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AGING TECHNIQUES & POPULATION DYNAMICS  
OF BLUE SUCKERS (*CYCLEPTUS ELONGATUS*)  
IN THE LOWER WABASH RIVER

By

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B.S. Environmental Biology

Eastern Illinois University

A thesis prepared  
for the requirements for the degree of  
Master of Science

Department of Biological Sciences

Eastern Illinois University

May 2020

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## THESIS ABSTRACT

The Blue Sucker, *Cycleptus elongatus*, occurs in large rivers of the Mississippi River basin, and also in rivers of the gulf coast slope, Texas, and Mexico. The imperiled status of this species has called attention to the need for its management and protection. Estimating age is crucial for directing management, but past studies have varied in their choice of hard structure, resulting in uncertainty regarding the basic life history of this species. Because the Wabash River Blue Sucker population may be one of few surveyable populations with high abundance and successful reproduction, the demographics of this population can provide a benchmark against which threatened populations can be compared. We harvested Blue Suckers ( $n = 168$ ) from the lower Wabash River and compared age estimations from scales, opercles, pectoral fin rays, and lapillus otoliths. Our results suggests that Blue Sucker otoliths yield more precise and credible age estimates than other structures, and we recommend the use of lapillus otoliths for aging this species. Specimens were assigned age estimates up to 42 years. We estimated annual mortality at 4.5%, and we modeled growth as  $TL = 680.29038 \cdot (1 - e^{(-0.15898 \cdot Age + 5.14037)})$ , where  $TL$  = total length (mm) and  $Age$  = otolith age (years). We estimated fecundity to average 110,933 eggs/female. The population length-weight regression was  $\text{Log}_{10}(WT) = 3.323 \cdot \text{Log}_{10}(TL) - 5.9592$  where  $WT$  = weight (g) and  $TL$  = total length (mm). We identified a declining trend in average relative weights from 2008 to 2019, and found this trend mirrored in the declining average conditions of four other benthic invertivorous fishes in the Wabash River. We suggest that Blue Suckers can serve as bioindicators for the Wabash River ecosystem and that their declining relative weights should be regarded as early symptoms of community level change.

## ACKNOWLEDGEMENTS

This research was completed thanks to the generous efforts of many people. I am thankful to the faculty (R. Colombo, E. Fanta-Effert, E. Bollinger) at Eastern Illinois University for their guidance and intellectual contributions to the project. I thank C. Moody-Carpenter for her leadership surveying fish on the Wabash River. My peers in the Center for Fisheries and Aquatic Sciences lab (S. Bogue, T. Faggin, R. Frey, K. Hanser, L. Herbert, T. Murray, K. Rempe, J. Rohr, S. Schaick, R. Sparks, K. Woods, and D. Yff) as well as C. Berlin, S. Birch, G. Gonzales, A. Monroe, and B. Monroe all assisted in surveying and dissecting Blue Sucker specimens. I greatly appreciate C. Moody-Carpenter and K. Rempe for their work estimating ages. I am indebted to the folks in A. R. Lackmann's laboratories at North Dakota State University and the University of Minnesota, Duluth, especially A. R. Lackmann and E. Bielak-Lackmann, for their assistance preparing and aging otoliths, and for help preparing Chapter 1 of this manuscript. Thank you to J. Hirst (Illinois Department of Natural Resources) for sharing data and specimens, and C. Jansen (Indiana Department of Natural Resources) for sharing anecdotal accounts and specimens, as well as J. McMurray (Indiana Department of Environmental Management) for sharing data. Financial support for this and research was provided by the U.S. Fish and Wildlife Service Sport Fish Restoration Program.

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## THESIS INTRODUCTION

The Blue Sucker (*Cycleptus elongatus*) is a unique fish, once abundant in the large rivers of its endemic North American range. It is a large-bodied fish (up to 93 cm, Carlander 1969), and only a century ago was an appreciated commercial and subsistence food source: “generally rated, where known, as the best of the suckers,” (Coker 1930). Today, however, Blue Suckers have declined in abundance in many portions of their range (Coker 1930, Smith 1979, Kay 1994, Pflieger 1997, Burr & Mayden 1999, NatureServe & Lyons 2019), leading to their status as a protected species in five states and a species of special concern in seven others. Known threats include impoundments (which bar migration patterns), channelization (which homogenizes available habitat), and reductions in water quality associated with siltation or aquatic pollution (Smith 1979, Vokoun 2003). The full extent of declines in Blue Sucker abundance and the range-wide conservation status of this species remain unknown due to insufficient documentation, which has prevented the protection of Blue Suckers at the federal level (Elstad & Werdon 1993).

The species has been understudied historically, as a result of several factors. In the past, research and management efforts were focused almost solely on economically important “game fish” (Reynolds et al. 2002), which has not included suckers as they are not highly susceptible to traditional angling. Suckers in general have historically been perceived as “rough fish” and thus devalued by anglers as well as biologists (Moyle 2002; Cooke 2005), even though the majority of catostomid species are already imperiled (Harris et al. 2014). Finally, Blue Suckers have long been under-documented due to the

difficulty of effectively sampling the deep, high-velocity channelized habitats that they often inhabit.

The Wabash River, where it forms the lower border between Illinois and Indiana, offers the unique opportunity to research a robust population of Blue Suckers. Benthic invertivorous fishes came to dominate this river system in the early 1990's (Broadway et al. 2015), and Blue Suckers were noted as increasing in abundance and expanding their range in the Wabash River in 1991 (Gammon as cited in Kay et al., 1994). Relatively shallow conditions in many stretches of this river allow Blue Suckers to be efficiently surveyed using electrofishing gear, and ten years of annual fish monitoring data have already been collected on this system, with Blue Suckers representing over 6% of the surveyed fish biomass.

The abundance of individuals in this population justified the lethal harvest of 168 specimens in 2018 and 2019, and enabled us to conduct valuable research that would not be possible in river systems in which the species was imperiled and/or restricted for lethal harvest. While investigating trends in Wabash River Blue Sucker data, we identified a significant pattern of declining average relative weights in the population from 2008-2019, and sought to associate this trend with community-level changes occurring in the river system. Our objectives in this research were to (a) identify the hard structure(s) that yields the most precise and credible age estimations; (b) describe the demographics of the Wabash River population; (c) explore the role of Blue Suckers as a bioindicator of change in the Wabash River.

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CHAPTER 1:  
COMPARISON OF MULTIPLE HARD STRUCTURES FOR ESTIMATING AGE IN  
BLUE SUCKERS, *CYCLEPTUS ELONGATUS*

*[Formatted in the style of: Transactions of the American Fisheries Society]*

ABSTRACT

The Blue Sucker is a catostomid endemic to North America. The imperiled status of this species has called attention to the need for its management and protection. Estimating age is crucial for directing management, but no hard structures have been validated for age estimation in this species. Past studies have varied in their choice of hard structure, resulting in uncertainty regarding the basic life history of this species. Our objective was to identify the most precise and credible structure with which to age Blue Suckers. We harvested Blue Suckers ( $n = 168$ ) from the lower Wabash River and compared age estimations from scales, opercles, pectoral fin rays, and lapillus otoliths. In our initial comparison, we found that pectoral fin rays were substantially more precise than scales or opercles, and identified non-parallel structure bias between all three structures. We then compared ages assigned from pectoral fin rays to ages assigned by lapillus otoliths and found that the otoliths were more precise and yielded a much higher range of ages (up to 42 years old). Relative to otolith age assignments, fin rays overestimated age in specimens  $\leq 7$  years old by as much as seven years, and underestimated age in specimens  $\geq 13$  years old by as much as 34 years. Also relative to otoliths, fin rays overestimated age in individuals  $< 550$  mm and underestimated age in

individuals > 625 mm total length. We were unable to identify any range of ages or total lengths in which fin ray age could be accurately corrected to otolith age. We identified a strong correlation between whole lapillus mass and estimated lapillus age ( $R^2 = 0.89$ ). Evidence suggests that Blue Sucker otoliths yield more credible age estimates than other structures, a finding that is consistent across numerous other fishes, including other age-validated catostomids. We recommend the use of lapillus otoliths for aging this species.

## INTRODUCTION

Blue Suckers *Cyprinella elongatus*, are large-bodied catostomids endemic to the lotic freshwater systems of North America. Blue Suckers occur in large rivers and are found in the Mississippi and Missouri River systems as well as Gulf Coast tributaries and west into Texas and Mexico (Elstad and Weldon 1993; Burr and Mayden 1999). Of the twenty-three U.S. states they naturally occurred in, Blue Suckers are now extirpated from Pennsylvania, are protected as a threatened or endangered species in five states (Ohio, New Mexico, Tennessee, Texas, and Wisconsin), and are listed as a species of concern in an additional seven states (Louisiana, Minnesota, Montana, North Dakota, Oklahoma, South Dakota, and West Virginia). The American Fisheries Society classifies Blue Suckers as a “vulnerable” species, indicating “...imminent danger of becoming threatened throughout all or a significant part of its range” (Jelks et al. 2008). With the growing need to sustain Blue Sucker populations via management and protection, research on this species has become crucial.

The successful management of vulnerable fish populations depends on biologists’ ability to accurately estimate population parameters such as mortality and growth. The

accuracy and precision of these estimates of dynamic rates are dictated by the accuracy and precision of the age estimates assigned to individual specimens. Therefore, age assignments are one of the most influential biological measures in fisheries management (Campana 2001). Unfortunately, a lack of unified aging methods and lack of any validated structure for Blue Sucker age estimation has led to general confusion regarding the longevity and basic life history of the species.

Estimates of Blue Sucker longevity have varied greatly among past aging studies (Table 1.1). Scales have long been known to underestimate age in large fishes including catostomids (Beamish and Harvey 1969; Beamish and McFarlane 1983; Casselman 1983; Beamish and McFarlane 1987; Scopettone 1988). Scales have been shown to underestimate age relative to pectoral fin rays and otoliths in White Suckers *Catostomus commersonii* (Quinn and Ross 1982; Sylvester and Berry 2006). Nonetheless, aging with scales dominated Blue Sucker research until the mid-2000s. The ten-year maximum longevity estimate made in the early 1980s based on scales (Rupprecht and Jahn 1980; Moss et al. 1983) has been hard to dispel and still appears in guidebooks and Blue Sucker species profiles. Since the mid-2000s researchers have recognized the inadequacy of scales and favored the use of pectoral fin rays for estimating age in this species. Based on pectoral fin rays, researchers have estimated Blue Sucker ages as high as 37 years (Bednarski and Scarnecchia 2006) and 34 years (Lyons et al. 2016).

Opercular bones (opercles) have previously been analyzed for marginal increment analysis and used to age the closely-related species, the Southeastern Blue Sucker *Cycleptus meridionalis*, assigning ages up to 33 years (Peterson et al. 1999) which some have suggested is likely the accurate age range for Blue Suckers (Burr and Maiden

1999). However, opercles may be problematic for aging catostomids because bone growth has been found to hide early annuli, and annulus edge-crowding can occur on older fish (Scopettone et al 1986; Scopettone 1988).

Within the last decade research has begun to compare Blue Sucker age estimates among structures. LaBay et al. (2011) reported that relative to fin rays, scales underestimated the ages of individuals  $\leq 7$  years old, and were less precise. Acre et al. (2017) compared Blue Sucker age estimates from scales, anal fin rays, dorsal fin rays, pelvic fin rays, pectoral fin rays, and lapillus otoliths. Though they found dorsal, anal, and pelvic fin rays to underestimate age relative to otolith ages, they did not identify any bias between the ages obtained from scales or pectoral fin rays relative to otoliths. The authors suggested that the absence of bias among these three structures was due in part to the predominance of young fish in their study. The small sample size of their study ( $n = 9$ ) also limits interpretation of their results.

The need for precise and accurate age estimates is paramount to successful fisheries management (Beamish and McFarlane 1983; Casselman 1983; Campana 2001; Quist et al. 2007). Casselman (1983) emphasized that detrimental systematic errors can result from age structures that do not continue to grow throughout the life of a fish, resulting in consistent under-aging of the species. Campana (2001) warned of the damage that can be inflicted on fish populations as a result of management decisions made based on optimistic growth rates calculated from under-estimated ages. The successful management and protection of Blue Sucker populations is currently impaired by uncertainty regarding the appropriate hard structure with which to age the species. Identifying the most accurate and precise aging structure would allow biologists to

manage populations based on the most accurate and reproducible estimates of longevity, growth, and mortality. Based on the imperiled status of the species, lethal aging techniques have largely been avoided in Blue Sucker research. There is valid concern in sacrificing substantial numbers of Blue Suckers in locations in which their status is unknown, and collection permits may be limited in states in which the species is protected. It is critically important, however, that we understand the value and limitations of different aging structures, lethal or non-lethal, to guide Blue Sucker management. Thus, the objectives of this research were to compare the precision and credibility of the age estimates using multiple structures (scales, opercles, pectoral fin rays, and lapillus otoliths) with which to estimate age in Blue Suckers, and to identify the structure(s) that yield the most precise and credible results.

## METHODS

*Site description.* – The Wabash River originates near Fort Recovery, Ohio, and flows approximately 764 river kilometers (rkm) southwest before its confluence with the Ohio River. It is the largest northern tributary of the Ohio River, with discharge ranging from 2,610 m<sup>3</sup>/s to 317,000 m<sup>3</sup>/s (river gauge data from New Harmony, IN, 2010-2019, USGS 2020).

Sampling for this research (excepting three specimens obtained from rkm 471) was restricted to the lower 322 rkm of the Wabash River, where it forms the border between Illinois and Indiana. This stretch of river will be referred to henceforth as the “lower Wabash River,” and spans from just south of Terre Haute, IN, to the confluence of the Wabash River with the Ohio River. In this study, rkm are counted northward, with

rkm 0 at the mouth of the Wabash River. The fish assemblage of the lower Wabash River has been annually surveyed since 2010 as part of the Illinois Natural History Survey's Long-term Electrofishing (LTEF) program, conducted by Eastern Illinois University since 2012.

*Sampling methods.* – The annual LTEF surveys employed standardized DC boat electrofishing at randomly selected sites along the shorelines of the navigable river and were conducted between June and October (Gutreuter et al. 1995; Fritts et al. 2014; Fritts et al. 2017). The majority of the Blue Sucker specimens used in this research were collected during the 2018 and 2019 LTEF surveys ( $n_{\text{LTEF}} = 132$ ). Additional specimens were collected by targeted DC electrofishing ( $n_{\text{target}} = 27$ ) and while sampling for other research projects ( $n_{\text{opportunistic}} = 9$ ). We dissected each specimen and collected multiple hard structures with the potential for yielding age estimates ( $n_{\text{total}} = 168$ , total length range = 189-774 mm, mean total length = 604.64 mm, median total length = 615 mm, Figure 1.1).

*Preparing hard structures.* – Methods of removal, preparation, and reading of each potential aging structure (scales, opercles, cleithra, pectoral fin rays, and lapillus otoliths) are addressed below. As these structures have not been validated for Blue Sucker, we will use the term “annuli” to refer to marks that we presumed to be annually formed.

At least 10 scales were collected from the left side of each 2018 specimen ( $n = 68$ ). Scales were taken from the region above the lateral line and below the dorsal fin of each fish, as suggested by Schneider et al. (2000). Regenerated scales and areas of scarring were avoided when apparent. We selected a single scale sample to represent each

specimen and three readers independently counted the number of annuli on each sample under an Olympus SZ51 dissecting microscope (8x to 40x) with transmitted light. Presumed scale annuli were identified via crowding and thinning of circuli in the anterior and lateral portions of the scale (Cable 1956); also annuli appeared as thin light-colored bands with manipulation of the angle of transmitted light and the focal depth of the microscope (Beamish and Chilton 1977).

We removed the left opercle from each 2018 Blue Sucker specimen ( $n = 68$ ) and boiled each opercle to remove the flesh. Opercles ranged in diameter from 17 to 59 mm (from dorsal to ventral point). Opercles were digitally photographed at 1x against a black background and enhanced in Adobe® Photoshop® software. Three readers independently viewed the images and recorded annulus counts. The opercles of the adult Blue Suckers were too thick for annuli to appear as translucent bands. The lateral surface of the bone was patterned in a series of fine circulus-like ridges, highly variable in their relative spacing and boldness. We experimented with cross-sectioning the opercles, but the cross sections were deemed more difficult to interpret than the whole structure. Presumed opercular annuli were identified with low confidence as appearing as relatively “bolder” ridges.

We removed the left cleithrum from each 2018 specimen ( $n = 68$ ) and then boiled and cleaned the bones. We attempted to estimate ages from whole cleithra, but were unable to do so because (1) annuli approaching the origin appeared hidden by a thickened build-up of bone in that region and (2) annuli approaching the edge of the posterior blade were lost due to the paper-thin and brittle qualities of the edge. We then attempted cross sectioning cleithra, but this revealed a porous bone core and did not improve annulus

readability. We therefore determined the cleithrum to be an unsuitable hard structure for estimating the age of this species, and their use in this study was discontinued.

