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Recruitment Sources and Spatial Patterns of Population Demographics of Spotted Bass in a Large River-Tributary Network

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Abstract

Understanding spatial patterns in population characteristics and the principal natal environments supporting riverine fish populations are important for fisheries management. Fin ray microchemistry was used to identify natal environment and age estimates from sectioned fin rays were used to estimate growth and mortality rates for spotted bass, *Micropterus punctulatus* (Rafinesque), in a segment of the Ohio River (Smithland Pool) and three tributaries. Differences in water Sr:Ca and Ba:Ca among the Ohio River and tributaries were reflected in fin ray edge Sr:Ca and Ba:Ca. Fourteen percent of spotted bass ≥ age 2 captured in the Ohio River originated in tributaries, whereas 10% captured in tributaries originated in the Ohio River. Spotted bass in the Ohio River reached larger maximum size ($L\infty = 448.7$) than conspecifics in tributaries ($L\infty = 324.4$), although mortality rates were not different. Although 86% of spotted bass were collected in their inferred natal environment, small tributaries may be a supplemental source of recruitment for the spotted bass stock in Smithland Pool.

KEYWORDS: fin rays, growth, microchemistry, *Micropterus punctulatus*, Ohio River, tributaries

Introduction

The extent to which fish move and disperse within and among rivers and streams can substantially influence species distributions and population dynamics (Cooke *et al*. 2016). Understanding patterns of movement and habitat use by fishes to fulfill life history requirements (including movement between rivers and tributaries) is key for assessing population dynamics and effects of riverscape fragmentation and for conservation of habitats that contribute to population persistence (Schlosser 1991; Fausch *et al.* 2002). Furthermore, knowledge of spatial patterns in population demographics and fish movement and dispersal patterns in river-tributary networks is important for identifying relevant spatial scales for population assessment and management (Cooke *et al.* 2016; Laughlin *et al.* 2016; Porreca *et al.* 2016). In many large rivers, channel geomorphology and hydrology have been severely altered through anthropogenic activities such as impoundments, channelization and bank stabilization (Nilsson *et al.* 2005), which has led to global declines in many riverine fish species (Rinne *et al.* 2005). However, channel geomorphology and hydrology of many smaller tributaries of large rivers remain relatively unaltered in comparison. Tributaries may be particularly important as recruitment sources for fishes and sustaining species richness in large, main-stem rivers through fish movement from tributary to main-stem habitats (Pracheil *et al.* 2009, Pracheil *et al.* 2013). Although tributaries can contribute substantially to recruitment of riverine fish stocks, the extent to which early life stage dispersal and movement of older juvenile and adult fishes result in exchange of individuals among main-stem river and tributary habitats can vary considerably among river-tributary networks and species (Pracheil *et al.* 2009; Humston *et al.* 2010; Benjamin *et al.* 2014; Humston *et al.* 2016; Laughlin *et al*. 2016; Sousa *et al*. 2016; Spurgeon *et al*. 2017). Thus, additional studies investigating the exchange of fishes among tributary and main-stem environments across river systems and among fish species are needed to broaden understanding of the influence of river-tributary connectivity on fish population dynamics and to inform spatially-explicit assessment and management of fish populations tailored to particular species and systems.

Spotted bass, *Micropterus punctulatus* (Rafinesque), is a recreationally important fish species native to the south-central and Midwestern USA and is common in lotic environments ranging from small, permanently-flowing streams to large rivers (Pflieger 1997). Several studies using radio telemetry (Horton & Guy 2002; Goclowski *et al*. 2013), mark and recapture (Funk 1957), and visual observation (Lewis & Elder 1953) have reported movement of spotted bass within small streams. In Otter Creek, Kansas, Horton & Guy (2002) reported variable home ranges of spotted bass and that most movement took place during spring and fall. In addition, Lewis & Elder (1953) and Goclowski et al. (2013) each reported increased movement of spotted during seasonal transition periods in Clear Creek, Illinois, and in the Flint River, Georgia. Funk (1957) reported that a subset of spotted bass (2 of 24) within Missouri streams demonstrated mobile behavior, with movements of mobile individuals ranging from 11 to 38 km. Although prior studies have provided information on spotted bass movement tendencies in small streams, there have been no published studies of spotted bass movement and dispersal patterns within large rivers or between large rivers and their smaller tributaries. There is also scant literature regarding population demographics of stream-dwelling spotted bass (Tillma *et al*. 1998; Johnson *et al*. 2009) and no published studies reporting population demographics of spotted bass in large rivers. Thus, management-relevant baseline information on movement, growth, recruitment and mortality rates for riverine spotted bass populations are generally lacking.

Elemental analysis of calcified structures has demonstrated utility as a tool for reconstructing fish environmental history where geologic variation provides persistent spatial differences in water chemistry (Pracheil *et al*. 2014), including large rivers and their tributaries in the Midwestern USA (Smith & Whitledge 2010; Laughlin *et al*. 2016). Concentrations of some chemical elements in calcified structures reflect elemental concentrations in the waters in which a fish lives (Pracheil *et al*. 2014). Associating changes in chemical composition with growth marks in calcified structures enables inference of timing of fish movement among chemically distinct locations when an individual has resided in those locations long enough to incorporate their respective chemical signatures (Pracheil *et al*. 2014).

Fin rays represent a non-lethal alternative to use of otoliths for elemental analysis when sacrificing fish for otolith removal is not appropriate due to conservation status or rarity of the species and sizes of fish being studied (Clarke *et al*. 2007; Allen *et al*. 2009; Smith & Whitledge 2010; Phelps *et al*. 2012; Rude *et al*. 2014). Fin rays have also been used for non-lethal age estimation of *Micropterus* species (Morehouse *et al*. 2013; Rude *et al*. 2013). This study used elemental composition of fin rays to infer natal environment and movement of spotted bass. In addition, age estimates derived from sectioned fin rays were used to estimate spotted bass growth and mortality in the Smithland Pool of the Ohio River, USA and three of its tributaries. Study objectives were to: 1) verify that water elemental compositions differed between the Ohio River and tributaries and assess whether differences in water chemistry between the Ohio River and tributaries were reflected in spotted bass fin rays; 2) determine the accuracy with which spotted bass ≤ age 1 could be assigned to their collection location using fin ray chemistry; 3) identify natal environment of spotted bass ≥ age 2 sampled from the Ohio River and tributaries to assess the extent to which tributaries and main stem river habitats contribute to spotted bass recruitment in the Ohio River system; 4) assess precision of age estimates among readers and between sectioned pectoral and pelvic fin rays from spotted bass and 5) compare growth and mortality of spotted bass sampled from the Ohio River and tributaries.

