropis stilbius*	45	184	0.95	0.15
3/21/2021	*Percina nigrofasciata*	60	120	1.29	0.34
3/24/2021	*Percina*	55	21	1.18	0.64

	nigrofasciata				
3/24/2021	*Percina nigrofasciata*	55	118	1.64	0.46
3/29/2021	*Percina nigrofasciata*	53	68		0.67
3/29/2021	*Percina nigrofasciata*	58	106		0.54
3/29/2021	*Percina nigrofasciata*	53	127	2.33	0.69
3/29/2021	*Percina nigrofasciata*	57	110		0.63
5/1/2021	*Percina nigrofasciata*	54	132	1.43	0.37
3/10/2021	*Percina palmaris*	57		2.00	1.13
3/14/2021	*Percina palmaris*	47	44		0.52
3/21/2021	*Percina palmaris*	51	21	1.76	0.96
3/21/2021	*Percina palmaris*	54	68	1.86	1.03
3/21/2021	*Percina palmaris*	53	112	1.65	0.66
4/28/2021	*Percina palmaris*	70	148		1.04
4/28/2021	*Percina palmaris*	72	143	1.81	0.87
4/28/2021	*Percina palmaris*	63	140	1.65	0.62