We collected the leading left and right pectoral fin rays of each 2018 and 2019 specimen ( $n = 168$ ). We used a scalpel to separate the lead fin ray from the rest of the pectoral fin and used cutting pliers to detach each lead fin ray at its base, as close to the body wall as possible. One pectoral fin ray from each specimen was sectioned on an IsoMet™ low-speed diamond-blade saw. Multiple sections were made from each ray, starting from the base end of the ray and working no more than 1 cm forward. Samples collected in 2018 were sectioned with a single-blade saw; section thicknesses ranging from 0.5-1.0 mm were tried experimentally, and we determined 0.7 mm to be the target thickness for subsequent sections. Sections were mounted onto glass slides and polished with a series of aluminum oxide sheets ranging from 250 grit to 60,000 grit. Samples collected in 2019 were thin-sectioned using twin diamond-embedded blades, which produced comparable results without the need for polishing.

Fin ray thin sections were viewed under a Leica S8 APO dissecting microscope (10x to 80x) using reflected light against a black background, with glycerin oil to enhance annulus visibility. Under the microscope, we selected a single representative fin ray section for each specimen based on clarity of visible annuli. Sections taken closest to the base of the fin ray, where it is flared, were frequently selected as having the best readability and focal integrity. Digital images were taken of each sample (35x to 80x) using a mounted Leica EC3 3.1MP microscope camera and enhanced in Adobe® Photoshop® software. Three readers independently viewed the original and enhanced images and assigned an estimated age to each specimen based on the number of apparent



annuli. Presumed pectoral fin ray annuli were identified as thin translucent/absorptive bands separated by thicker opaque/reflective intervals (Figure 1.2 A-E).

During dissection, we attempted to extract both lapillus otoliths from all 2018 and 2019 specimens ( $n = 168$ ). In some cases we were unsuccessful at locating one or both lapilli, but our rate of success improved with repetition. Asteriscus and sagittal otoliths were extracted from some 2019 specimens for experimental use in age estimation, but these structures proved to be fragile and limited in their usefulness. We successfully collected at least one lapillus otolith from each of 155 specimens. Otoliths were air-dried whole and weighed to the nearest 0.001 g using a Cahn Electrobalance®. They were then prepared and aged following the protocol used by Lackmann et al. (2019) on the lapilli of Bigmouth Buffalo *Ictiobus cyprinellus*, a closely-related catostomid for which otolith age-estimates were validated via bomb radiocarbon dating. Blue Sucker lapilli were set in epoxy and were thin-sectioned through the core of the otolith at approximately 300  $\mu\text{m}$  using an IsoMet™ low-speed diamond-blade saw with twin blades.

Lapillus otolith thin-sections were coated in mineral oil and viewed under an Olympus® BH-2 or CX31 or a Nikon Eclipse 80i compound microscope. Samples were digitally photographed (40x to 100x) using SPOT Imaging™ or Swift® photo-microscopy software and enhanced in Adobe® Photoshop® software. Three readers estimated ages by digitally marking annuli on each otolith image. Viewed with transmitted light, the nucleus and the slow-growth bands appeared dark and opaque, whereas the fast growth periods were translucent and light. The repetitive and consistently spaced opaque bands were interpreted as annuli (Figure 1.2). These presumed annuli were immediately

recognizable and consistent with other validated catostomid lapillus annuli (Terwilliger et al. 2010; Bettinger and Crane 2011; Lackmann et al. 2019).

*Age estimation.* – Three readers examined each hard structure and recorded an estimated age equal to annuli counted. To minimize bias, estimates were made independent of other readers and without knowledge of the specimen (e.g. total length) or of the relationship between multiple structures from the same specimen. Two of the three readers who read scales, pectoral fin rays, and opercles differed from those who read otoliths; the corresponding author read all structures.

All structures were read with the presumption that edge annuli would be more closely spaced than central annuli. For pectoral fin rays, opercles, and otoliths, readers agreed that rings that did not appear continuous across most of the structure would be considered checks and would not be counted. Due to difficulty interpreting the opercular bones, readers were forced to employ the presumption that annual ridges would appear bolder than checks and would occur at reasonably-spaced intervals. Readers agreed that thin-sectioned lapillus otoliths increments beyond the innermost 4-5 annuli occurred at notably even intervals (Figure 1.2 *G-J*).

Interpretation of the edge of each structure was standardized based on collection date. Birthdates were set at April based on local Blue Sucker spawning observations (Daugherty et al. 2008; C. Jansen, Indiana Department of Natural Resources, personal communication), and annuli were estimated to be formed around January, on average the coldest month of the year in the research locality and presumably least-optimal for growth (Casselman 1983). The edge of either structure was not counted as an annulus for specimens collected June-December, as these samples were thought to have exhibited

marginal growth since the formation of the last (winter) annulus. Conversely, the edge of each structure was counted as an annulus for specimens collected January-May, as these samples likely did not exhibit noticeable growth past the formation of the most recent (winter) annulus.

*Comparing structures.* – The relative precision of scales, opercles, and pectoral fin rays was compared using Blue Sucker samples collected in 2018 ( $n = 68$ ). For this comparison, samples that were deemed unreadable by at least two of the three age readers were removed from that structure’s sample set.

We addressed structure bias by comparing age estimates from pectoral fin rays to those from lapillus otoliths using Blue Sucker specimens collected in both 2018 and 2019 ( $n = 168$ ). For each sample, agreement among readers was based on a tiered-allowance system, in which specimens in their first decade (0-9 years) were given a 1-year allowable discrepancy (AD) among readers, specimens in their second decade (10-19 years) given a 2-year AD, and so on. A decade was assigned to each sample of each structure based on the decade assigned by at least two of three readers. The minimum discrepancy (MD) among readers was used to determine the final assigned age: (a) when  $MD = 0$ , the consensus was accepted; (b) when  $MD = 1$ , we selected the median age estimate; (c) when  $MD \geq 2$  we assigned age as the rounded average of the three estimated ages. When the MD among the three readers was  $\leq$  the AD assigned to the sample, the sample was deemed to have produced “acceptable agreement.” Re-sections were attempted for pectoral fin ray and lapillus otolith samples that were deemed unreadable by two or more readers, or that failed to reach an acceptable agreement (if a backup

sample was available for the specimen). If a readable sample could not be obtained, the specimen was excluded from the sample set for that structure.

*Statistical analysis.* – Statistical measures were calculated using Microsoft® Excel (2007), Fishery Analysis and Modeling Simulator (FAMS © v1.0), and R (R Core Team, 2018) software. Each specimen was assigned to a year class based on its year of capture minus its estimated age. Significant bias between readers or structures was determined with Microsoft® Excel add-in Real Statistics Resource Pack using methods published by Howell (2010) for comparing the slopes of two independent samples. The mean coefficient of variation (CV) and average percent error (APE) were calculated for each structure based on equations published by Campana (2001). Fishery Analysis and Modeling Simulator 1.0 software was used to estimate mortality based on each structure's catch curve of assigned ages; underrepresented early age classes were omitted from the catch curve. Nonlinear growth models were compared in R (package: fishmethods), and von Bertalanffy growth models were calculated (packages: FSA, nlstools) using methods described by Ogle (2016) for model selection and comparing parameters between sexes. The significance of all statistical tests was defined by  $\alpha = 0.05$ .

## RESULTS

Our comparison of three readers' age estimates based on structures from 68 Blue Sucker specimens revealed that pectoral fin rays (mean CV = 13.69%, APE = 10.10%) produced more precise results than scales (mean CV = 35.09%, APE = 25.65%) or opercles (mean CV = 21.07%, APE = 15.57%, Table 1.2). As previous studies have

suggested, scales in this study assigned lower ages (3-16 yrs) than pectoral fin rays (4-20 yrs, Figure 1.3). Readers were able to assign age estimates to all but one scale sample ( $n = 67$ , 98.5%) but reported difficulty defining the innermost annuli and interpreting closely-spaced edge annuli on older specimens. Opercles were highly variable in appearance and several were damaged by scarring and regeneration, allowing readers to estimate ages for 65 samples (95.5%); readers reported this structure to be the most difficult to interpret with any confidence. Pectoral fin rays required more processing time for thin sectioning and also varied highly in their readability; age estimates were assigned to 64 samples (94.1%) and though there was great variation among samples, a portion were able to be read with relative confidence. Reader-bias plots revealed significant reader bias in two-out-of-three comparisons between readers of scales, in three-out-of-three comparisons for opercles, and in one-out-of-three comparisons for fin rays ( $\alpha = 0.05$ , Figure 1.4). We also identified significant non-parallel structure-bias among all three structures, based on the average of the three age estimates assigned to each specimen by each structure ( $\alpha = 0.05$ , Figure 1.5).

As fin rays were found to be substantially more precise (mean CV and APE) than scales or opercles and have already been favored among Blue Sucker researchers as a non-lethal aging structure, we proceeded to analyze this structure further by comparing fin ray age estimates to lapillus otolith age estimates. Based on an augmented sample size ( $n = 168$ ), pectoral fin rays yielded a greater proportion of readable samples ( $n = 167$ , 1 was unreadable) compared to lapillus otoliths ( $n = 128$ , 13 failed extraction completely (i.e. no otoliths extracted), and 27 failed thin-sectioning or were unreadable, Table 1.4). Though otoliths proved more difficult to obtain and prepare, our methods improved with

practice and the majority of these losses occurred early in the research mostly due to inadequate processing methods prior to collaboration with A. R. Lackmann. Some otolith samples proved too dark and opaque after thin-sectioning, but the majority of samples had distinct and regularly-spaced annuli that could be read with moderate-to-high confidence (Figure 1.2). Fin rays were found to be less precise (CV = 18.45%, APE = 13.61%, 94.6% acceptable agreement) than lapillus otoliths (CV = 12.26%, APE = 9.09%, 97.7% acceptable agreement, Table 1.3). Age estimates based on otoliths resulted in a remarkably higher range of ages (1-42 years) compared to fin rays (1-20 years, Figure 1.6). Assigned otolith age was strongly correlated with whole lapillus mass with no significant effect of sex ( $R^2 = 0.89$ , Figure 1.7). This correlation was calculated using the heavier lapillus when two were available for a specimen, in case the lighter lapillus was incomplete. Lapilli from the same specimen differed by >1 mg in only 5.2% of instances. Lapilli from one specimen did differ dramatically, with one lapillus 30% smaller than the other (4.2 mg difference).

We compared the ages assigned to individual specimens and identified significant non-parallel structure bias between pectoral fin rays and lapillus otoliths ( $P < 0.0005$ , Figure 1.8). We were unable to identify any range of fin ray ages that could be accurately corrected to otolith age. Relative to otolith age assignments, fin rays overestimated age in 7-year-olds and younger, by as much as seven years, and underestimated age in 13-year-olds and older, by as much as 34 years in the oldest fish (Figures 1.8 and 1.9). Similarly, no range of total lengths could be identified for which fin ray ages could be accurately corrected to otolith age. Relative to otolith age assignments, fin rays overestimated age in individuals < 550 mm and underestimated age in individuals > 625 mm (Figure 1.10).

We hypothesize that this relationship is a result of the high inconsistency of annulus-like marks on the pectoral fin ray thin-sections. Smaller and younger individuals may exhibit more prominent checks in the fin rays, leading to erroneously high estimations of age (e.g. Figure 1.2, comparison of the spacing of scored annuli in fin ray *B* and *C* versus *D* and *E*). As individuals grow larger and older, banding seems to become less pronounced in the fin rays. In addition, annuli become clustered on the edges of the structure and the fin rays may even eventually cease to grow, following the somatic growth of the species and leading to under-estimations of age (e.g. Figure 1.2, fin rays *D* and *E*). In contrast, annuli-like marks in the lapillus otoliths were highly consistent across all samples (Figure 1.2, otoliths *F-J*).

Catch curve analyses estimated a population annual mortality rate of 22.9% based on fin ray ages versus 4.5% based on otolith ages. We modeled the data using von Bertalanffy, Gompertz, and logistic growth models and found the von Bertalanffy model to have the best fit, based on residual sum-of-squares. The von Bertalanffy growth model parameters [with bootstrapped 95% upper and lower confidence intervals] were calculated from fin ray ages ( $L_{\infty} = 674.6811$  [650.4, 710.9],  $K = 0.2408$  [0.1760, 0.3081],  $t_0 = -1.0523$  [-2.5131, -0.2274]) and from otolith ages ( $L_{\infty} = 680.2904$  [663.9, 698.2],  $K = 0.1590$  [0.1222, 0.2059],  $t_0 = -5.1404$  [-7.2992, -3.3905], Figure 1.11). Model selection indicated (based on the highest log-likelihood value and the lowest Akaike information criterion value) that parameters  $K$  and  $t_0$  differed significantly between males and females based on both fin rays ( $K_{\text{female}} = 0.3333$  [0.1154, 0.6108],  $t_{0\text{-female}} = 1.1869$  [-5.7265, 2.9674];  $K_{\text{male}} = 0.0932$  [0.0247, 0.4125],  $t_{0\text{-male}} = -10.9044$  [-28.8206, 0.6056]) and otoliths ( $K_{\text{female}} = 0.1082$  [0.0721, 0.1502],  $t_{0\text{-female}} = -8.2634$  [-13.1748, -5.2266];  $K_{\text{male}} =$

0.0519 [0.0267, 0.0802],  $t_{0\text{-male}} = -22.0704$  [-36.5128, -14.7590]). The  $t_0$  parameter is negative in all but one of these models, which is likely due to an underrepresentation of young age classes in our data. The male-specific otolith  $t_0$  parameter (-22.0704) is extreme and reflects the inadequacy of the data in the sex-specific models. The mixed-sex models include data from individuals of indeterminate sex (22% of 168 specimens), and these specimens make up the underrepresented young age classes, which are therefore absent from the sex-specific models.

## DISCUSSION

This study identified lapillus otoliths as being the most precise structure for estimating Blue Sucker ages. Specimens were aged up to 42 years with otoliths, greatly exceeding age ranges assigned by other structures in the comparison. The population age structure based on otolith ages contains a high proportion of adult specimens, as would be expected in an unexploited stock (Goedde and Coble 1981; Figure 1.6).

This study, like the majority of Blue Sucker research efforts, is hindered by a scarcity of young/small specimens (< 500 mm, Figure 1.1) which could have an influence on comparisons between aging structures and on growth curve analyses. Young Blue Suckers have been documented to occupy shallow riffles and gravel bars, vegetated shorelines, side channels, and inundated floodplains (Cross and Collins 1975; Moss et al. 1983; Semmens 1985; Eder 2009; Steffensen et al. 2014). This has led to speculation that young Blue Suckers may segregate from adults (Morey et al. 2003). Alternatively, it has been suggested that young Blue Suckers may associate with adults but are less susceptible to electrofishing gear (LaBay 2008; Mayes 2015).



It is worth considering that the value of particular aging structures may not be consistent across the geographical range of the species. Images of thin-sectioned pectoral fin rays published by Bednarski and Scarnecchia (2006) showed more clearly-defined annuli for Blue Suckers in the Milk River, Montana, compared to those analyzed in this study of Blue Suckers in the lower Wabash River, Illinois/Indiana. Differences in latitude and associated seasonal temperature extremes could impact the clarity of annuli in pectoral fin rays, and possibly other structures, but whether or not that is occurring in this species has not been explored.

Though no aging structure has been validated for this species, otoliths can be expected to provide the most accurate ages; however, this expectation demands future confirmation via validation. The tight correlation between whole lapillus mass and otolith age assignment ( $R^2=0.89$ , Figure 1.7) indicates that lapilli experience consistent growth with age, despite asymptotic somatic growth. In contrast, hard structures which mirror the diminishing increments of somatic growth in long-lived fishes (e.g. fin rays, opercles, scales) can be biased toward under-aging (Casselman 1983; Beamish and McFarlane 1987). Because otoliths grow acellularly and are metabolically inert, they are not subject to resorption and vascularization the way scales, opercles, and fin rays are; thus otoliths retain annuli that could be lost in other structures (Casselman 1983; Secor et al 1995).

Studies of many fishes have revealed otoliths to generally be the most reliable aging structure (Casselman 1983). Lapillus otoliths have been validated for aging a variety of catostomid species including the White Sucker (Thompson and Beckman 1995), Lost River Sucker *Deltistes luxatus*, and Shortnose Sucker *Chasmistes*

*brevirostris*, (Hoff et al. 1997; Terwilliger et al. 2010), Notchlip Redhorse *Moxostoma collapsum*, and Brassy Jumprock *Moxostoma sp.*, (Bettinger and Crane 2011), and Bigmouth Buffalo (Lackmann et al. 2019). Otoliths were found to have higher precision than other aging structures in Razorback Suckers *Xyrauchen texanus* (McCarthy and Minckley 1987), and in Bluehead Suckers *Catostomus discobolus*, Flannelmouth Suckers *Catostomus latipinnis*, White Suckers, Roundtail Chub *Gila robusta*, Creek Chub *Semotilus atromaculatus*, White Sucker x Bluehead Sucker hybrids, and White Sucker x Flannelmouth Sucker hybrids (Quist et al 2007). Fin rays, in contrast, have been found to be inadequate for aging multiple long-lived species including Cui-ui *Chasmistes cujus*, (Scoppettone 1986), Pallid Sturgeon (Hurley et al. 2004) and White Sturgeon *Acipenser transmontanus* (Rien and Beamesderfer 1994). Our range of fin ray ages (1-20 yrs) is in line with fin ray ages assigned to Blue Suckers in the Wabash River in 2009 (3-16 yrs, Bacula et al. 2009) but otolith data suggest these fin rays may be severely underestimating the ages of some specimens.