Methods

Study Area

The study area encompassed Smithland Pool, an impounded 115.9 km section of the lower Ohio River between Illinois and Kentucky, USA and three tributaries of the Ohio River (Figure 1). This section of the Ohio River supports a recreational fishery for spotted bass, along with largemouth bass, *Micropterus salmoides* (Lacepède), and smallmouth bass *Micropterus dolomieu* Lacepède. Tributaries included in this study (Lusk Creek, Big Grand Pierre Creek and Big Creek) are relatively small in comparison to the Ohio River. Ohio River mean annual discharge was 5,795 m^3/s from 2014 through 2015 at Smithland Lock and Dam, while Lusk Creek mean annual discharge was 1.76 m^3/s from 2014 through 2015 near Eddyville, IL (USGS 2017). Big Creek and Big Grand Pierre Creek are similar to Lusk Creek in size but do not have stage and discharge gauges. The lowest portion of each tributary consists of an embayment where Ohio River and tributary water mix. The Lusk Creek embayment extends approximately 1.2 km upstream from its confluence with the Ohio River; the downstream sections of Big Creek and Big Grand Pierre Creek where Ohio River water mixes with creek water are shorter (< 0.5 km).

Water Collection and Analyses

Application of calcified structure microchemistry for inferring fish environmental history requires confirmation of spatial differences in water chemistry within the study area. Therefore, duplicate 20-ml water samples for analysis of strontium (Sr), barium (Ba), and calcium (Ca) concentrations were collected from the Ohio River at Golconda, Illinois, Lusk Creek, Big Grand Pierre Creek, Big Creek and embayments at creek mouths during 2014-2016 to assess differences in Sr:Ca and Ba:Ca among the Ohio River (including embayments) and tributaries. Water sampling began in early June, as age-0 spotted bass were expected to be present by this time of year (Smith 1979) and was generally conducted monthly through September each year to encompass most of the growing season when nearly all fin ray growth occurs (Whitledge 2017). However, not all sites were sampled during each month. Water samples were filtered using acid-cleaned polypropylene syringes and Whatman Puradisc (GE Healthcare Life Sciences, Pittsburgh, PA, USA) 0.45-μm polypropylene syringe filters (Shiller 2003) and stored in acid-cleaned polypropylene bottles until overnight shipment and analysis at the Center for Trace Analysis, University of Southern Mississippi. In the laboratory, water samples were acidified to pH 1.8 using ultrapure HCl and allowed to sit acidified for at least 1 week before analysis. Samples were then diluted 11x in ultrapure 0.16 M HNO_3. The HNO_3 contained 2 μg/L scandium, indium, and thorium as internal standards. Externally certified reference standards were also prepared using the same HNO_3 used for sample dilutions. Samples were analyzed for ^{44}Ca, ^{88}Sr, and ^{137}Ba in medium resolution using a Thermo-Finnigan Element 2 (Thermo Fisher Scientific, Waltham, MA, USA) inductively coupled plasma mass spectrometer (ICPMS). Precision of analyses based on repeated measurements of standards was better than ±2% (2

Standard Deviations). Elemental concentration data from water samples were converted to Sr:Ca and Ba:Ca ratios (mmol/mol).

Fish Collection and Fin Ray Analyses

Spotted bass were collected from the main-stem Ohio River throughout Smithland Pool, although the majority were collected within 2 km of tributary confluences where sampling effort was highest. Spotted bass were also collected from embayments (within 1 km upstream of the mouth of Lusk Creek; within 0.5 km upstream of the mouths of Big Grand Pierre and Big creeks). Fish were collected from the main-stem Ohio River and embayments (hereafter collectively referred to as “Ohio River” fish) during June-October 2014-2016 using boat-mounted pulsed DC electrofishing. Spotted bass were collected from tributaries at sites ranging from 8 to 30 km upstream of tributary mouths (hereafter collectively referred to as “tributary” fish) by angling and seine net during June-August 2014 and by pulsed DC boat electrofishing during August 2015. In September 2016 and May 2017, angling was used to increase fish sample size from Big Creek, as there were few accessible areas in this stream where boat electrofishing was feasible.

After capture, total length (TL) of each spotted bass was measured to the nearest mm and a leading pectoral and pelvic fin ray were removed (only a leading pectoral fin ray was removed from fish collected during 2014) as close to the body as possible. Fish collection, handling and fin ray collection procedures were conducted following protocols approved by the Southern Illinois University Animal Care and Use Committee (protocols 12-009 and 15-009). Fin rays were embedded in epoxy, sectioned at the widest part at the base of the fin ray into 1.3 mm sections using a Buehler ISOMET^{TM} low-speed saw (Buehler Inc., Lake Bluff, IL, USA), sanded using silicon carbide sandpaper (800 and 1000 grit) and polished with lapping film. The majority of fin ray sections were then jointly observed under a dissecting scope by the same two readers to estimate age of each spotted bass. A subsample of 200 fish were aged independently to evaluate age agreement among readers. When age discrepancies among readers occurred, or if age estimates differed between pectoral and pelvic fin rays, fin ray sections were concert aged and a consensus was reached. Fish were removed from analysis if an age estimate could not be obtained from either fin ray or if a consensus age could not be reached. After age estimation, fin ray sections were mounted on acid-washed glass slides using double-sided tape and then stored in acid washed polypropylene Petri dishes.

A subsample of aged fin ray sections was analyzed for Sr, Ba and Ca concentrations using a Perkin-Elmer DRC II (Perkin-Elmer Life and Analytical Sciences, Shelton, CT, USA) inductively coupled plasma mass spectrometer coupled with a CETAC Technologies (Teledyne CETAC Technologies, Omaha, NE, USA) LSX-213 laser ablation system. Pelvic fin ray sections were used if the pectoral fin ray from the same fish was broken or separated from the epoxy during the sanding and polishing process. The laser was used to ablate a spot at the core (primordium) and at the edge of each fin ray section (laser beam diameter = 25 µm, laser pulse rate = 20 Hz, laser energy level = 75%, 150 shots/spot). A standard developed by the U. S. Geological Survey (MACS-3) was analyzed by laser ablation every 15-20 samples to adjust for possible instrument drift. Each sample was preceded and succeeded by a 30 second gas blank measurement. Isotopes assayed included ^{86}Sr, ^{137}Ba, and ^{43}Ca. A Microsoft Excel macro developed at the University of Massachusetts-Boston’s Environmental Analytical Facility was used to calibrate elemental data to the standard reference material, correct for instrument drift, subtract background concentrations of elements and convert raw isotopic counts to elemental concentrations (μg/g) (Pracheil *et al*. 2014). Sr and Ba concentrations were normalized to Ca concentration within each sample based on the consideration of Ca as a pseudo-internal standard (Pracheil *et al*. 2014) and the stoichiometric concentration of calcium in fin ray hydroxyapatite (27%; Allen *et al*. 2009). Molar Sr:Ca and Ba:Ca ratios (μmol/mol) were then calculated from Sr, Ba, and Ca concentration data for laser ablation spots at the fin ray core (reflecting a fish's natal environment) and edge (reflecting a fish's recent environmental history) for each fish.