We recommend the mixed-sex otolith-age von Bertalanffy growth model (Figure 1.11) as being more descriptive of our data than the sex-specific models, because a significant proportion (22%) of our samples were of unknown sex. The rapid early growth in total length depicted by this model may be driven by the evolutionary advantage of escaping predation-vulnerability as early as possible. Our von Bertalanffy growth model predicts an individual to average 424 mm at age-1, 462 mm at age-2, and 494 mm at age-3. This growth is more rapid than that found by Moss et al (1983), who found 1-year olds to average 266 mm and 2-year olds to average 323 mm based on scale ages, and by Eitzmann and Makinster (2007), who found 2-year olds to be around 200

mm based on pectoral fin rays, but in line with LaBay et al. (2008) who found age-0 specimens up to 461 mm total length based on pectoral fin rays. Differences in these estimations of length at age may illustrate the influence that the choice of aging structure has on subsequent conclusions about growth. However, our growth model contains only a few 1-year olds, ranging from 189 mm to 439 mm total length, and no age-0 fish. The overall lack of very young fish in our otolith-based von Bertalanffy growth model may result in overestimates of early growth (stemming from the negative  $t_0$  parameter), and estimations of growth at these early ages should be considered with caution.

Aging Wabash River Blue Suckers with otoliths yielded outcomes in contrast to those of Bacula et al. (2009), who aged the same population a decade earlier using pectoral fin rays. Whereas Bacula et al. estimated population mortality between 22% and 25%, our findings based on otolith ages estimate population mortality at only 4.5%. The previous team described rapid growth up to age-6 (48-141 mm/yr), but our model supports rapid growth only up to age-1 (424 mm). Though we found our mixed-sex growth model to be most appropriate due to the high proportion of specimens of unknown sex, we did detect significant differences in the  $K$  and  $t_0$  parameters by sex, which suggested that males grew more slowly than females but eventually achieved comparable lengths at older ages. Sex-specific growth has also been detected in other Blue Sucker studies (Ruppertch and Jahn 1980; Moss et al. 1983; Vokoun et al. 2003; Bednarski and Scarnecchia 2006; Lyons et al. 2016; Acre 2019).

Although the use of lethal aging structures has been largely avoided in past Blue Sucker research, it is important for biologists to understand the limitations of non-lethal structures for this species. A lethal structure that offers greater precision yields more

reproducible data (Campana 2001) and a lethal structure that offers greater accuracy can better inform species management (Beamish and McFarlane 1983). The dramatic discrepancy between the estimated population mortality rate from fin rays (22.9%) versus from otoliths (4.5%) demonstrates the impact that choice-of-aging-structure can have on population parameters that are crucial to management decisions. Though we sought to recommend a range of fin ray ages or specimen lengths that could be used with correction-to-otoliths for non-lethally aging Blue Suckers, we were unable to identify any such range (Figures 1.9 and 1.10). We recommend aging this species with lapillus otoliths and suggest that the prudent harvest of Blue Suckers for lethal aging is a necessary sacrifice to inform the management of threatened and endangered populations.

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TABLES

Table 1.1: Literature review of published Blue Sucker maximum ages based on various hard structures used for aging, presented chronologically.

Age structure	Maximum age	Sample size ( <i>n</i> ) & maximum total length	Reference
Scales Pectoral fin rays	10 yrs 13 yrs	<i>n</i> = 153 817 mm	Ruppretch and Jahn 1980
Scales	9 yrs	<i>n</i> = 84 763 mm	Moss et al. 1983
Scales	22 yrs	<i>n</i> = 103 800 mm	Vokoun et al. 2003
Scales	9 yrs	<i>n</i> = 102 717 mm	Morey et al. 2003
Scales	11 yrs	<i>n</i> = 264 700 mm	Hand et al. 2003
Pectoral fin rays	37 yrs	<i>n</i> = 253 806 mm	Bednarski and Scarnecchia 2006
Pectoral fin rays	16 yrs	<i>n</i> = 101 782 mm	Eitzmann and Makinster 2007
Scales Pectoral fin rays	7 yrs 7 yrs	<i>n</i> = 511 <i>n</i> = 584 650 mm	LaBay et al. 2008
Pectoral fin rays	16 yrs	<i>n</i> = 250 775 mm	Bacula et al. 2009
Scales Pectoral fin rays	16 yrs 22 yrs	<i>n</i> = 230 797 mm	LaBay et al. 2011
Pectoral fin rays	34 yrs	<i>n</i> = 173 822 mm	Lyons et al. 2016
Scales Pectoral fin rays Lapillus otoliths	9 yrs 9 yrs 11 yrs	<i>n</i> = 9 720 mm	Acre et al. 2017
Scales Opercles Pectoral fin rays Lapillus otoliths	11 yrs 15 yrs 20 yrs 42 yrs	<i>n</i> = 68 769 mm <i>n</i> = 168 774 mm	This study

Table 1.2: Comparison of age estimates and measures of precision between scales, opercles, and pectoral fin rays for specimens collected in 2018 ( $n = 68$ ).

	Scales	Opercles	Fin rays
Sample size	67	65	64
Range of total lengths (mm)	497-769	497-769	499-769
Mean total length (mm)	614.5	617.0	616.1
Range of age estimates (yrs)	5-11	5-15	4-16
Mean age estimate (yrs)	8.2	8.3	9.3
Median age estimate (yrs)	8	8	9
Maximum discrepancy between readers (yrs)	11	11	11
Mean discrepancy between readers (yrs)	3.6	2.2	1.6
Mean CV (%)	35.09	21.07	13.69
Average percent error (%)	25.65	15.57	10.10

Table 1.3: Comparison of age estimates and measures of precision between pectoral fin rays and lapillus otoliths for specimens collected in 2018 and 2019 ( $n = 168$ ).

	Fin rays	Otoliths
Sample size	167	128
Range of total lengths (mm)	189-774	189-774
Mean total length (mm)	604.7	600.0
Range of age estimates (yrs)	1-20	1-42
Mean age estimate (yrs)	9.6	13.5
Median age estimate (yrs)	10	12
Maximum discrepancy between readers (yrs)	11	7
Mean discrepancy between readers (yrs)	2.1	1.5
Acceptable agreement (%)	94.6	97.7
Mean CV (%)	18.45	12.26
Average percent error (%)	13.61	9.09

Table 1.4: Based on 168 Blue Sucker specimens, frequency of age-assignment, minimum discrepancy values, and acceptable agreement achieved by three readers' age estimations using pectoral fin rays and lapillus otoliths.

	Pectoral fin rays		Lapillus otoliths	
0 samples obtained from specimen	0	<i>0% of 168</i>	13	<i>7.7% of 168</i>
≥1 sample obtained from specimen	168	<i>100% of 168</i>	155	<i>92.3% of 168</i>
Samples unreadable	1	<i>0.6% of 168</i>	27	<i>17.4% of 155</i>
Samples assigned ages	167	<i>99.4% of 168</i>	128	<i>82.6% of 155</i>
Minimum discrepancy among readers:				
0 yrs	71	<i>42.5% of 167</i>	72	<i>56.3% of 128</i>
1 yrs	71	<i>42.5% of 167</i>	48	<i>37.5% of 128</i>
≥ 2 yrs	25	<i>15% of 167</i>	8	<i>6.3% of 128</i>
Failed agreement	9	<i>5.4% of 167</i>	3	<i>2.3% of 128</i>
Achieved acceptable agreement	158	<i>94.6% of 167</i>	125	<i>97.7% of 128</i>



FIGURES

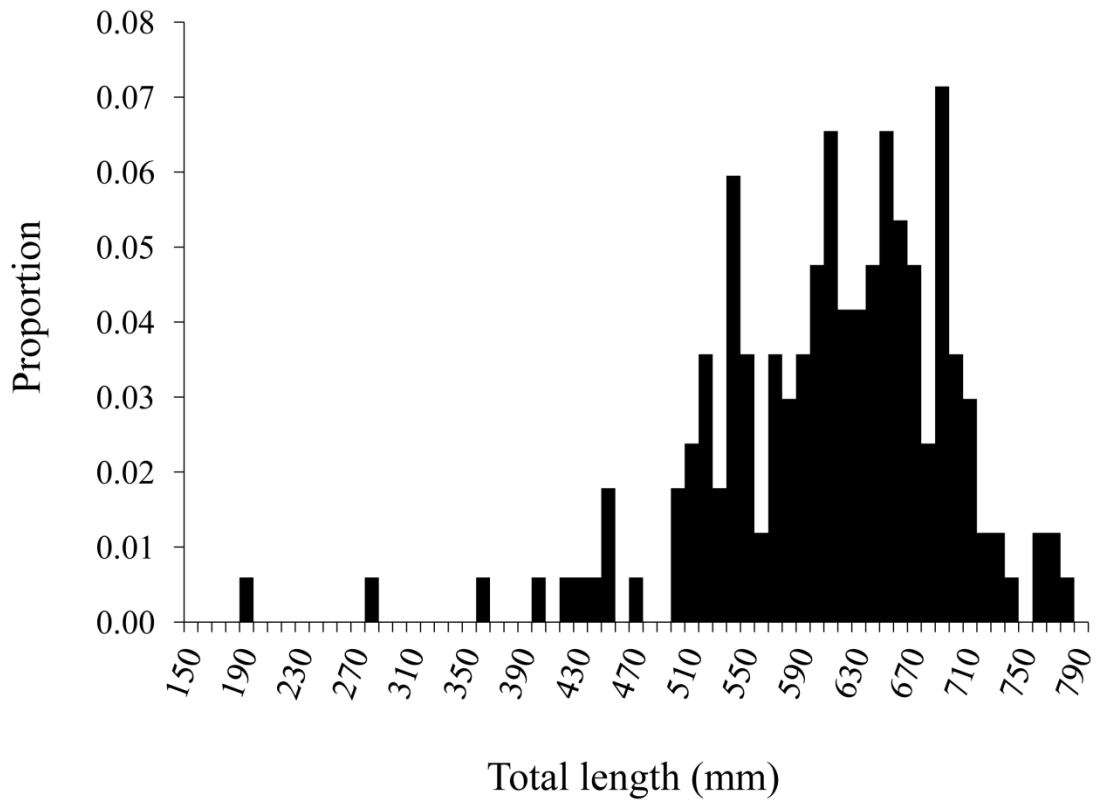


Figure 1.1. Total length frequency histogram of Blue Sucker specimens ( $n = 168$ ) collected in 2018 and 2019 from the lower Wabash River. Average total length = 604.6 mm; median total length = 615 mm.

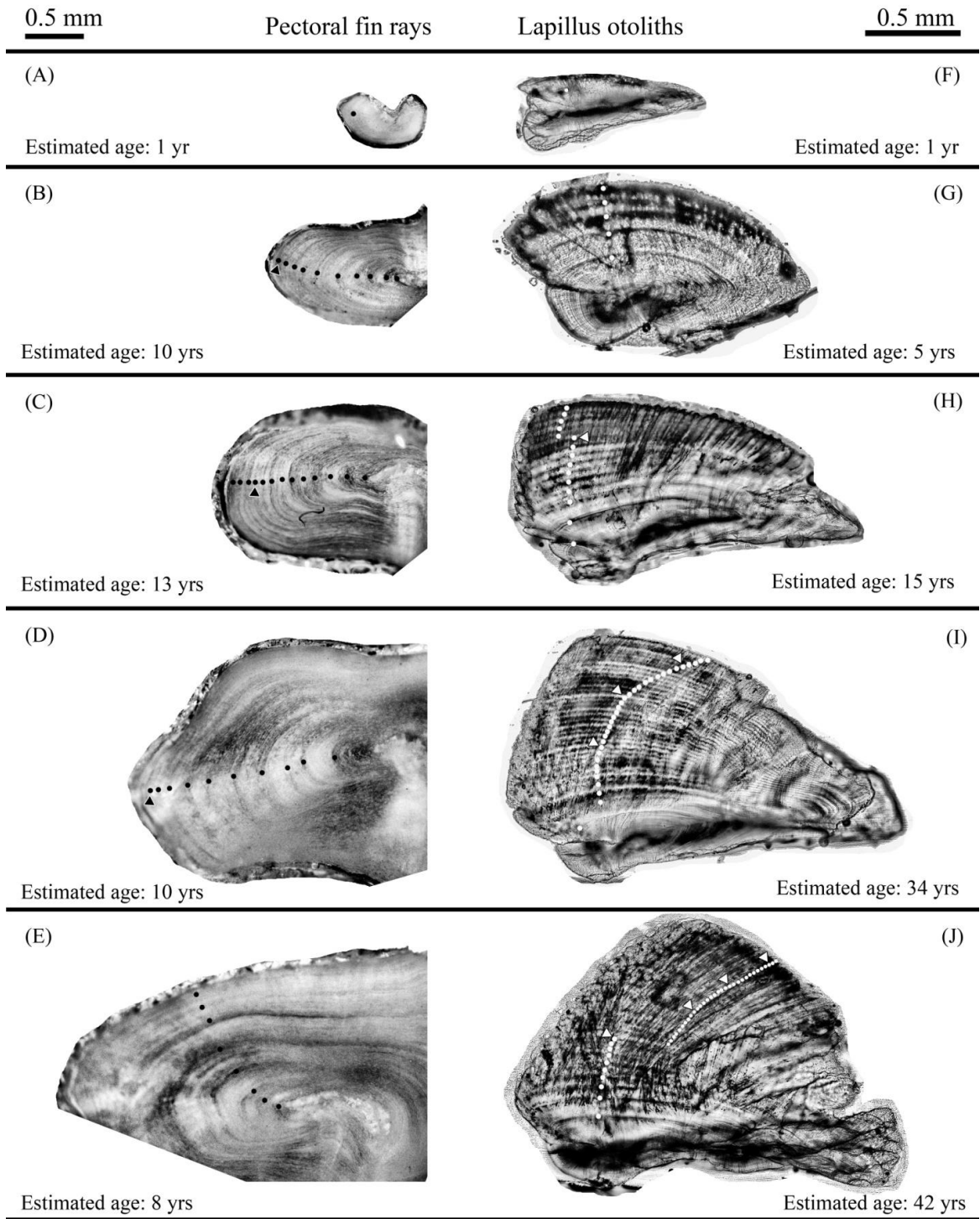


Figure 1.2. Thin-sectioned pectoral fin rays compared to thin-sectioned lapillus otoliths from the same specimens ( $n = 5$ ), with estimated ages. Within each row, both structures are from the same Blue Sucker specimen. Dots indicate presumed

annuli and triangles designate decades. All pectoral fin ray photos are set to the same 0.5 mm scale bar (upper left) and all lapillus otolith photos are set to the same 0.5 mm scale bar (upper right), and the scale is different between the two structures. Note: otolith thin-section *G* was cut on a different plane than the other otoliths.

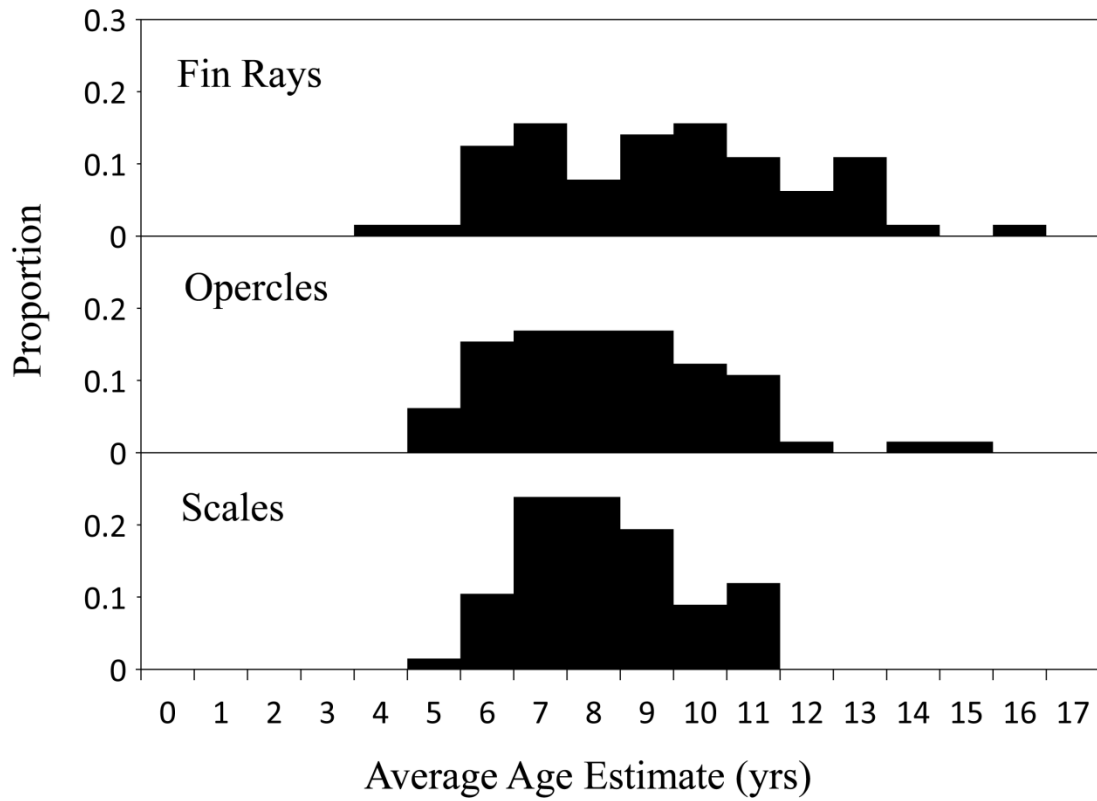


Figure 1.3. Age distribution for Blue Sucker specimens collected in 2018 ( $n = 68$ ) as aged by scales ( $n = 67$ ), opercles ( $n = 65$ ), and pectoral fin rays ( $n = 64$ ). Age estimates were averaged across three readers.

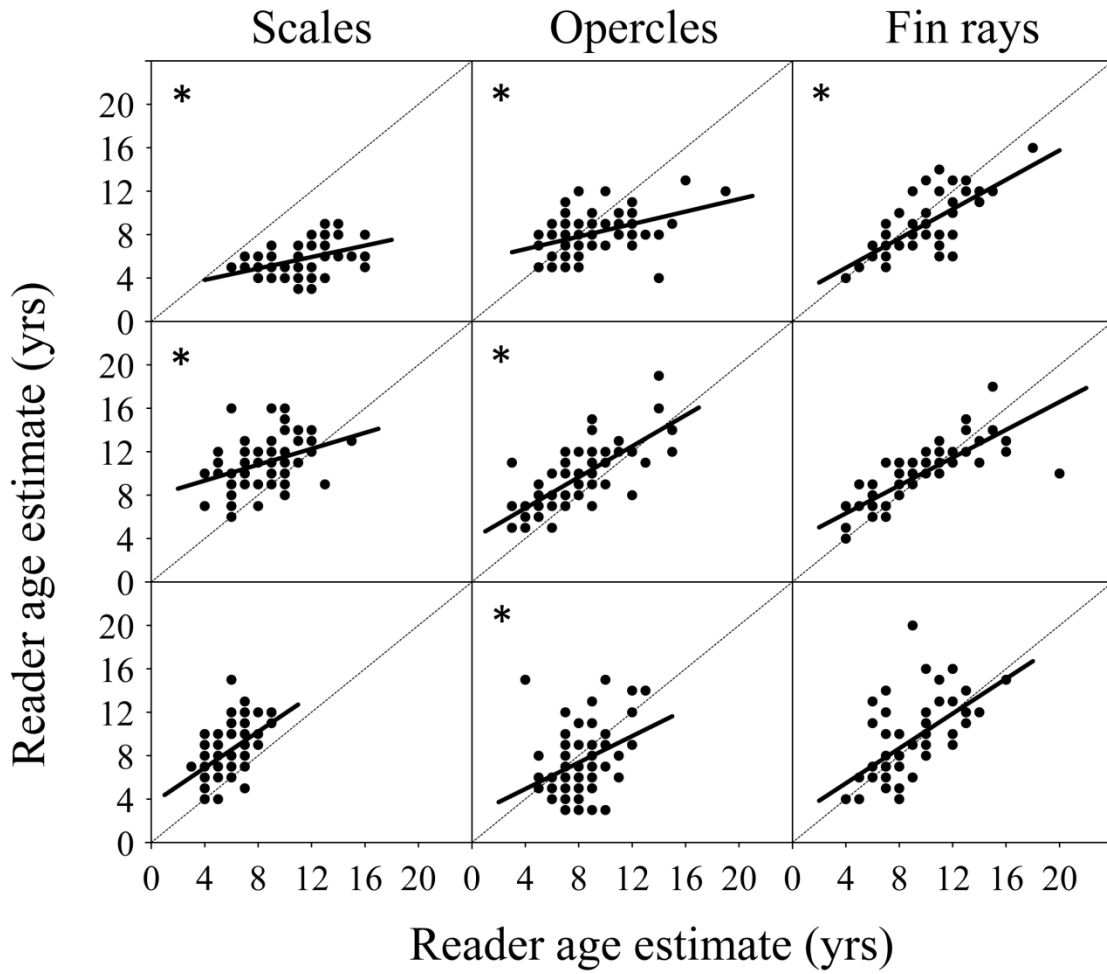


Figure 1.4. Reader bias plots comparing ages estimated by three independent readers using Blue Sucker scales, opercles, and pectoral fin rays. Asterisks indicate data trends with slopes differing significantly from 1 ( $\alpha = 0.05$ ).

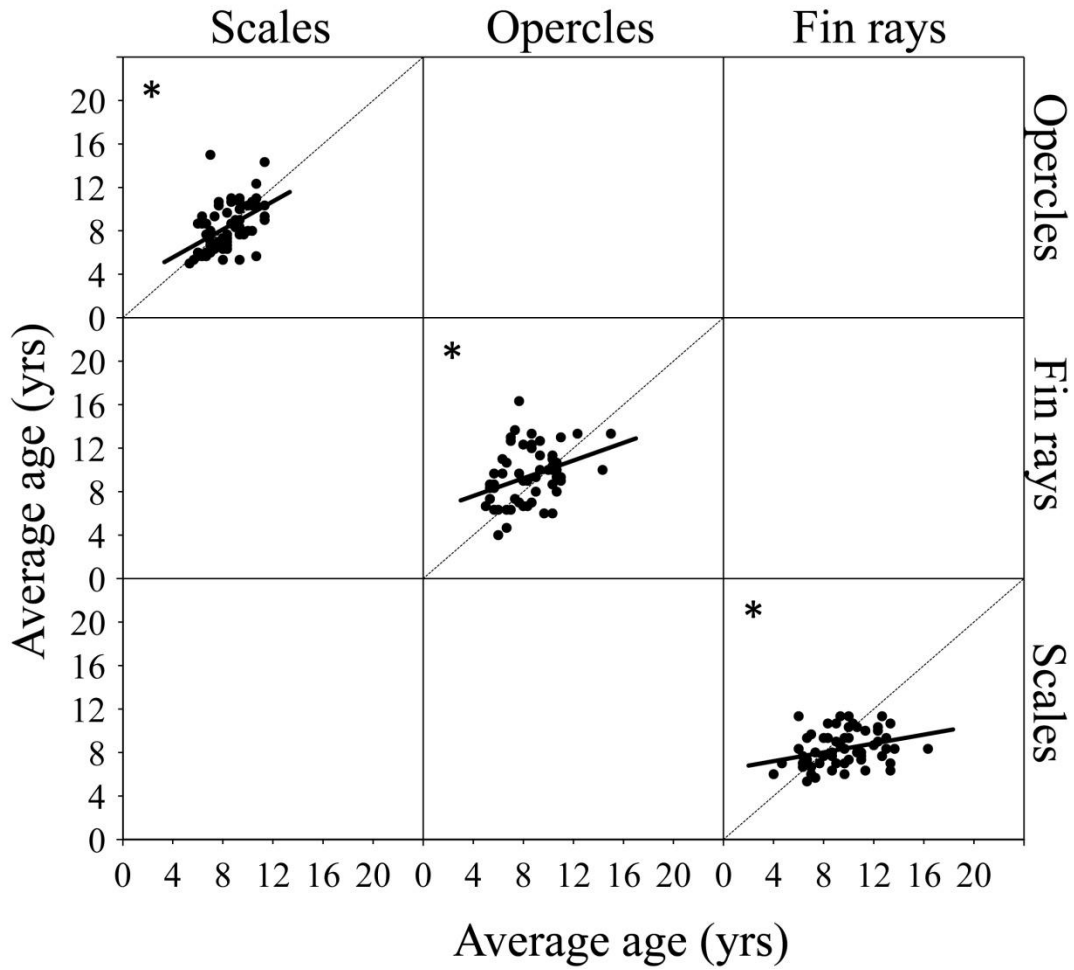


Figure 1.5. Structure bias plots comparing average age estimated by three independent readers using Blue Sucker scales, opercles, and pectoral fin rays. Asterisks indicate data trends with slopes differing significantly from 1 ( $\alpha = 0.05$ ).

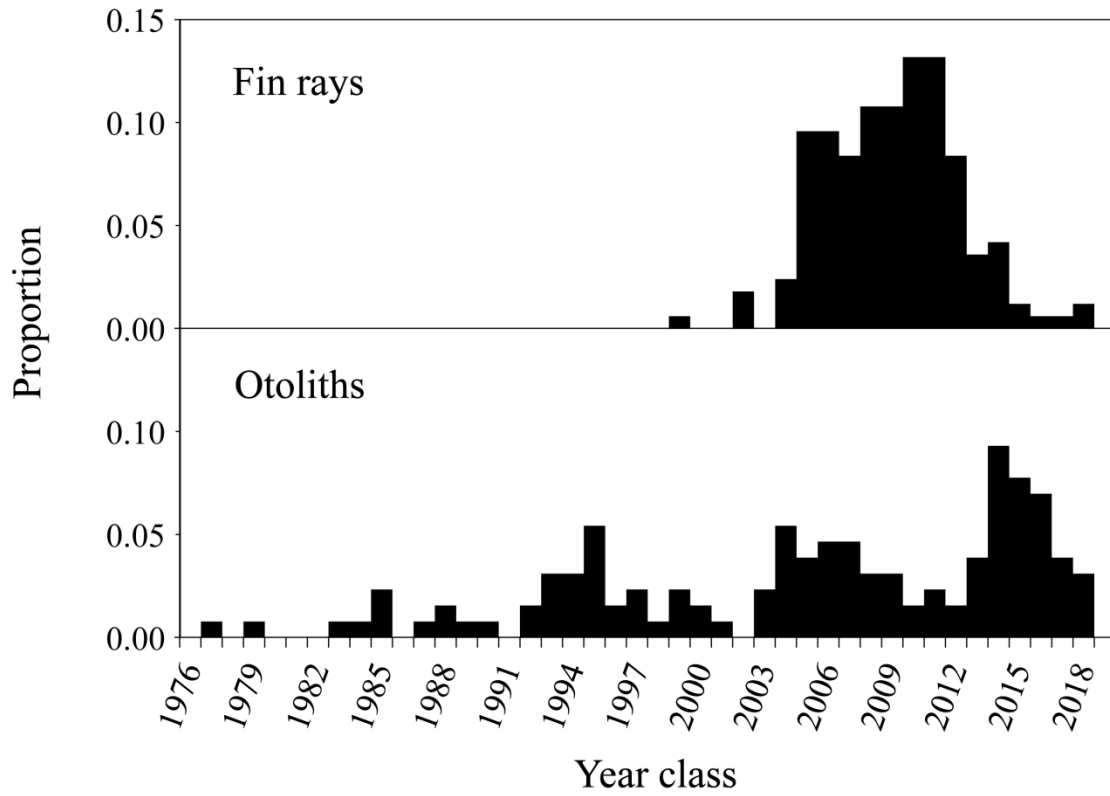


Figure 1.6. Year-class distribution for Blue Sucker specimens collected in 2018 and 2019 ( $n = 168$ ) as aged by fin rays ( $n = 167$ ) and otoliths ( $n = 128$ ).

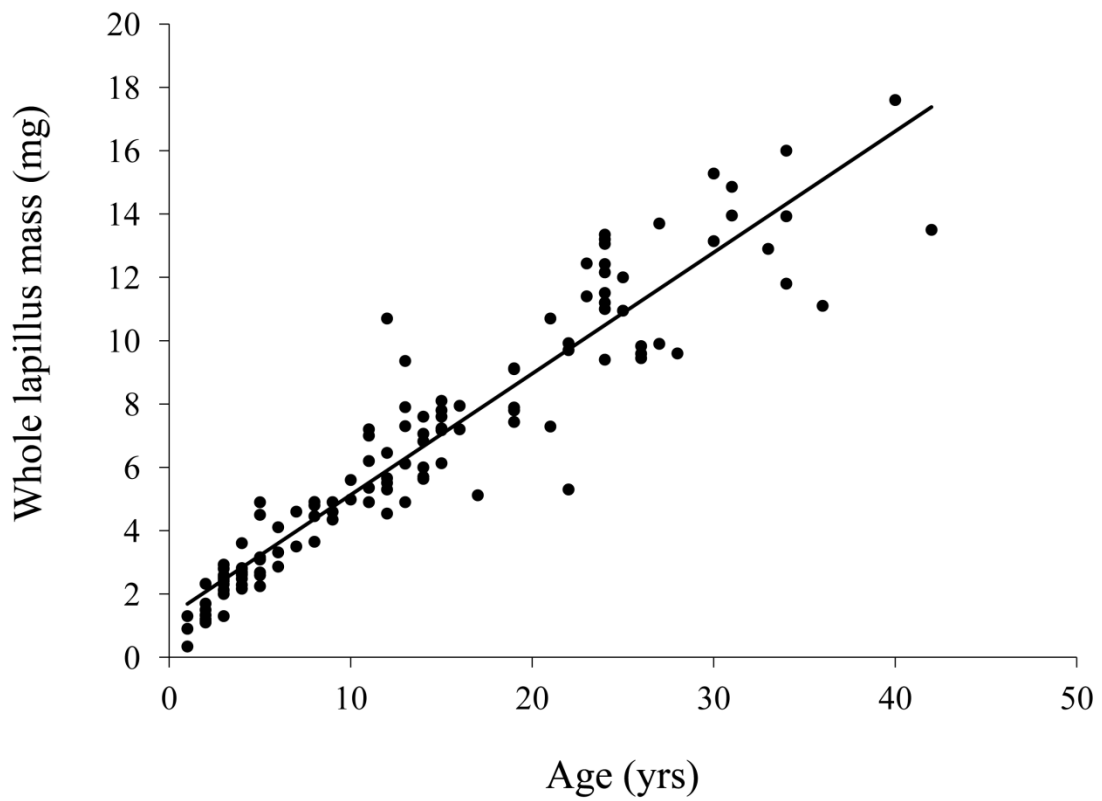


Figure 1.7. Correlation between Blue Sucker whole lapillus mass and otolith age  
assignment:  $\text{Mass}(\text{mg}) = 0.3827 \cdot \text{Age}(\text{yrs}) + 1.3008$ ,  $R^2 = 0.89$ ,  $n = 128$ .



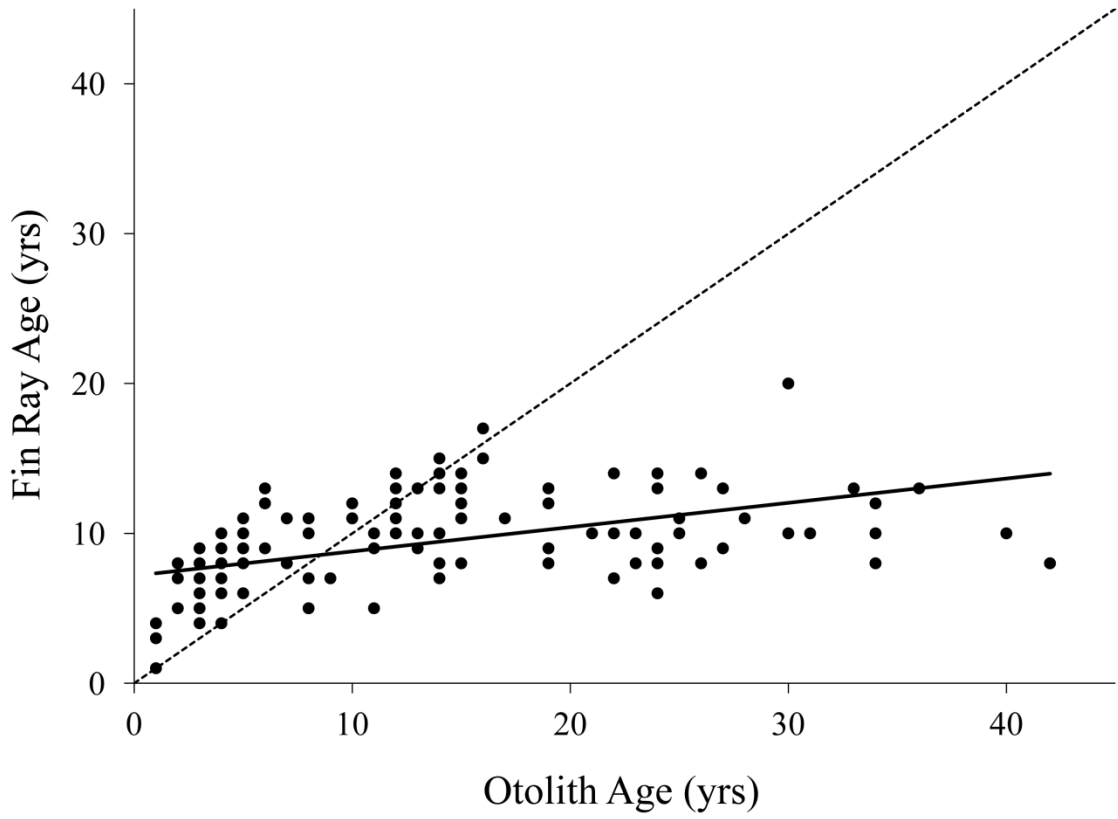


Figure 1.8. Structure bias plot comparing Blue Sucker ages assigned from pectoral fin rays and lapillus otoliths. The slope of the data differed significantly from 1 ( $P < 0.0005$ ).