Data Analysis

Growth coefficients (K), theoretical mean maximum length (L_∞), and annual mortality (A) were estimated for spotted bass captured in the Ohio River and in tributaries to compare population demographics between these environments. Growth and mortality were estimated for spotted bass from all tributaries collectively rather than from individual tributaries due to the physical similarity and proximity of tributary streams and the limited range of age classes (i.e., rarity of old fish) sampled in each tributary. Growth was estimated by fitting Gompertz growth models to length and age at capture data (written for asymptotic L_∞; Quinn & Deriso 1999):

$$L(t) = L_\infty \exp\left[-\frac{1}{K} e^{-K(t-t_0)}\right]$$

as the Gompertz model provided estimates for L_∞ and K most similar to those reported in prior studies (Olmsted & Kilambi 1978); L_∞ estimated for spotted bass collected in the Ohio River using the von Bertalanffy growth model was also unrealistically large based on maximum size reported for this species (Table 1). Differences in mean length at capture between fish sampled in the Ohio River and tributaries were assessed for each age class common to fish sampled from the Ohio River and tributaries using Wilcoxon rank sum tests. Age-0 fish were not included in this analysis due to differences in dates of fish collections between the Ohio River and its tributaries; age-5 fish were also excluded from this analysis due to low abundance in samples. Annual mortality was estimated for fish captured with electrofishing using weighted regression catch curves (Miranda & Bettoli 2007). Age-5 fish were not included in mortality rate estimates due to low abundance in samples.

Generalized linear models (gamma distribution, log link) followed by Tukey's pairwise comparisons were used to assess differences in both water Sr:Ca and Ba:Ca (Satterthwaite's adjustment to correct for heteroscedasticity) among tributaries, embayments and the Ohio River; the discrete covariates year and month were also included in each model to assess temporal variability in Sr:Ca and Ba:Ca within tributaries and the Ohio River. Relationships between mean water Sr:Ca and fin ray edge Sr:Ca for fish ≤ age 1 and between mean water Ba:Ca and fin ray edge Ba:Ca for fish ≤ age 1 were evaluated using standard linear regressions. Fin ray type (pectoral or pelvic) was included as a covariate in linear regression models to assess whether relationships between water and fin ray Sr:Ca and Ba:Ca differed between pectoral and pelvic fin rays.

Quadratic discriminant function analysis (QDFA) was used to determine the accuracy with which spotted bass ≤ age 1 could be assigned to their collection location using fin ray edge Sr:Ca and Ba:Ca. A second QDFA using fin ray core Sr:Ca and Ba:Ca data from fish ≤ age 1 was used to determine whether location assignment (Ohio River or tributary) would differ for individual fish depending on whether fin ray core or edge data were used. Results from QDFAs indicated consistency of fin ray core and edge Sr:Ca and Ba:Ca signatures for spotted bass ≤ age 1; therefore, fin ray edge Sr:Ca and Ba:Ca data from spotted bass ≤ age 1 were considered indicative of fin ray chemistry signatures for resident fish in tributaries and the Ohio River.

A modification of the QDFA developed using fin ray edge Sr:Ca and Ba:Ca data from spotted bass ≤ age 1 was subsequently used to infer natal environment for spotted bass ≥ age 2 collected from the Ohio River and tributaries. The QDFA was not 100% successful in assigning fish ≤ age 1 to their collection environment (Ohio River or tributary) due to a small range of overlap in fin ray Sr:Ca and Ba:Ca signatures of fish ≤ age 1 between individuals collected in the Ohio River and its tributaries. Therefore, an additional QDFA containing three natal environment categories (Ohio River, tributary and uncertain) was developed using fin ray edge Sr:Ca and Ba:Ca data from spotted bass ≤ age 1; the uncertain category included individuals whose collection location was not correctly identified by the original QDFA. This additional QDFA enabled incorporation of uncertainty in environment classification assignments when the model was subsequently applied to infer natal environment for fish ≥ age 2. Fin ray core Sr:Ca and Ba:Ca for each fish ≥ age 2 were entered into the modified discriminant function to assign a natal environment for each individual. River-tributary movements by fish ≥ age 2 were inferred by comparing each fish’s environment of capture with its assigned environment of origin. A loglinear model (negative binomial distribution, log link) was used to assess whether the frequency of Ohio River-origin and tributary-origin fish ≥ age 2 differed both within and among fish sampled from each of the two collection areas (Ohio River and tributaries). Factors in the model included collection location (Ohio River or tributary), inferred natal environment (Ohio River or tributary), and the interaction of natal environment and collection location. Significance of all pairwise combinations of origin-collection location interactions were evaluated using least squares means with Tukey-Kramer adjustment for multiple comparisons. All statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina). *P*-values ≤ 0.05 were considered significant for all statistical tests.

Results

Water and Fin Ray Microchemistry

Both water Sr:Ca ($F_{4,38} = 144.24$, $p < 0.001$) and Ba:Ca ($F_{4,25} = 473.49$, $P < 0.001$) differed among the Ohio River, tributaries, and embayments, but did not differ within or among years for either Sr:Ca ($F_{4,38} = 0.74$, $P = 0.54$; $F_{4,38} = 0.53$, $P = 0.67$) or Ba:Ca ($F_{4,25} = 0.86$, $P = 0.48$; $F_{4,25} = 1.89$, $P = 0.17$). Water Sr:Ca and Ba:Ca for embayments at tributary mouths fell within ranges of Sr:Ca and Ba:Ca for Ohio River channel samples; mean water Sr:Ca of Ohio River channel ($n=11$) and embayment ($n=9$) samples did not differ ($t = -0.32$, $P = 0.99$), nor did mean water Ba:Ca of embayment ($n=6$) and Ohio River channel ($n=9$) samples ($t = 0.61$, $P = 0.10$). The Ohio River and embayments collectively differed from Lusk Creek in Ba:Ca ($t_{25} = 35.28$, $P < 0.001$), Big Creek in Sr:Ca ($t_{38} = -20.50$, $P < 0.001$), and Big Grand Pierre Creek in Sr:Ca ($t_{38} = -7.12$, $P < 0.001$) and Ba:Ca ($t_{25} = 15.96$, $P < 0.001$) (Figure 2).