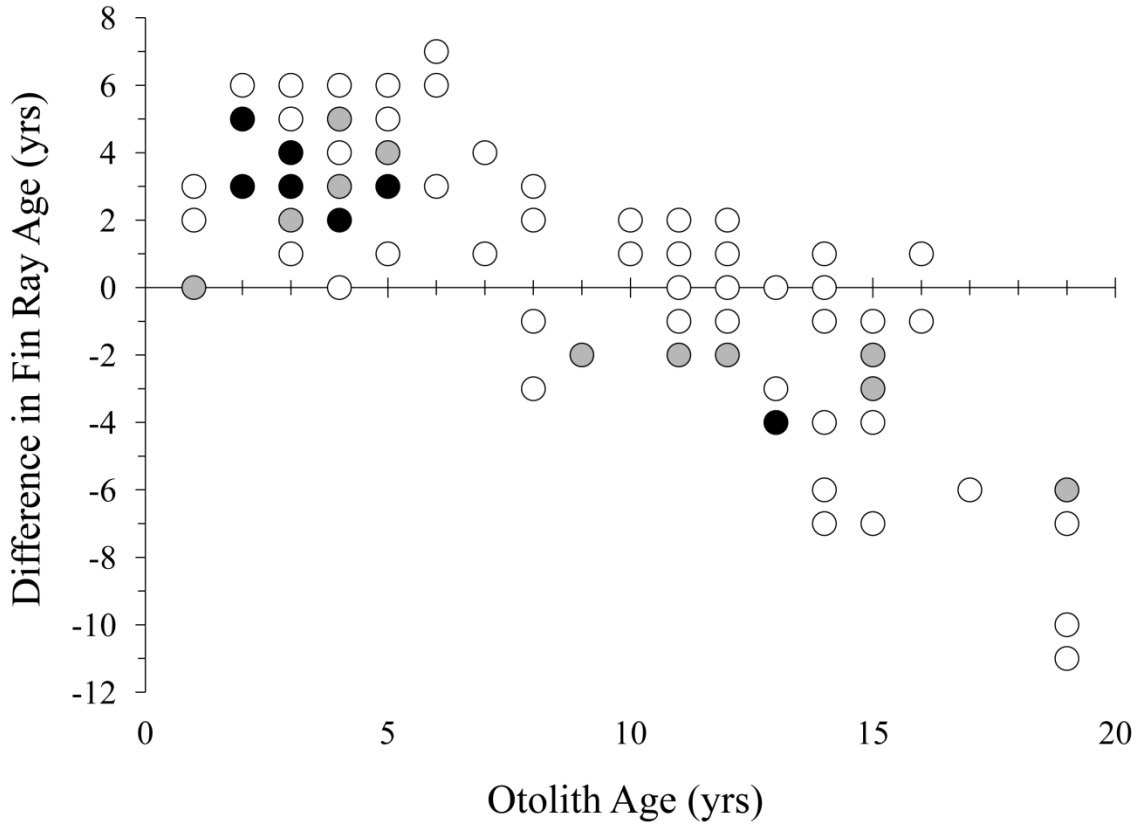


Figure 1.9. Frequency of difference in Blue Sucker fin ray age assignments relative to otolith age assignments at otolith age. Figure includes otolith ages 1-19 yrs ( $n = 46$  comparisons), white points = 1 occurrence, gray points = 2 occurrences, black points = 3-4 occurrences.

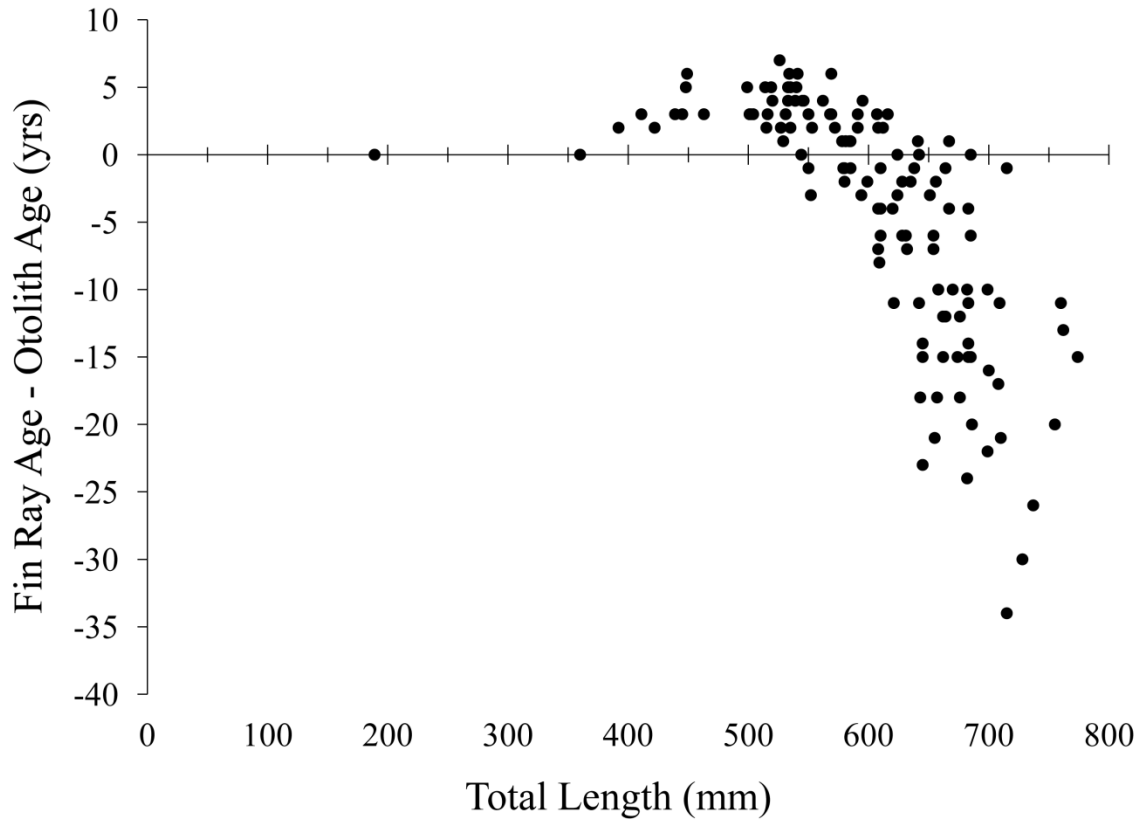


Figure 1.10. Difference in Blue Sucker fin ray age assignments relative to otolith age assignments at specimen total length ( $n = 127$  comparisons). Relative to otoliths, fin rays over-estimated age in individuals  $< 550$  mm and under-estimated age in individuals  $> 625$  mm.

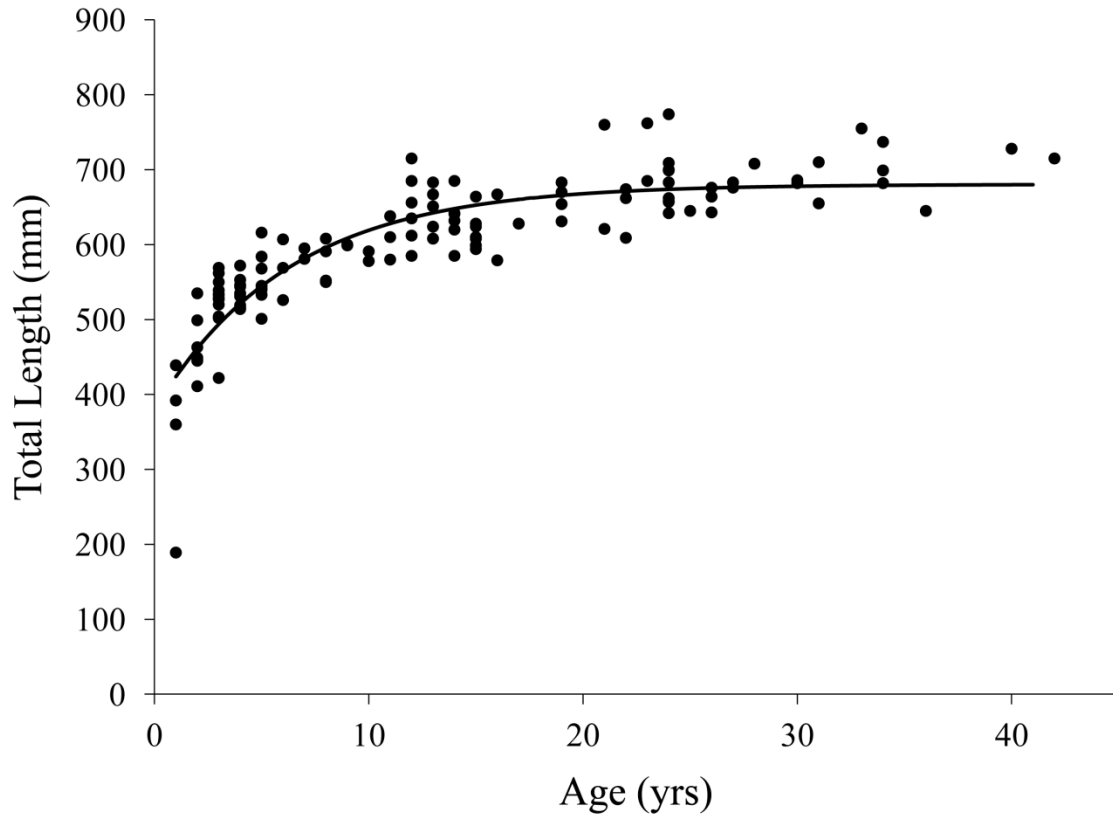


Figure 1.11. Blue Sucker total length at otolith age and von Bertalanffy growth curve:  $TL = 680.29038 \cdot (1 - e^{(-0.15898 \cdot Age + 5.14037)})$ , where  $TL$  = total length (mm) and  $Age$  = otolith age (years) ( $n = 128$ ).

CHAPTER 2:  
BLUE SUCKER (*CYCLEPTUS ELONGATUS*, LESUEUR, 1817)  
POPULATION DYNAMICS AND INDICATORS OF CHANGE  
IN THE WABASH RIVER

*[Formatted in the style of: Ecology of Freshwater Fish]*

ABSTRACT

The Blue Sucker (*Cycleptus elongatus*, Lesueur, 1817) is an imperiled North American fish, declining in abundance in much of its range. Research and management interest in Blue Suckers has been growing in response to their recognition as an imperiled species, and the Wabash River Blue Sucker population may be one of only a few surveyable populations with high abundance and successful reproduction. The demographic parameters of this population can provide a benchmark against which threatened populations can be compared. Specimens were assigned age estimates up to 42 years. We estimated annual mortality at 4.5%, and we estimated fecundity to average 110,933 eggs/female. The population length-weight regression was  $\text{Log}_{10}(WT) = 3.323 \cdot \text{Log}_{10}(TL) - 5.9592$ , where  $WT$  = weight (g) and  $TL$  = total length (mm). We identified a declining trend in average relative weights from 2008 to 2019, and found this trend mirrored in the declining average conditions of four other benthic invertivorous fishes in the Wabash River. We suggest Blue Suckers are bioindicators for the Wabash River ecosystem and that their declining relative weights should be regarded as early symptoms

of community level change, potentially driven by invasive Asian carp, substrate degradation, or climate change.

## INTRODUCTION

The Blue Sucker (*Cycleptus elongatus*, Lesueur, 1817) is an endemic North American fish, documented to grow up to 927 mm in total length (Carlander, 1969). The species inhabits medium to large rivers of the Mississippi and Missouri River basins as well as tributaries of the Gulf Coast and rivers in Texas and Mexico. They require a diversity of annual habitats for their adaptive life history strategies, seeking channelized shorelines in the summer, deep pools and areas of reduced current in the fall, and tributaries and unchannelized portions of the mainstem river in spring (Neely et al., 2010). Blue Suckers are benthic foragers, associated with exposed gravel, cobble, and bedrock substrates in deep riffles (1-2 m) with strong and constant flows (Elstad & Werdon, 1993; Moss et al., 1983). Where river connectivity allows, the species has been tracked making annual migrations of up to 545 km, associated with synchronous spring spawning events (Bednarski & Scarnecchia, 2006).

Blue Sucker abundance has declined in portions of their range due to poor water quality, the construction of impoundments that alter hydrology, and from siltation associated with agricultural practices (Smith, 1979). In the early 1990s Blue Suckers were assessed as a candidate species for federal protection, but inadequate records were available for the species at that time and the listing category was soon after eliminated (Elstad & Werdon, 1993). Today, they are classified by the American Fisheries Society as a “vulnerable” species, indicating “...imminent danger of becoming threatened

throughout all or a significant part of its range” (Jelks et al., 2008). Blue Suckers are already extirpated from Pennsylvania, are a state threatened or endangered species in Ohio, New Mexico, Tennessee, Texas, and Wisconsin, and are a species of special concern in Louisiana, Minnesota, Montana, North Dakota, Oklahoma, South Dakota, and West Virginia.

Interest in researching and managing Blue Suckers has been growing in response to their recognition as an imperiled species (Cooke, 2005; NatureServe & Lyons, 2019). Though historically underappreciated, there is growing recognition of the intrinsic and extrinsic value of studying and managing Blue Suckers and other catostomid fishes (Lackmann et al., 2019). Blue Suckers were once abundant and a valuable commercial species; they were captured during their spawning migrations with nets collecting as much as 360-400 kg of Blue Sucker per night and annual commercial harvests from the upper Mississippi River exceeding 1 million kg of (mixed) sucker flesh in 1899 (Coker, 1930). Today, Blue Suckers are used as cut bait by commercial fishermen in Arkansas (Layher, 2007) and have been anecdotally reported on at least two occasions in the harvest accounts of commercial fishermen on the Wabash River (C. Jansen, personal communication, May 3, 2019), though overall harvest is probably negligible. Blue Suckers are also valuable as bioindicators of the overall health of aquatic systems (Hesse et al., 1989; Hesse & Mestl, 1993; Neely et al., 2008), and due to similarities in habitat use can be used as a surrogate species to gain insights into endangered Pallid Sturgeon (*Scaphirhynchus albus*, Forbes & Richardson, 1905) and Shovelnose Sturgeon (*Scaphirhynchus platorynchus*, Rafinesque, 1820) populations (Lyons et al., 2016; Quist et al., 2004). Furthermore, assessing and managing native “non-game” fishes is now

being recognized as an important component in supporting the healthy and biodiverse ecosystems that game fish depend upon (Cooke, 2005; Moyle, 2002; Richter, 2007).

The objectives of this research are to (a) describe the demographics of an unimpounded and successful population of Blue Suckers to provide a benchmark against which threatened populations can be compared, and (b) to explore the role of Blue Suckers as bioindicators of change for the Wabash River fish community and river ecosystem.

## METHODS

*Study area & sampling methods.* – The Wabash River flows approximately 764 river kilometers (rkm) southwest, from its headwaters in Ohio to its confluence with the Ohio River. It is the largest south-flowing tributary of the Ohio River, with discharge ranging from 2,610 m<sup>3</sup>/s to 317,000 m<sup>3</sup>/s (river gauge data from New Harmony, Indiana, 2010-2019, USGS 2020). Despite a long history of anthropogenic modifications (Pyron & Neumann, 2008), the Wabash River is currently a relatively free-flowing system. It hosts a single dam in its upstream portion (rkm 661), below which flows the longest stretch of unimpounded river east of the Mississippi River.

Sampling for this research (excepting three specimens obtained from rkm 471, as described below) was restricted to the lower 322 rkm of the Wabash River, where it forms the border between Illinois and Indiana. This stretch of river will be referred to as the “lower Wabash River,” and spans from just south of Terre Haute, Indiana, to the confluence of the Wabash River with the Ohio River. In this study, rkm are counted northward, with rkm 0 at the river mouth. The fish assemblage of the lower Wabash



River has been annually surveyed since 2010 as part of the Illinois Natural History Survey's Long-term Electrofishing (LTEF) program, conducted by Eastern Illinois University since 2012.

The LTEF surveys employed standardized DC boat electrofishing at randomly selected sites along the shorelines of the navigable river. Each year, sample sites ( $n = 66$  in 2010 to 2012,  $n = 102$  in 2013 to 2019) were divided evenly into three time periods: mid-June to July, August to mid-September, and mid-September to October. Sites were randomly generated each period using Esri© ArcMap™ (v10.8) software, with the proximity of each randomized point to the river banks determining if the Illinois (west) or Indiana (east) shoreline was surveyed (Fritts et al., 2014). Sampling for LTEF was performed using the standardized DC electrofishing protocol described by Gutreuter et al. (1995) and survey methods described by Fritts et al. (2017). Sampling crews included two dip netters and one operator, and effort was held constant at 15 minutes per site. All sampled fish were held in an onboard livewell before being identified to species, weighed (g), measured for total length (mm), and released. The presence of structures at each sample site (e.g. snags, rip-rap, etc.) was noted, and substrate was qualified as one of four categories: gravel/rock/hard clay, silt/clay/little sand, sand, and silt. Additional measures including water velocity and site depth were recorded at each sample site.