Analysis of covariance indicated that the water Sr:Ca-fin ray type interaction term was non-significant ($F_{1,65} = 0.16$, $P = 0.69$), indicating that relationships between water and fin ray Sr:Ca did not differ between pelvic and pectoral fin rays. Non-significance of the water Ba:Ca-fin ray type interaction term ($F_{1,65} = 0.44$, $P = 0.51$) also indicated that relationships between water and fin ray Ba:Ca did not differ for pelvic and pectoral fin rays. For the combined set of pelvic and pectoral fin ray chemistry data, fin ray edge Sr:Ca for spotted bass ≤ age 1 was strongly related to mean water Sr:Ca of fish collection locations ($F_{1,67} = 159.18$, $r^2 = 0.70$, $P < 0.001$; Figure 3a). Likewise, fin ray edge Ba:Ca for spotted bass ≤ age 1 was strongly correlated with mean water Ba:Ca ($F_{1,67} = 97.07$, $r^2 = 0.59$, $P < 0.001$; Figure 3b).

Differences in water Sr:Ca and Ba:Ca among the Ohio River and tributaries, combined with strong relationships between water and fin ray Sr:Ca and Ba:Ca, resulted in separation of fin ray chemistry signatures among fish ≤ age 1 captured in the Ohio River and its tributaries (Figure 4). The strong relationship between fin ray and water Sr:Ca and Ba:Ca was also reflected by QDFA results, as 90% (73 of 81) of fish ≤ age 1 were correctly assigned to their collection environment based on fin ray edge data (Table 2). Quadratic discriminant function analysis of mean fin ray core data resulted in 96% of fish ≤ age 1 being assigned to the same location as when using fin ray edge data, indicating that fin ray core Sr:Ca of most fish ≤ age 1 also reflected their collection location.

A total of 162 aged fin rays from spotted bass ≥ age 2 were analyzed for Sr:Ca and Ba:Ca. Fin ray Sr:Ca and Ba:Ca were obtained from 80 spotted bass ≥ age 2 in the Ohio River, 58 of which were captured in the main channel and 22 of which were captured in embayments; 75% (60 of 80) were classified as Ohio River origin, 14 % (11 of 80) were classified as tributary origin, and 11% (9 of 80) were of uncertain origin. Of the 22 spotted bass collected in embayments, 10 were classified as Ohio River origin, 7 were classified as tributary origin, and 5 were of uncertain origin. Of the 58 spotted bass collected in the Ohio River main channel, 50 were classified as Ohio River origin, 4 were classified as tributary origin, and 4 were of unknown origin. Fin ray Sr:Ca and Ba:Ca were measured for 82 spotted bass ≥ age 2 in tributaries; 72% (59 of 82) were classified as tributary origin, 10% (8 of 82) were classified as Ohio River origin, and 18% (15 of 82) were of uncertain origin. Based on loglinear analysis (Pearson Chi-Square/d.f. = 0.80), counts of spotted bass natal environment allocations differed by catch location (Ohio River or tributaries; $F_{1,80} = 17.34$, $p < 0.0001$). Tukey's pairwise test indicated that there were significantly more Ohio River-origin fish caught in the Ohio River than in tributaries ($t_{80} = 2.69$, $p = 0.0419$), and there were significantly more tributary-origin fish captured in tributaries than in the Ohio River ($t_{80} = -3.20$, $p = 0.0103$). Among fish ≥ age 2 collected from tributaries, the proportion of individuals that originated in tributaries was significantly higher than the proportion of individuals that originated in the Ohio River (t_{80} = -2.95, p = 0.0210). Likewise, a significantly higher proportion of fish ≥ age 2 collected from the Ohio River were classified as having originated in the Ohio River compared to the proportion of fish ≥ age 2 caught in the Ohio River that were inferred to have originated in tributaries (t_{80} = 2.95, p = 0.0214).

Age Estimates and Demographics

A total of 268 spotted bass were collected from the Ohio River and 363 from tributaries for age estimation and demographic analysis. Agreement of estimated spotted bass ages among readers was 60%, with 90% of fish assigned ages differing by no more than 1 year among readers. Age estimates were obtained from both the pectoral and pelvic fin ray for 175 spotted bass, of which 77% had matching age estimates for both fin rays. Of the 23% of fish whose age estimates did not match (n=40), 95% (n=38) of age estimates were within 1 year, with the majority (83%) of fish assigned older age estimates using pelvic fin rays.

The Gompertz growth model estimated that theoretical maximum total length (L_∞, mm) of spotted bass captured in tributaries (L_∞ = 324.4; CI_{95} = 293.4, 355.4; K = 0.58; CI_{95} = 0.48, 0.68) was lower than that of spotted bass captured in the Ohio River (L_∞ = 448.7; CI_{95} = 366.1, 531.2; K = 0.40; CI_{95} = 0.30, 0.50; Figure 5). Significant differences in mean length at age occurred between spotted bass sampled from the Ohio River and its tributaries for age classes 1 (p = 0.0015) and 3 (p = 0.0022), but not for age classes 2 (p = 0.1213) or 4 (p = 0.3999). Mortality did not differ between fish collected in the Ohio River (A = 0.36; CI_{95} = 0.20, 0.49) and its tributaries (*A* = 0.70; CI_{95} = 0.27, 0.88; Figure 6), as estimated *A* for fish collected from the Ohio River was within the 95% confidence interval of *A* for fish collected from tributaries.

Discussion

Applicability of fin ray microchemistry

Persistent differences in water Sr:Ca and Ba:Ca between the Ohio River and tributaries and corresponding differences in fin ray edge Sr:Ca and Ba:Ca between spotted bass ≤ age 1 demonstrated the applicability of fin ray microchemistry for inferring spotted bass natal environment and movement between the Ohio River and tributaries entering Smithland Pool. Differences in water chemistry between the Ohio River and each of the three tributaries were detected from water samples collected during summer and fall over a three-year period despite some temporal variability in water Sr:Ca and Ba:Ca within each tributary and the Ohio River. Thus, observed differences in water chemistry among locations appear to be persistent across years. Classification of embayments at the mouths of tributaries as part of the Ohio River for the purpose of inferring spotted bass natal environment was warranted by water chemistry data that indicated water Sr:Ca and Ba:Ca in embayments were within the range of Ohio River channel water Sr:Ca and Ba:Ca. The presence of these embayments (“drowned river mouths”) and their differences in water Sr:Ca and Ba:Ca from upstream reaches of tributaries are a consequence of local topography and geology, as these tributaries quickly descend from the Shawnee Hills uplands into the Ohio River floodplain near their mouths. Observed differences in water chemistry between the Ohio River and tributaries suggest that calcified structure microchemistry will be applicable to studies of environmental history of other fish species (e.g., largemouth bass) present in Smithland Pool of the Ohio River and tributaries. However, additional water sampling will be needed to verify that differences in water chemistry between the Ohio River and tributaries reported herein persist in future years.

Results of this study add to the growing body of literature supporting the utility of fin ray microchemistry as a nonlethal alternative to otolith microchemistry in a variety of fish species (Veinott *et al*. 1999; Clarke *et al*. 2007; Allen *et al*. 2009; Smith & Whitledge 2010; Phelps *et al*. 2012; Rude et al. 2014). No published studies have applied calcified structure microchemistry to spotted bass; relationships between water and fin ray Sr:Ca and Ba:Ca reported in this study may be useful for assessing potential applicability of fin ray microchemistry to this species in other locations where spatial differences in water Sr:Ca or Ba:Ca are present. Relationships between water and fin ray Sr:Ca and water and fin ray Ba:Ca did not differ between spotted bass pectoral and pelvic fin rays, suggesting that either structure could be used for microchemistry studies. However, additional comparisons of trace elemental compositions among rays or spines obtained from different fins for individual fish would be useful to test the generality of this finding. A potential limitation of using fin rays for microchemical studies is that they are potentially subject to partial resorption during periods of high mineral demand, although calcium and phosphorus tend to be preferentially resorbed from scales rather than bones (Whitledge 2017). However, strong relationships between water and fin ray Sr:Ca and Ba:Ca and 90% accuracy in assigning fish ≤ age 1 to their collection locations suggest that either resorption of fin ray material was minimal, or that some reabsorption may have occurred but fin ray Sr:Ca and Ba:Ca of fish ≤ age 1 reflected collection location due to residency in locations with temporally consistent water chemistry. Elemental signatures of natal environments have been shown to be retained for >7 years in muskellunge, *Esox masquinongy* Mitchill, pelvic fin rays (Rude *et al*. 2014); but additional studies are needed to assess persistence of natal environment chemical signatures in fin rays for other fish species. Results of this study support the use of fin ray microchemistry for studies where sacrificing fish for otolith removal is undesirable.

Movement and Population Connectivity

Consistency of fin ray core and edge Sr:Ca and Ba:Ca and ≥ 90% of assignment of individuals to collection location using fin ray core or edge data implied that nearly all spotted bass ≤ age 1 in the study area had not dispersed from their natal environments. Thus, use of fin ray edge Sr:Ca and Ba:Ca data from these age groups to characterize location-specific chemical signatures was justified. Although spotted bass ≤ age 1 were not assigned to their collection locations with 100% accuracy due to minor overlap in fin ray edge chemical signatures between fish collected in the Ohio River and tributaries, imperfect assignment using QDFA did not substantially affect the ability to infer natal environment for fish ≥ age 2, as fin ray core Sr:Ca and Ba:Ca for most spotted bass ≥ age 2 fell within ranges that were definitively representative of Ohio River- or tributary-origin individuals.

Fin ray microchemistry indicated that most spotted bass sampled from the Ohio River and tributaries originated in the environment in which they were collected. However, an estimated 14% of spotted bass ≥ age 2 collected in the Ohio River were immigrants from tributaries and 10% of individuals ≥ age 2 collected in tributaries were immigrants from the Ohio River; accounting for individuals of uncertain origin, the percentage of immigrants in each location could have been as high as 25-28%. Similar percentages of immigrants in both the Ohio River and tributaries suggest that spotted bass movement between the river and tributaries was not unidirectional. Although there have been no prior investigations of spotted bass movement in large river systems, studies that have provided insights regarding the magnitude and frequency of spotted bass movement in small streams have indicated that movement tendencies are variable among streams and individuals within streams, likely dependent on habitat conditions (Lewis & Elder 1953; Funk 1957; Horton & Guy 2002; Goclowski *et al*. 2013). Further studies are needed to elucidate factors influencing movement of spotted bass between main-stem rivers and tributaries and develop a greater predictive capacity regarding the extent to which tributary and main-stem environments support recruitment and may influence population dynamics of spotted bass in river-tributary networks.

Fin ray core microchemistry provided some evidence that the proportion of immigrants from tributaries differed between spotted bass sampled from the Ohio River channel and tributary embayments. Seven of 22 fish collected in tributary embayments were classified as being of tributary origin, whereas only four of 58 fish captured in the Ohio River channel were inferred to have originated in a tributary. Thus, fish that move out of tributaries to the Ohio River may tend to disperse minimal distances upon entering embayment and main channel habitats. The presence of a higher proportion of tributary-origin fish in embayments than in the main channel may also reflect seasonal movement of some spotted bass from headwater areas to downstream sections of tributaries as water levels drop during summer and autumn (USGS 2017). This pattern of seasonal movement by spotted bass was reported by Lewis & Elder (1953) within the Clear Creek drainage, a tributary of the Mississippi River in southern Illinois. There are also reports of spotted bass undertaking seasonal movements in other small streams (Horton & Guy 2002; Goclowski *et al*. 2013). Future studies are needed to describe intra-annual patterns of seasonal movement exhibited by spotted bass in river-tributary networks. In particular, the potential influences of prolonged periods of flooding or low discharge on movement patterns of spotted bass among main-stem rivers and their tributaries should be investigated.

Population Demographics and Age Estimation

Limited exchange of spotted bass between the Ohio River and tributaries may have contributed to some differences in estimates of population demographic parameters between fish sampled from these environments. The Gompertz growth model provided estimates of L_∞ and K similar to those reported by Olmsted & Kilambi (1978) for spotted bass and more reasonable than those from the von Bertalanffy model, suggesting that spotted bass growth in both the Ohio River and tributaries may be slow early in life (Quinn & Deriso 1999). Spotted bass sampled from the Ohio River had a larger proportion of older individuals compared to fish sampled from tributaries, and estimated maximum size of spotted bass was greater for Ohio River fish than those found in tributaries. Differences in age structure and growth of fish between the Ohio River and tributaries may have been partially influenced by downstream movement of older fish into tributary embayments and the Ohio River or greater growth rate for fish in Ohio River and embayment habitats relative to upper reaches of tributaries. Estimated annual mortality rate (A) was higher for spotted bass sampled from tributaries in comparison to fish collected from the Ohio River, although confidence limits around A overlapped among these two groups. Relative scarcity of fish ≥ age 3 sampled from tributaries may have contributed to higher estimated mortality rate for tributary fish compared to the Ohio River, although whether relatively low abundance of older fish in samples from tributaries resulted from a higher A in tributaries or emigration of older fish to the Ohio River is unknown.