From 2010 to 2019, the Wabash River LTEF surveys documented 563 Blue Suckers (Table 2.1, Figure 2.1). Sampling and collection methods were compliant with the Eastern Illinois University Institutional Animal Care and Use Committee for the humane handling of research animals. During the 2018 and 2019 surveys, Blue Suckers were retained and humanely euthanized by immersion in an ice-slurry ( $n_{2018} = 68$ ,  $n_{2019} =$

64). Thirty-six supplemental Blue Sucker specimens were collected and euthanized in 2019, for a total of 168 harvested specimens (Table 2.1, Figure 2.1). We collected 14 supplemental specimens on May 23, 2019, using targeted electrofishing over submerged remnants of a historic low-head dam structure in water depths of 2.4 to 4.3 m (rkm 156). We also employed targeted electrofishing at a known riffle/run location with cobble substrate in a water depth of 0.5 to 1.0 m (rkm 207.5 to 209) on October 8, 2019, and collected 13 specimens. Five specimens were opportunistically collected using electrified trawling gear. Our smallest harvested specimen (189 mm) was collected by the Illinois Department of Natural Resources using DC boat electrofishing near the mouth of the Wabash River, and was contributed to this research. Three Blue Sucker specimens were collected with the Indiana Department of Natural Resources while gillnetting for Shovelnose Sturgeon on April 29, 2019, in Lafayette, IN, at rkm 471 (notably 149 rkm north of the main study reach).

*Age & fecundity estimation.* – Blue Sucker specimens collected in 2018 and 2019 ( $n = 168$ ) were kept frozen until thawed for laboratory dissection. A comparison of hard structures (scales, opercles, pectoral fin rays, and lapillus otoliths) for aging these specimens indicated that lapillus otoliths yielded the most precise and credible results. Otolith age estimates were assigned to 128 specimens (Chapter 1).

The gonads of each harvested specimen were photographed in the body cavity, weighed, and preserved in formalin. The histological examination of 42 gonadal sets informed us in defining five reproductive stages in adult female Blue Suckers and four reproductive stages in adult male Blue Suckers, based on general stages for wild fishes described by Blazer (2002). We used insights from the histological assessment to then

assign reproductive stages to the majority of our specimens, including eight females with ovaries in maturation stage. We estimated the fecundity of seven of these specimens by counting the eggs in 1.0-gram subsamples from the anterior, midsection, and posterior of each ovary. Eggs-per-gram were averaged within each ovary and extrapolated to the total weight of the organ. The sum of eggs estimated in both ovaries yielded the fecundity estimate per individual.

*Statistical analysis.* – Statistical measures were calculated using Microsoft® Excel (2007) and R (R Core Team, 2018) software. Catch-per-unit-effort (CPUE) for LTEF data was calculated as fish-per-site rather than fish-per-hour, to more accurately reflect our 15-minute sampling efforts. Observed probabilities were tested against predicted probabilities using chi-squared tests and Fisher’s exact test, as specified in the results. Predicted probabilities for habitat variables and hydrological conditions were based on observed habitat availability (912 surveyed sites). Daily average discharge values from the most downstream hydrological gauge on the Wabash River (New Harmony, Indiana) were obtained from the U.S. Geological Survey. Predicted probabilities for group sizes were based on the Poisson distribution generated from the 2010 to 2019 average of 0.647 Blue Suckers per site. The significances of catch rates for co-occurring species were calculated using two-tailed tests comparing species catch rates at sites that yielded Blue Suckers ( $n = 228$ ) to species catch rates at all surveyed sites ( $n = 912$ ). We tested for significant differences between linear trends using the Microsoft® Excel add-in Real Statistics Resource Pack and methods published by Howell (2010) for comparing the slopes of two independent samples. Relative weight values ( $W_r$ , scaled to 100) were calculated based on the Blue Sucker 75<sup>th</sup>-percentile linear equation  $\text{Log}_{10}(W_s) - (-6.301)$

+ 3.456 \*  $\text{Log}_{10}(TL)$  proposed by Neely et al. (2008, where  $W_s$  = standard weight,  $TL$  = total length (mm)). Standardized relative weight equations are not yet available for all species, so relative condition ( $K_n$ , scaled to 1) values were calculated for other LTEF-surveyed fishes based on their length-weight regressions within the 2010-2019 LTEF dataset. A multiple regression test with backward selection (based on Akaike's information criterion) was used to identify models of best fit relating Blue Sucker  $W_r$  to potential predictor variables. A multivariate analysis of variance (MANOVA) test was employed to detect trends in  $W_r$  within individual moths across years. Additional datasets were sourced from the Illinois Department of Natural Resources, the Indiana Department of Environmental Management, and the National Oceanic and Atmospheric Administration; their content and applications are described in the results. The significance of all statistical tests was defined by  $\alpha=0.05$  except where otherwise specified.

## RESULTS

Trends in Blue Sucker occurrence in the lower Wabash River were identified based on data from the annual LTEF surveys, 2010-2019. Across this decade, Blue Sucker CPUE ( $\pm$ SE) averaged 0.65 (0.07) with a slight downward trend but no significant regression over time (Table 2.2). The species ranked 5<sup>th</sup> (6.14%) in proportional biomass within the surveyed fish community, outranked by Common Carp (25.74%, *Cyprinus carpio*, Linnaeus, 1758), Silver Carp (14.22%, *Hypophthalmichthys molitrix*, Valenciennes in Cuvier & Valenciennes, 1844), Smallmouth Buffalo (11.90%, *Ictiobus*

*bubalus*, Rafinesque, 1818), and Freshwater Drum (6.90%, *Aplodinotus grunniens*, Rafinesque, 1819).

The long-term surveys offered insights into Blue Sucker habitat preference and intra- and inter- species associations. Wabash River LTEF-surveyed Blue Suckers ( $n = 563$ ) demonstrated a significant preference for sites with snags (70.5% of specimens, chi-squared test  $P < 0.0005$ ). No significant preference for substrate was identified, based on our four substrate categories (chi-squared test  $P = 0.49$ ). No trend in Blue Sucker relative location (rkm) by month was identified in the data. Blue Suckers exhibited a significant bias to be sampled at lesser discharge volumes (Fisher's exact test  $P < 0.0005$ ), with CPUE inversely related to river discharge (average CPUE 0.99 when discharge was 0 to 9,999 m<sup>3</sup>/s, average CPUE 0.59 when discharge was 10,000 to 19,999 m<sup>3</sup>/s, and average CPUE 0.12 when discharge was 20,000 to 29,999 m<sup>3</sup>/s). Of the Blue Suckers surveyed in LTEF, 75.2% were collected in water velocities  $\leq 1$  m/s and 71.5% were collected at sites 1 to 4 m deep.

Blue Suckers were often in groups, and up to thirteen individuals were sampled from a single site (October 2018). Of the sites at which Blue Suckers were sampled ( $n = 228$ ), 52.2% yielded more than one specimen and 20.6% yielded four or more. Of the Blue Suckers surveyed in LTEF ( $n = 563$ ), 80.6% were sampled in groups of two or more, 50.1% in groups of four or more, and 18.5% in groups of eight or more. Observed probabilities for group sizes 0 to 13 differed significantly from expected probabilities generated from the Poisson distribution (chi-squared test  $P < 0.0005$ ). For example, we observed 1.8% of all surveyed sites produced Blue Suckers in groups of 4 or more, compared to the expected Poisson probability of only 0.4% of all sites. The proportion of

specimens surveyed in groups of four or more increased each month from July to October, as did the CPUE (Table 2.3). Within groups of four or more specimens, males and females co-occurred at 9 out of 10 sites in which sex was known, and total lengths ranged from 117 mm to 775 mm with multiple instances of small (117 mm, 210 mm, 273 mm, 276 mm) individuals occurring with large adults. Within survey sites at which Blue Suckers were sampled, ten additional species were identified as more likely to co-occur than not: Freshwater Drum (77.2%), Smallmouth Buffalo (75.0%), Common Carp (73.2%), Emerald Shiner (*Notropis atherinoides*, Rafinesque, 1818, 67.5%), Spotted Bass (*Micropterus punctulatus*, Rafinesque, 1819, 64.0%), Gizzard Shad (*Dorosoma cepedianum*, Lesueur, 1818, 61.0%), River Carpsucker (*Carpionodes carpio*, Rafinesque, 1820, 55.3%), Channel Catfish (*Ictalurus punctatus*, Rafinesque, 1818, 54.8%), Shortnose Gar (*Lepisosteus oculatus*, Winchell, 1864, 54.4%), and Spottfin Shiner (*Cyprinella spiloptera*, Cope, 1867, 50.4%). Other benthic invertivorous species-of-interest also co-occurred at sites in which Blue Suckers were sampled: Shorthead Redhorse (25.0%), Black Buffalo (19.3%), and Shovelnose Sturgeon (15.8%). Of the thirteen co-occurring species mentioned above, four occurred at significantly higher average rates at Blue Sucker sites compared to all sites (t-critical = 1.97): Freshwater Drum (t-stat = 2.37), Smallmouth Buffalo (t-stat = 2.56), Shorthead Redhorse (t-stat = 2.77), and Shovelnose Sturgeon (t-stat = 3.31). The other nine referenced species showed no significant difference in occurrence rates at Blue Sucker sites compared to all sites.

We examined the gonads of the harvested Blue Sucker specimens ( $n = 168$ ) and identified 71 females (42.3%) and 60 males (35.7%); the remaining 37 individuals were of unknown sex (22.0%), of which five were immature. Immature individuals ranged

from 189 mm to 411 mm, and individuals as small as 422 mm exhibited some stage of sexual reproduction. Three specimens < 500 mm total length were identified as adult females (422 mm, 448 mm, 439 mm), and one specimen < 500 mm total length was identified as an adult male (492 mm). Average gonadosomatic indices (GSI,  $\pm$ SE) began to rise in August (1.58, 0.49,  $n = 25$ ) and September (1.96, 0.60,  $n = 11$ ) with accelerated development in October (6.20, 0.41,  $n = 91$ , Figure 2.2). Tubercles were observed on live specimens in October 2018 and 2019, and also in May 2019 during supplemental sampling. Maturation-stage females ( $n = 8$ ) were collected in October of both years, and maturation-stage (“late spermatogenic” stage) males ( $n = 38$ ) were generally collected in October, though one was sampled in August 2019 and another in September 2019. Spent (“post-ovulatory” stage) females ( $n = 4$ ) were sampled in May 2019 and a single post-spawn male was sampled in April 2019. The females collected in maturation stage ranged in total length from 608 mm to 762 mm (average 673 mm) and estimated to be age-6 to age-28 (average age-15). Fecundity estimates ranged from 87,217 eggs to 126,696 eggs (average 110,933 eggs); GSI ranged from 8.8% to 10.7% (average 9.7%). No significant trend in fecundity versus total length or versus weight was identified, presumably due to the small sample size.

The length-weight regression for this Blue Sucker population was calculated using all surveyed specimens:  $\text{Log}_{10}(WT) = 3.323 \cdot \text{Log}_{10}(TL) - 5.9592$ , ( $R^2 = 0.95$ ,  $n = 599$ ) where  $WT$  = weight (g) and  $TL$  = total length (mm). No significant difference in the slope of the regression was detected between sexes. As described in Chapter 1, we assigned age estimates to harvested specimens ( $n = 168$ , 2018-2019) using thin-sectioned lapillus otoliths (Figure 2.3) and used those ages to estimate the population mortality rate

(4.5%) and von Bertalanffy growth model:  $TL = 680.29038 \cdot (1 - e^{(-0.15898 \cdot Age + 5.14037)})$ , where  $TL$  = total length (mm) and  $Age$  = otolith age (years) (Figure 1.11).

We calculated the 75<sup>th</sup>-percentile  $W_r$  score for all LTEF-surveyed Blue Sucker specimens and used these values (individuals of total length > 240 mm and  $W_r$  values  $\pm 3$  standard deviations from the mean,  $n = 548$ ) to identify a significant declining trend in the population's average  $W_r$  across the 2010 to 2019 decade:  $W_r = -1.5262 \cdot Yr + 3168.6924$ , ( $P = 0.0015$ ,  $R^2=0.73$ ) where  $Yr$  = calendar year (Table 2.2). We tested for potential confounding factors that could be influencing the declining  $W_r$  trend. A multiple regression test with backward selection dropped the variables average-total-length by year and Blue-Sucker-CPUE by year and concluded the model-of-best-fit to include only the effect of years ( $R^2 = 0.79$ ,  $P = 0.018$ ). A MANOVA test of Blue Sucker average  $W_r$  by month across years indicated significant declining trends within August ( $P = 0.0075$ ), September ( $P = 0.030$ ), and October ( $P = 0.046$ ), but not July ( $P = 0.21$ ). A multiple regression test with CPUE's of multiple invasive carp species (Silver Carp, Common Carp, Grass Carp [*Ctenopharyngodon idella*, Valenciennes in Cuvier & Valenciennes, 1844], and the combined total all three carps) from the LTEF surveys as variables did not produce a significant model and none of the predicting variables were significant ( $R^2 = 0.09$ ,  $P = 0.89$ ).

To explore a broader time span of  $W_r$  trends for this population, we combined Blue Sucker data from 2010-2019 LTEF surveys ( $n = 548$ ) with Blue Sucker data from 1996-2015 lower Wabash River surveys conducted by the Illinois Department of Natural Resources ( $n = 597$ ), and calculated average  $W_r$  per year. This dataset revealed no trend in  $W_r$  from 1996 to 2006 (mean  $W_r = 105.7$ ) but identified a significant declining trend



from 2008 to 2019:  $W_r = -1.3182 \cdot Yr + 2749.5061$ , ( $P < 0.0005$ ,  $R^2 = 0.76$ ) where  $Yr$  = calendar year. The year 2007 was omitted from the timeframe of the identified declining trend, as we felt the high  $W_r$  value associated with this year ( $W_{r2007} = 116.1$ ) would disproportionately influence the results (Figure 2.4).

We sought to compare the declining trend in Blue Sucker average  $W_r$  to trends in other species of similar and dissimilar trophic guilds. Relative condition was calculated per individual for multiple well-represented species in the 2010-2019 LTEF dataset. We relaxed our criteria for significance to  $\alpha = 0.10$  for this series of regression tests, to detect more subtle trends. Within the benthivore guild, a significant declining trend in average relative condition was identified for Shorthead Redhorse ( $-0.020 K_n/\text{yr}$ ,  $P = 0.0098$ ,  $R^2 = 0.59$ , *Moxostoma macrolepidotum*, Lesueur, 1817), Shovelnose Sturgeon ( $-0.014 K_n/\text{yr}$ ,  $P = 0.053$ ,  $R^2 = 0.39$ ), Black Buffalo ( $-0.0074 K_n/\text{yr}$ ,  $P = 0.093$ ,  $R^2 = 0.31$ , *Ictiobus niger*, Rafinesque, 1819), and Smallmouth Buffalo ( $-0.0064 K_n/\text{yr}$ ,  $P = 0.064$ ,  $R^2 = 0.37$ ). Quillback was the only species found to have a positive  $K_n$  trend ( $+0.0084 K_n/\text{yr}$ ,  $P = 0.068$ ,  $R^2 = 0.36$ , *Carpionodes cyprinus*, Lesueur, 1817), and no significant trend was identified in River Carpsucker ( $P = 0.12$ ) or Freshwater Drum ( $P = 0.31$ ). Significant trends were also not identified in the  $K_n$  of two piscivorous fishes: Spotted Bass ( $P = 0.53$ ) and White Bass ( $P = 0.31$ , *Morone chrysops*, Rafinesque, 1820), nor in two planktivorous species: Bigmouth Buffalo ( $P = 0.14$ , *Ictiobus cyprinellus*, Valenciennes in Cuvier & Valenciennes, 1844) and Gizzard Shad ( $P = 0.71$ ).

The Indiana Department of Environmental Management (P. D. McMurray, Jr., personal communication, February 5, 2019) shared data from macroinvertebrate surveys they conducted on the lower Wabash River in 1993 and 1997 (riffle kick & Hester-Dendy

methods) and in 2009 and 2016 (multi-habitat methods). Between 1993 and 1997 three fixed sites all exhibited declines in the number of macroinvertebrate taxa present (from 9:10:12 to 6:4:8). The average abundance of taxa per site in 2016 ( $n = 8$  sites) versus 2009 ( $n = 9$  sites) suggested dramatic declines had occurred in orders Diptera and Hemiptera (from 100/site to 16/site and from 219/site to 46/site, respectively).

Finally, precipitation trends within the Wabash River basin were approximated using 2010-2019 data from the Indianapolis International Airport, a central location within the watershed (N.O.A.A.). Total annual precipitation followed a (statistically non-significant but nonetheless notable) increasing trend across the decade (+2.1 cm/yr,  $R^2 = 0.25$ ,  $P = 0.14$ ), particularly precipitation from February to August (+2.7 cm/yr,  $R^2 = 0.38$ ,  $P = 0.056$ ).