Validity of growth and mortality estimates depends on the accuracy of spotted bass ages estimated from fin ray annuli counts. Fin rays have been demonstrated to be suitable nonlethal alternatives to otoliths for age estimation in some fishes (Cass & Beamish 1983; Sikstrom 1983; Phelps *et al*. 2007; Walsh *et al*. 2008; Rude *et al*. 2013). In this study, obtaining age estimates from sectioned fin rays from spotted bass proved problematic for some fish due to limited contrast among structural growth bands when viewing fin ray sections. Age estimates for both pectoral and pelvic fin rays were obtained for only 34% (*n* = 175) of fish from which both structures were taken. All other fish were aged using only one of the two fin rays. Thus, removal of two leading pelvic or pectoral fin rays from each fish is recommended when using fin rays to age spotted bass in case a section from one of the two structures does not exhibit clearly identifiable annuli. Precision of age estimates among readers was similar to that reported for fin ray age estimates of largemouth bass and smallmouth bass (Morehouse *et al.* 2013; Rude *et al.* 2013). However, there have been no studies that have validated fin ray age estimates from spotted bass. Thus, additional studies should validate spotted bass age estimates using pectoral and pelvic fin rays obtained from known-age fish. Comparison of age estimates derived from spotted bass otoliths and fin rays would also be beneficial when sacrificing fish is not a concern. Otolith age estimates have not been validated for spotted bass but otoliths are considered to be the most accurate and precise (Long & Fisher 2001) structures for age estimation.

Management Implications

Currently, fishing regulations for spotted bass in the Ohio River and its tributaries in Illinois are identical. Most spotted bass within the study area were collected in their inferred natal environment, but a subset of fish moved between the Ohio River and tributaries, especially after age 1. Lotic spotted bass populations that contain a subset of mobile individuals (Funk 1957) may benefit from management at a relatively large scale. In contrast, more localized management strategies may be appropriate where populations consist of sedentary individuals. Due to the study area being restricted to one pool of the Ohio River, it is unknown if inferred movement patterns of spotted bass occur elsewhere in the Ohio River or in other main-stem rivers. Furthermore, there are few reports of growth and mortality rates for spotted bass, especially in lotic environments. Considering the popularity of spotted bass with anglers, as well as the paucity of demographic and life history data, investigators should seek to determine the influence of tributary and main-stem habitats on spotted bass populations and their demographics within riverine environments, especially in fisheries where exploitation rate is high.

Although most spotted bass were collected in their inferred natal environment, an estimated 14% of fish sampled in the Ohio River originated in tributaries, suggesting the potential for tributaries to supplement recruitment of the spotted bass stock in the Ohio River. Contribution of tributary-origin spotted bass to the Ohio River stock may vary over time depending on relative year-class strengths of Ohio River-origin and tributary-origin fish. However, results suggest at least some potential for buffering of within-river fluctuations in spotted bass recruitment by contributions of recruits from tributaries. Maintaining spotted bass stocks and their habitats in tributaries may be influential in supporting the Ohio River spotted bass stock and the fishery it supports. Considering the potential for fish exchange among tributary and main-stem environments, assessments of spotted bass stocks in the Ohio River and potentially other river systems should examine population demographic data from both main-stem rivers and their tributaries.

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Table 1. Estimates and 95% confidence limits of $L\infty$ and K for fish captured in the Ohio River and tributaries generated by the Von Bertalanffy, Logistic, and Gompertz growth models.

Model	Ohio River			
	L_∞		K	
	Estimate	95% CL	Estimate	95% CL
Von Bertalanffy	948.7	74.9, 1822.6	0.0801	-0.0136, 0.1738
Logistic	376.5	337.0, 415.9	0.7347	0.6222, 0.8471
Gompertz	448.7	366.1, 531.2	0.3995	0.3006, 0.4984

Model	Tributaries			
	L_∞		K	
	Estimate	95% CL	Estimate	95% CL
Von Bertalanffy	421.5	335.0, 508.0	0.2386	0.1533, 0.3239
Logistic	300.3	278.9, 321.7	0.8907	0.7709, 1.0106
Gompertz	328.5	296.6, 360.5	0.5673	0.4673, 0.6673

Table 2. Results of quadratic discriminant function analysis showing classification accuracy for spotted bass ≤ age 1 to environment of collection based on fin ray edge Sr:Ca and Ba:Ca.

Source location	Assigned location		
	Ohio River	Tributaries	% correct
Ohio River	22	2	91.6
Tributaries	6	51	89.5

Figure Captions.

Figure 1. Study area showing Smithland Pool of the Ohio River and tributary creeks in Illinois, USA. Filled diamond symbols indicate water sampling locations in tributaries; open diamond symbols indicate water sampling locations in the Ohio River and embayments at tributary mouths.

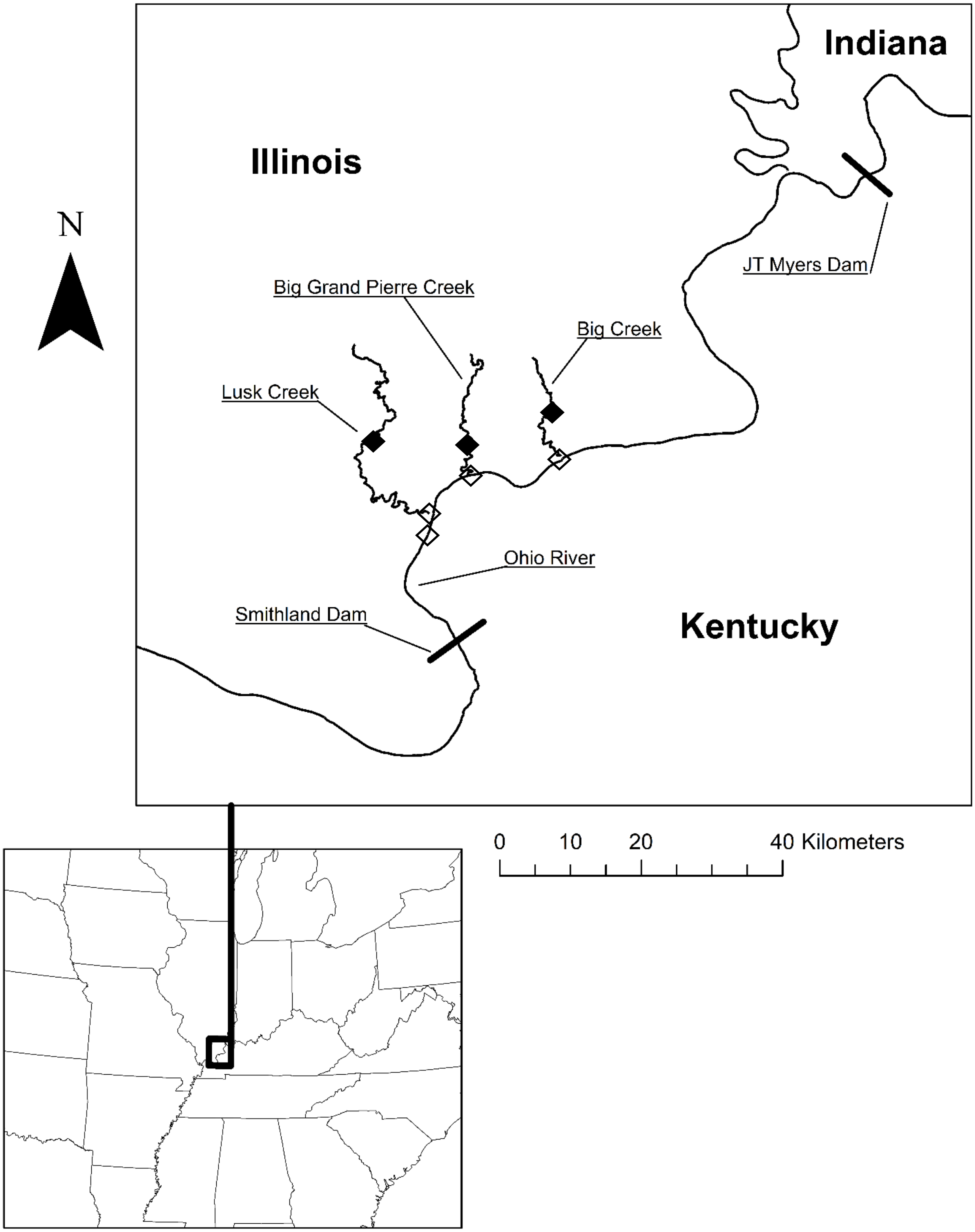
Figure 2. Boxplots displaying the ranges, medians, and interquartile ranges for water (a) Sr:Ca and (b) Ba:Ca from sites within the study area. OHIO = Ohio River and tributary embayments; LUSK = Lusk Creek; BGP = Big Grand Pierre Creek; BIG = Big Creek. Mean water Sr:Ca or Ba:Ca differ among locations with different letters above boxplots ($p < 0.05$). n=number of samples from each site.