## DISCUSSION

Blue Suckers in the Wabash River are abundant, long-lived, and appear to be experiencing low mortality and successful recruitment (Bacula et al. 2009, Figure 2.3). Relative weights were high across all surveyed years (Figure 2.4), although these values are currently trending downward. This population seems to be one of the few enduring Blue Sucker populations experiencing such success (Gammon, 1998). Their resilience may be due in part to the high degree of connectivity in the Wabash River system, which supports the needs of migratory species (Sheilds et al., unpublished). The demographics described for this population should serve as a benchmark against which threatened populations can be compared to assess their relative status and to direct their management.

The lower Wabash River is dominated by hard substrates (clay, gravel, and bedrock, Bacula et al., 2009) which support a robust population of Blue Suckers despite relatively shallow conditions (0.5 meter depth) in some stretches of the river. These shallow waters make the Wabash River Blue Sucker population more susceptible to electrofishing surveys when compared to populations occupying deeper rivers (e.g. the Mississippi River and Ohio River). Due to difficulties sampling fish in the high-velocity channels of deep rivers, it is difficult to assess the status of populations in such systems, whereas the Wabash River provides a unique opportunity to survey Blue Suckers with relative efficiency. This study found hydrological conditions to be an important predictor of Blue Sucker catch rates, and the bias this population exhibited for being sampled at lesser discharges can inform future targeted efforts to sample this species. Blue Sucker CPUE was inversely related to river discharge volume, presumably because at lesser discharge volumes Blue Suckers were restricted to shallower site depths (71.5% of specimens collected at sites 1 to 4 m deep) and slower water velocities (75.2% of specimens collected in water velocities  $\leq 1$  m/s), factors that generally improve the efficiency of DC electrofishing sampling methods.

The abundance of Blue Suckers in the lower Wabash River system was evidenced by their ranking 5<sup>th</sup> in fish biomass based on 2010-2019 LTEF community surveys. This may reflect a relatively recent increase in abundance as Broadway et al. (2015) identified a community shift in the Wabash River between 1989 and 1996, during which time trophic guild dominance shifted from planktivores to benthic invertivores. In 1991, Gammon described the Wabash River Blue Sucker population as increasing in range and abundance (as cited in Kay et al., 1994), and in 2002 the species was delisted as a species

of special concern in Indiana after consistent documentation of high CPUE's (Bacula et al., 2009). Blue Suckers in the Wabash River appear to be abundant, long-lived, and successfully reproducing (Figure 2.3). The high proportion of specimens we sampled in intraspecific groups (50% in groups of four or more) exceeded Poisson probability expectations and was probably underestimated due to sampling inefficiency. The increasing occurrence of these groups in the fall months (Table 2.3) aligns with published observations of Blue Suckers traveling in groups during synchronous spring and fall migrations (Bednarski & Scarnecchia, 2006; Neely et al., 2009). The Wabash River LTEF surveys documented small individuals associated with the adult groups, which lends support to the hypothesis that immature Blue Suckers do not segregate from adults. Small specimens (< 500 mm) are nonetheless underrepresented in most Blue Sucker research efforts including this one (Figure 2.1), which suggests a possible bias of the electrofishing gear (LaBay, 2008; Mayes, 2015).

Across ten years of LTEF surveys, 70.5% of Blue Suckers occurred at sites with snags. This significantly exceeds the expected probability based on habitat availability and suggests a Blue Sucker preference for affiliating with snags, possibly for macroinvertebrate resources associated with these structures. No preference for substrate (based on four qualitative categories) was observed in our data. At sites at which Blue Suckers were sampled, Freshwater Drum, Smallmouth Buffalo, Shorthead Redhorse, and Shovelnose Sturgeon were all sampled at higher rates than average, suggesting that these four species are selecting similar habitats and conditions as the Blue Sucker.

Based on examinations of Blue Sucker whole gonads and sub-sampled gonad histology, we identified immature Blue Suckers as large as 411 mm and adult Blue

Suckers as small as 422 mm. Our findings suggest sexual maturity may occur at total lengths between 400 to 500 mm in this population. This would suggest an estimated age-at-maturation of only 0-3 years based on our von Bertalanffy growth model, but we are cautious of this estimate as we suspect early growth is skewed in the model and acknowledge that our data insufficiently represents young specimens. Our size-at-maturation estimate is smaller than those estimated for populations on the upper Mississippi River (minimum 503 mm, Ruppertch & Jahn, 1980), the Wisconsin River (minimum 495 mm, Lyons et al., 2016), or from a previous study on the Wabash River population (minimum 515 mm, Daugherty et al., 2008). We suggest visual examinations used in other studies may have over-estimated size-at-maturity due to the nearly identical appearance of the immature gonads to the adult gonads (male or female) in early reproductive stages; these stages are indistinguishable without histological examination. The gonads of some large individuals did not appear to be progressing in reproductive stage even in October when others were reaching maturation stage, suggesting individuals may not spawn every year (Figure 2.2). Our surveys collected both males and females in the maturation stage in October, a spent male in April, and spent females in May. These observations are consistent with an April spawn date for the Wabash River population. A pre-spawning congregation has been documented in the mainstem near Lafayette, Indiana, in March and April 2006 (Daugherty et al., 2008) and a spawning congregation was observed in a tributary near Williams, Indiana, in April 2019 (C. Jansen, personal communication, May 3, 2019). Our estimate of female fecundity averaged 110,932 eggs/individual, much higher than fecundity estimates for Blue Sucker populations in South Dakota (average 61,008 eggs, Beal, 1967) and Arkansas (21,000 to

24,000 eggs, Layher, 2007), but within range of previous estimates from the Wabash River (26,829 to 267,471 eggs, Daugherty et al., 2008).

The 2010-2019 Blue Sucker specimens from the lower Wabash River modeled a length-weight regression of  $\text{Log}_{10}(WT) = 3.323 \cdot \text{Log}_{10}(TL) - 5.9592$  ( $R^2 = 0.95$ ) where  $WT$  = weight (g) and  $TL$  = total length (mm), with no apparent difference between sexes. The slope (3.323) was similar to that calculated for Blue Sucker populations in the upper Mississippi River (3.59, Ruppretch & Jahn, 1980), the James River (3.37) and Sioux River (3.50, South Dakota, Morey & Berry, 2003) and the Red River (3.12<sub>males</sub>, 3.01<sub>females</sub>, Arkansas, Layher, 2007), but much higher than that calculated for the Neosho River population (1.83, Kansas, Moss et al., 1983). Ages assigned to our harvested specimens by lapillus otoliths yielded a higher estimate of longevity (up to 42 years, Figure 2.3) than any previous studies. Previous studies have used scales and pectoral fin rays to estimate Blue Sucker ages, but both structures under-estimate the ages of older specimens relative to otoliths, although otoliths still require validation in this species (Chapter 1). Population mortality was estimated at 4.5% based on otolith ages (Chapter 1), much lower than the 22% to 25% previously estimated for this population based on pectoral fin ray ages (Bacula et al. 2009). Age-1 specimens ranged in total length from 189 mm to 439 mm ( $n = 4$ ), but the absence of age-0 specimens and under-representation of age-1 and age-2 specimens may have biased early growth in the von Bertalanffy growth model:  $TL = 680.29038 \cdot (1 - e^{(-0.15898 \cdot \text{Age} + 5.14037)})$  where  $TL$  = total length (mm) and  $\text{Age}$  = otolith age (years) (Figure 1.11, Chapter 1).

Long-term Wabash River survey data revealed a significant declining trend in Blue Sucker average  $W_r$  from 2008 to 2019, at an average rate of -1.3  $W_r$ -points/year:  $W_r$

$= -1.3182 \cdot Yr + 2749.5061$  where  $Yr$  = calendar year (Figure 2.4). Should this trend continue, the population is likely to experience negative consequences associated with reductions in  $W_r$ , including reduced fecundity and increased susceptibility to disease (Anderson & Neumann, 1996; Murphy & Willis, 1996). This trend was hinted at by Gammon (1998), who noted that the average weight of Blue Suckers in the Wabash River seemed to be declining gradually over time. Though we modeled the downward trend with a linear regression, it is worth considering that Blue Sucker  $W_r$  could be following a non-linear trajectory, the nature of which might be more apparent if adequate data from a longer timeframe (pre-1990s) were available. Multiple regression model selection indicated that the linear trend we identified was driven by the effect of time (years) rather than the effect of a changing size structure (annual average Blue Sucker total length) or intraspecific competition within the population (annual average CPUE). Our data indicated a weak negative trend in CPUE from 2010-2019 ( $R^2 = 0.19$ ) and a previous study on the Wabash River identified a weak positive trend in Blue Sucker CPUE from 1974-2015 ( $R^2 = 0.17$ , Shield et al., unpublished), indicating that Blue Sucker density is changing over time, but at a subtle scale.

Though our data included specimens sampled across various months and thus across multiple reproductive stages, the trend holds true within the standardized timeframe of LTEF surveys and also holds true when restricted to specimens sampled in the individual months of August, September, or October, but not July. The absence of a  $W_r$  trend across years in the month of July could be an outlier and somatic weight-at-length could be declining for this species. Alternatively, the declining trend in  $W_r$  could be driven by declining gonadal growth, which would predict the effect to be most

pronounced across the months of gonad development (August to October, Figure 2.2). In either scenario, declining  $W_r$  is the consequence of a diminished annual energy budget and indicates a reduction in Blue Sucker foraging resources.

Stomach content analyses have indicated Blue Sucker diets consist primarily of insect larvae, chiefly from the orders Diptera and Trichoptera but also including Ephemeroptera, Coleoptera, Odonata, Lepidoptera, and small mollusks (Bock et al., 2011; Cowley & Sublette, 1987; Eastman, 1977; Moss et al., 1983; Rupprecht & Jahn, 1980). Studies on macroinvertebrate assemblages in the lower Wabash River are lacking, but data from the Indiana Department of Environmental Management provided evidence that taxa diversity declined between 1993 and 1997 and that the dipteran density was dramatically reduced between 2009 and 2016. Blue Suckers are believed to spend the summer months foraging heavily to prepare for migration and reproduction (Adams et al., 2017). Diminishing summer forage resources could be reducing the individual energy surpluses and in turn reducing growth.

Blue Suckers are not alone in experiencing a trend of declining average condition, as this trend is mirrored in other benthic invertivores in the Wabash River: Shorthead Redhorse, Shovelnose Sturgeon, Black Buffalo, and Smallmouth Buffalo. The declining condition of Shovelnose Sturgeon in the Wabash River has been previously documented by Thornton et al. (2019), who identified the trend in female specimens and attributed it to pressures from commercial harvest. However, the fact that all five species experiencing the decline in condition (including Blue Suckers) are similar in their foraging behaviors and diets is a strong indicator that the trends may be driven by a common variable.



The identity of the hypothesized underlying variable is not known at this time, but we suggest that invasive Asian carp, substrate degradation, and climate change should be considered as likely drivers. The Wabash River is now home to a host of invasive carp species (Common Carp, Grass Carp, Goldfish [*Carassius auratus*, Linnaeus, 1758], Silver Carp, Bighead Carp [*Hypophthalmichthys nobilis*, Richardson, 1845] and recently Black Carp [*Mylopharyngodon piceus*, Richardson, 1846]), with Common Carp and Silver Carp occupying the majority of biomass within the fish community (25.7% and 14.2%, respectively). Although our multiple regressions test did not reveal a significant relationship between Blue Sucker  $W_r$  and invasive carp CPUE's, we suspect that may be because our LTEF data underestimates Silver Carp density as the species is not efficiently sampled by the standardized electrofishing methods we employed. Silver carp invaded the Wabash River around 1995 (Broadway et al., 2015), but were a rare species in the system until around 2006, when they began to rapidly increase in abundance (Shields et al., unpublished). In the Illinois River, Silver Carp have been shown to have caused reductions in the conditions of two planktivore competitors, Gizzard Shad and Bigmouth Buffalo (Irons et al., 2007). Although not in direct competition with benthic invertivores, Silver Carp could be altering food web dynamics in the Wabash River by competing with macroinvertebrates for plankton resources. Some studies have suggested that Silver Carp feces may invigorate benthic productivity (Shields et al., unpublished; Yallaly et al., 2015), but their actual impacts on the food webs of natural systems are still largely unknown.

Due to their need to forage over hard substrates, Blue Suckers are believed to be resilient against turbidity but not against accumulating siltation (Elstad & Werdon, 1993;

Tomelleri & Eberle, 1990). Fine substrates like silt are associated with smaller and shorter-lived macroinvertebrate food resources (Berkman & Rabeni, 1987).

Approximately 65% of the Wabash River watershed is row-crop agriculture and in 1998 Gammon commented on the inadequacies and absence of riparian buffer zones between the river and the surrounding agriculture fields. This problem is still readily apparent and, along with other factors, is contributing to erosion and siltation. Mueller & Pyron (2010) predicted that lithophilic and specialized fishes would be negatively impacted or even extirpated if further hydrological disturbances were to increase siltation and substrate degradation in the Wabash River. However, the connection between Blue Suckers and substrate quality in the river is unclear; we are lacking historical substrate quality data for the river, and there is no clear temporal connection between ongoing substrate degradation and the onset of the declining trend in Blue Sucker  $W_r$  in 2008.

When speculating on drivers of change in a modern river ecosystem, we must consider the potential influence of climate change. Precipitation records from within the Wabash River watershed indicate an increasing trend in annual precipitation (+2.1 cm/yr) and especially spring and summer precipitation (+2.7 cm/yr) over the past decade. The river's average annual discharge has been on an increasing trend since 1928 (Pyron & Lauer, 2004), as have the magnitude and duration of annual extremes (Pyron et al., 2006). Further research will be necessary to explore how changing hydrological conditions may be influencing the Wabash River Blue Sucker population.

Blue Suckers are believed to be an environmentally sensitive species with the potential to serve as ecosystem bioindicators. The declining trend in Blue Sucker average  $W_r$  from 2008 to present, coupled with similar trends in the relative conditions of other

benthic invertivores, should be regarded as early symptoms of changes occurring at the community level. Between 1989 and 1996 the benthic invertivore guild came to dominate the Wabash River fish community in terms of abundance, and energy sequestered in large-bodied invertivores like the Blue Sucker is hypothesized to limit other trophic levels in the system (Broadway et al., 2015). Pyron et al. (2017) have suggested that the sequestration of energy in benthic fishes of the Wabash River could be restricting the energy available to invasive Silver Carp, preventing Silver Carp from achieving the extreme level of abundance they exhibit in the nearby Illinois River. If the Wabash River fish community structure is now beginning to shift away from benthic invertivores, it may shift in favor of invasive species like Silver Carp (Pyron et al., 2017; Stuck et al., 2015).

The ecosystem-wide implications of a community shift that could benefit Silver Carp cannot be overstated. Future efforts should continue to monitor Blue Suckers and associated fishes in the Wabash River, and should be alert for indications of changes occurring at other trophic levels. It would be valuable to further document changes in the macroinvertebrate assemblages and substrate quality in this river system. Interactions between Blue Suckers, invasive carp, and climate change should be considered priorities for future research.

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TABLES

Table 2.1: Sample sets of Blue Suckers surveyed in the lower Wabash River. Note that 132 of the harvested specimens also occur in the LTEF surveyed specimens sample set. Standard error is included in parentheses where applicable.

	LTEF surveyed specimens	Harvested specimens	Combined specimens
Sample size	563	168	599
Sampled (yrs)	2010-2019	2018-2019	2010-2019
Total length range (mm)	66-775	189-774	66-775
Mean total length (mm)	615.1 (3.9)	604.6 (6.8)	613.6 (3.8)

Table 2.2: Annual average Blue Sucker statistics, based on LTEF surveyed specimens ( $n = 563$ , 2010-2019) of the lower Wabash River. Note that CPUE is reported as fish-per-site, with 66 annual sites surveyed 2010-2012 and 102 annual sites surveyed 2013-2019. Standard error is included in parentheses where applicable.

Year	Sample size	CPUE	Mean total length (mm)	$W_r$
2010	53	0.80 (0.26)	642.4 (9.8)	102.75 (1.56)
2011	66	1.00 (0.22)	624.4 (6.7)	103.17 (1.28)
2012	59	0.89 (0.24)	613.2 (13.5)	95.06 (1.25)
2013	39	0.38 (0.10)	628.5 (13.2)	98.07 (1.59)
2014	36	0.35 (0.10)	620.7 (16.9)	91.20 (1.86)
2015	84	0.82 (0.18)	633.0 (7.8)	92.97 (1.16)
2016	50	0.49 (0.12)	595.0 (13.1)	88.96 (1.42)
2017	44	0.43 (0.12)	625.8 (8.4)	91.94 (1.96)
2018	68	0.67 (0.16)	609.5 (9.8)	88.49 (1.00)
2019	64	0.63 (0.16)	607.8 (11.2)	92.20 (1.56)

Table 2.3: Average monthly statistics for Blue Suckers, based on LTEF surveyed specimens ( $n = 563$ , 2010-2019) from the lower Wabash River. The survey occurs June-October annually, but June has been omitted as few sites (3.3%) were sampled during this month across the years. Note that CPUE is reported as fish-per-site. Standard error is included in parentheses where applicable.