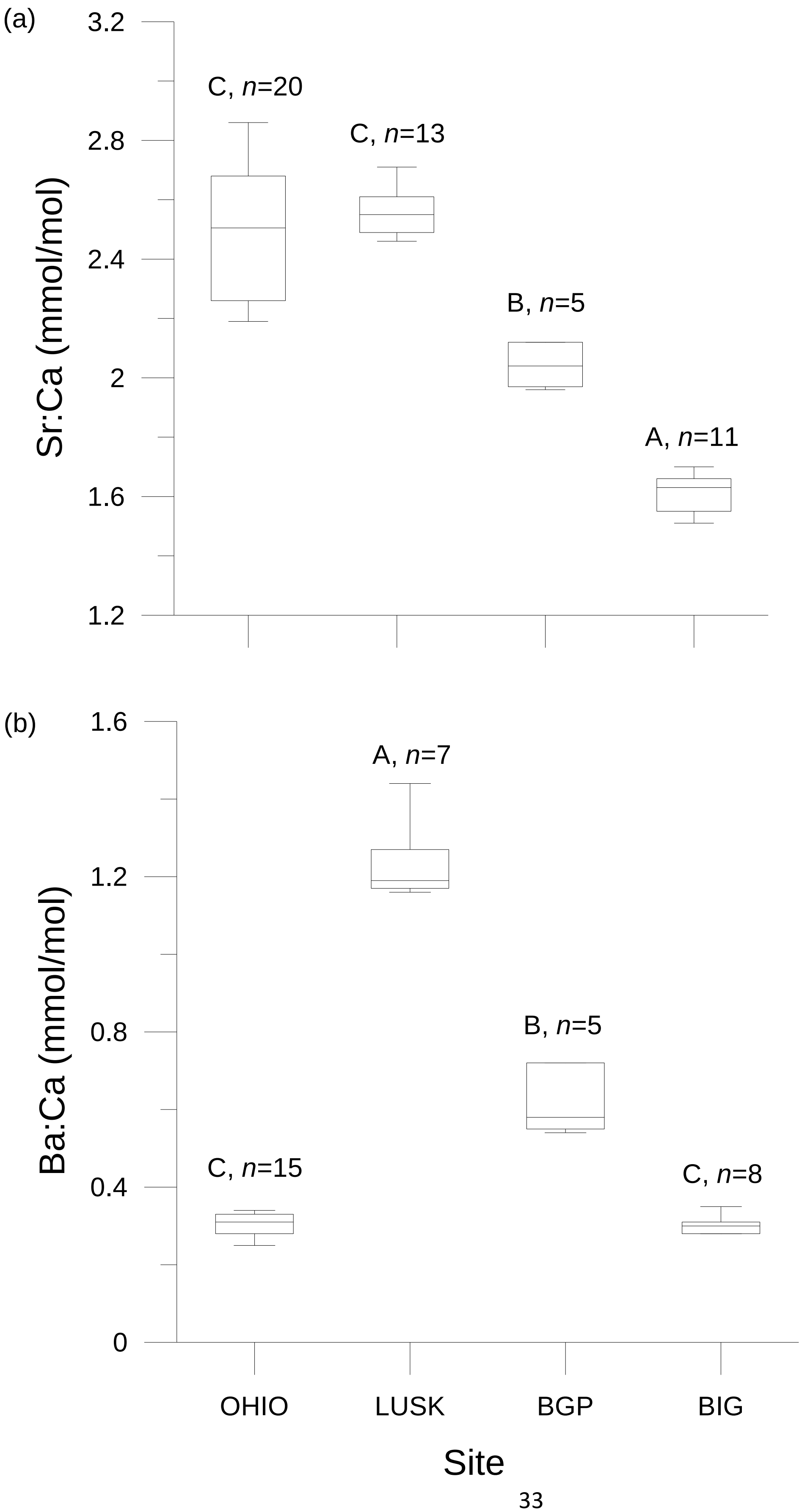
Figure 3. Relationships between (a) water Sr:Ca and fin ray edge Sr:Ca and (b) water Ba:Ca and fin ray edge Ba:Ca for spotted bass ≤ age 1 (n=69) collected from the Ohio River and tributaries.