Month	Sample size	CPUE	$W_r$	Groups $\geq 4$ (% specimens)
July	273	0.20 (0.04)	86.79 (1.53)	18.2
August	216	0.48 (0.08)	91.94 (1.02)	31.7
September	141	0.87 (0.14)	98.89 (1.17)	47.2
October	252	1.08 (0.14)	94.86 (0.65)	66.3

FIGURES

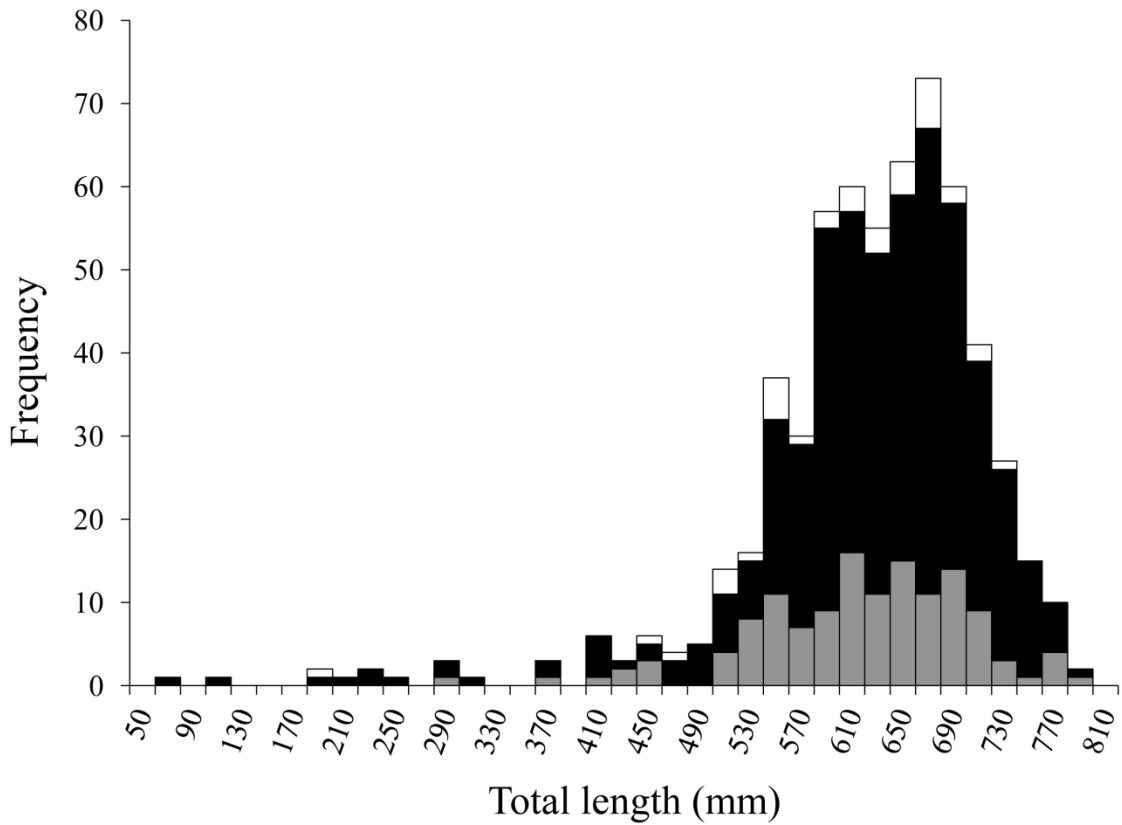


Figure 2.1: Size-frequency histogram of Blue Sucker specimens surveyed in the lower Wabash River, 2010-2019 ( $n = 599$ ). Black bars include specimens surveyed but not collected from LTEF surveys 2010-2017 ( $n = 431$ ); gray bars include specimens surveyed and collected from LTEF surveys 2018-2019 ( $n = 132$ ); white bars include specimens surveyed and collected via supplemental efforts in 2019 ( $n = 36$ ). Black bars and gray bars together form the LTEF survey sample set ( $n = 563$ ). Gray bars and white bars together form the harvested specimens sample set ( $n = 168$ ).



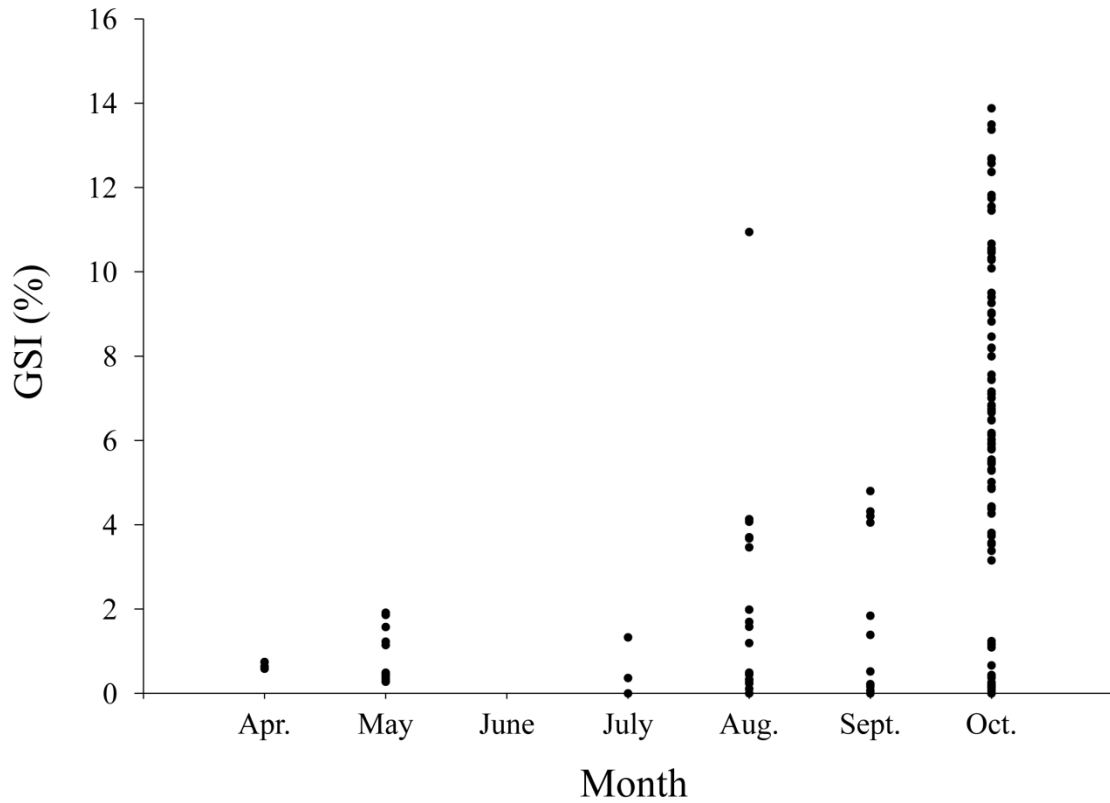


Figure 2.2: Gonadosomatic index (GSI) values by month, based on Blue Sucker specimens harvested from the lower Wabash River ( $n = 168$ , 2018-2019). Average GSI ( $\pm$ SE) for April = 0.65 (0.047,  $n = 3$ ), May = 0.81 (0.16,  $n = 14$ ), July = 0.071 (0.057,  $n = 24$ ), August = 1.58 (0.49,  $n = 25$ ), September = 1.96 (0.60,  $n = 11$ ), October = 6.20 (0.41,  $n = 91$ ).

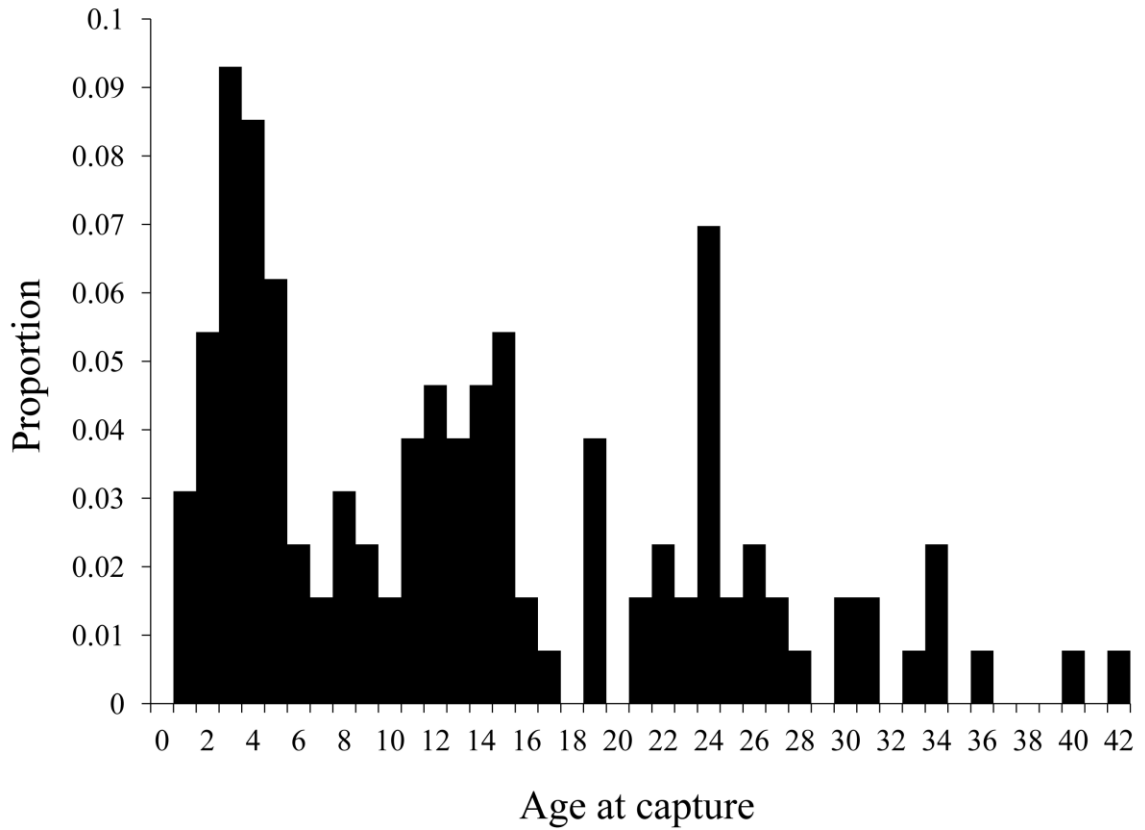


Figure 2.3: Proportional histogram of lapillus otolith age estimates assigned to Blue Sucker specimens harvested from the Wabash River, 2018-2019 ( $n = 128$ ). Age assignments ranged from 1 to 42 years (average 13.5 years).

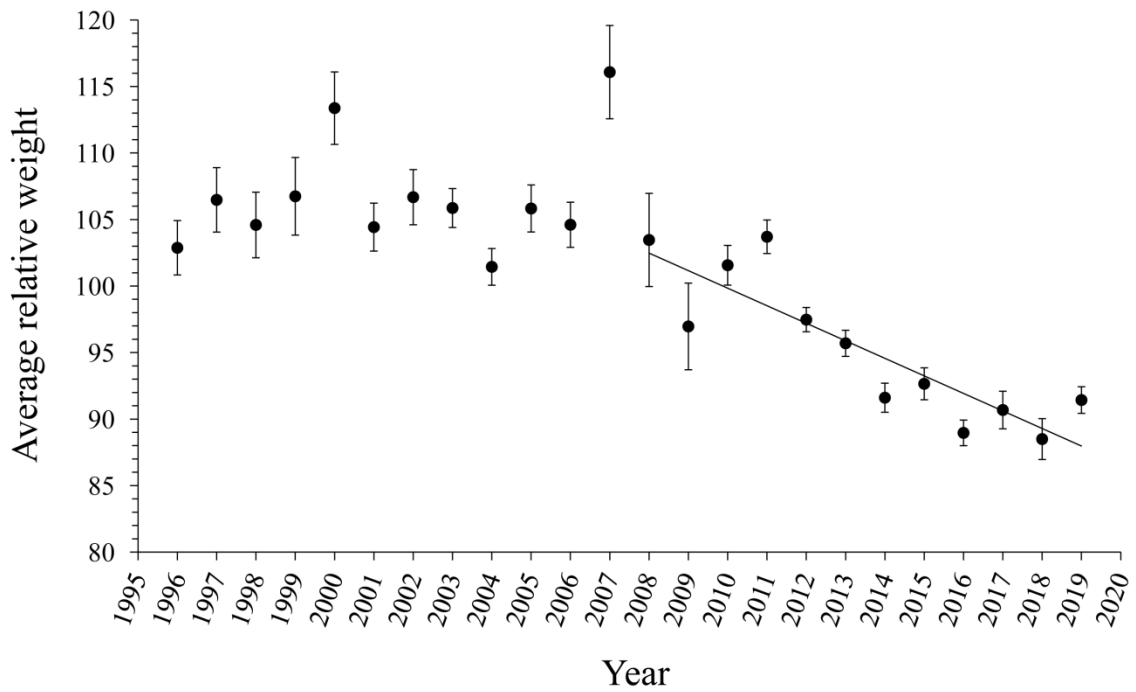


Figure 2.4: Blue Sucker average relative weight ( $W_r$ ) by year, 1996-2019. Based on specimens from the lower Wabash River LTEF surveys ( $n = 548$ ) and Illinois Department of Natural Resources surveys ( $n = 597$ ). Error bars show standard error. There is no significant trend in average  $W_r$  from 1996 to 2006 (average  $W_r = 105.7$ ); the trend line illustrates a significant declining trend in average  $W_r$  from 2008 to 2019:  $W_r = -1.3182 \cdot Yr + 2749.5061$  ( $P < 0.0005$ ,  $R^2 = 0.76$ ) where  $Yr =$  calendar year. The year 2007 was omitted so as to not disproportionately affect the downward trend.

## THESIS CONCLUSION

There is a growing interest in managing and protecting Blue Sucker populations, especially in states in which they are recognized as a threatened or endangered species. Blue Suckers have been historically understudied, limiting our understanding of their basic life history and conservation status and restricting our ability to evaluate population demographics against clear management goals. The Wabash River Blue Sucker population is rather unique in its abundance and reproductive success, and in its susceptibility to electrofishing gear (due to shallow river conditions). It provided our team with an opportunity to conduct valuable research to help inform the management and protection of threatened populations of Blue Suckers.

Inconsistencies in the choice of age-structure used in past studies of Blue Suckers have led to confusion regarding their life history (including estimated longevity, growth, and mortality). A species cannot be effectively managed without knowledge of these basic parameters, and we sought to identify the age structure that would yield the most precise and credible results for Blue Suckers in the absence of any validated structure. We compared age-estimations made from Blue Sucker scales, opercles, pectoral fin rays, and lapillus otoliths, and identified lapillus otoliths as being both the most precise and the most credible of these structures. Lapillus otolith age estimations suggested greater longevity (up to 42 years) than has been previously reported for this species and estimated a dramatically lower rate of annual mortality (4.5%) than was previously estimated for this population. We recommend aging this species with lapillus otoliths, and suggest that the prudent harvest of Blue Suckers for lethal aging is a necessary

sacrifice to inform the management of threatened and endangered populations. Validation of lapillus otoliths for aging Blue Suckers should be a top priority for future research.

In contrast to Blue Suckers in other populations, those in the Wabash River are abundant, long-lived, and appear to be experiencing low mortality and successful recruitment. The demographics described for this population should serve as a benchmark against which threatened populations can be compared. We estimated average female fecundity at 110,933 eggs (ranging from 87,217 to 126,696 eggs), and identified adult individuals as small as 422 mm in total length. The population length-weight regression was  $\text{Log}_{10}(WT) = 3.323 \cdot \text{Log}_{10}(TL) - 5.9592$ , where  $WT$  = weight (g) and  $TL$  = total length (mm). The von Bertalanffy growth curve fit to this population based on lapillus otolith ages was  $TL = 680.29038 \cdot (1 - e^{(-0.15898 \cdot \text{Age} + 5.14037)})$  where  $TL$  = total length (mm) and  $\text{Age}$  = otolith age (years) (Figure 1.11).

We identified a declining trend in average relative weights from 2008 to 2019, with an average loss of  $-1.5 W_r/\text{yr}$  (on a scale of 100). Similar downward trends were identified in the relative conditions of four other benthic invertivores fishes in the Wabash River. We suggest that Blue Suckers are serving as bioindicators in this system and that the declining trend in their condition should be regarded as an early symptom of community-level changes.

The Wabash River Blue Sucker population will no doubt remain valuable to researchers as a model of a relatively healthy population occurring in a relatively connected and biodiverse river system. Future efforts should continue to monitor Blue Suckers and associated fishes in the Wabash River and should be alert for indications of changes occurring at other trophic levels. It would be valuable to further document

changes in the macroinvertebrate assemblages and substrate quality in this river system. Explorations into the food web dynamics in the Wabash River may yield interesting insights into the potential direct and indirect pathways between benthic invertivores, macroinvertebrates, and Silver Carp. A subsample of Blue Sucker stomachs have been preserved from the harvested specimens used in this research, and a project addressing the dietary habits of this population would be worth pursuing in light of the declining relative weights trend.

Blue Suckers are a fascinating species, found nowhere except the freshwater rivers of North America. This once-abundant species is now imperiled, and the preservation of such a unique large-bodied, long-lived, potandromous fish serves to in turn preserve the integrity of the small- and large-scale ecosystems of which they are a part. This research seeks to support the management of sustainable Blue Sucker populations throughout their range.