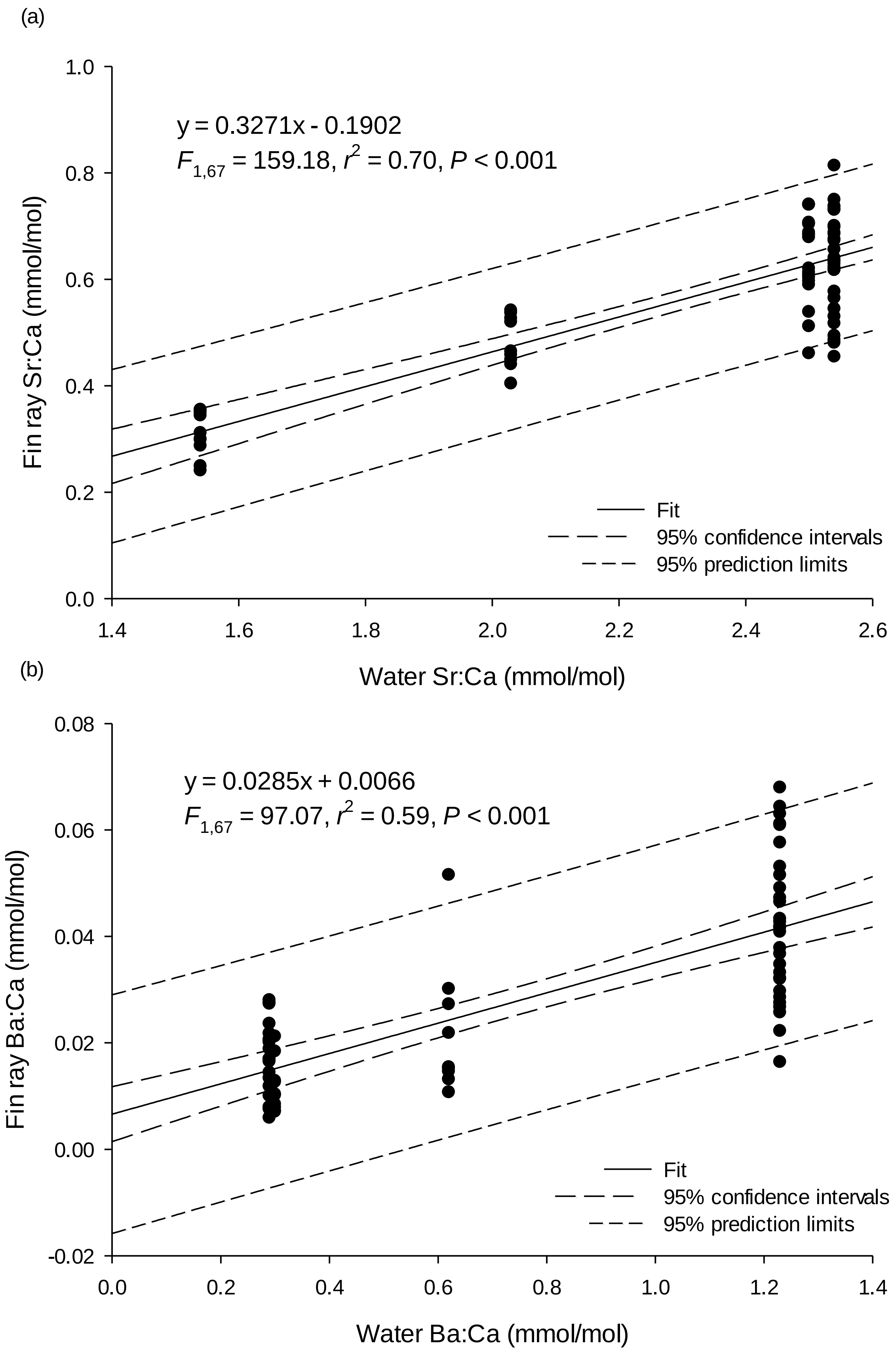
Figure 4. Plot of fin ray edge Sr:Ca and Ba:Ca for spotted bass ≤ age 1 (n=69) collected from the Ohio River and tributaries.

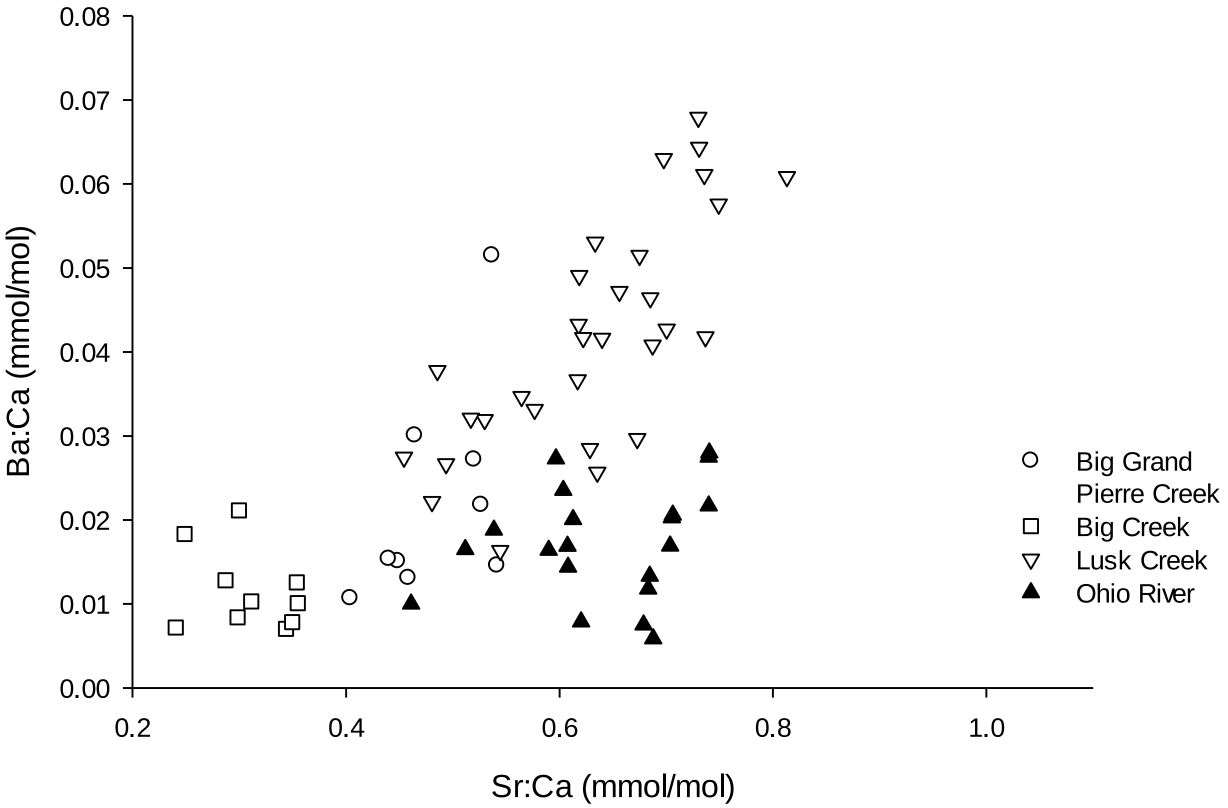
Figure 5. Total length at age for spotted bass collected from (a) the Ohio River (n=161) and (b) tributaries (n=306) and Gompertz growth model predictions of spotted bass length at age (with 95% confidence intervals) for spotted bass collected from the Ohio River and tributaries.

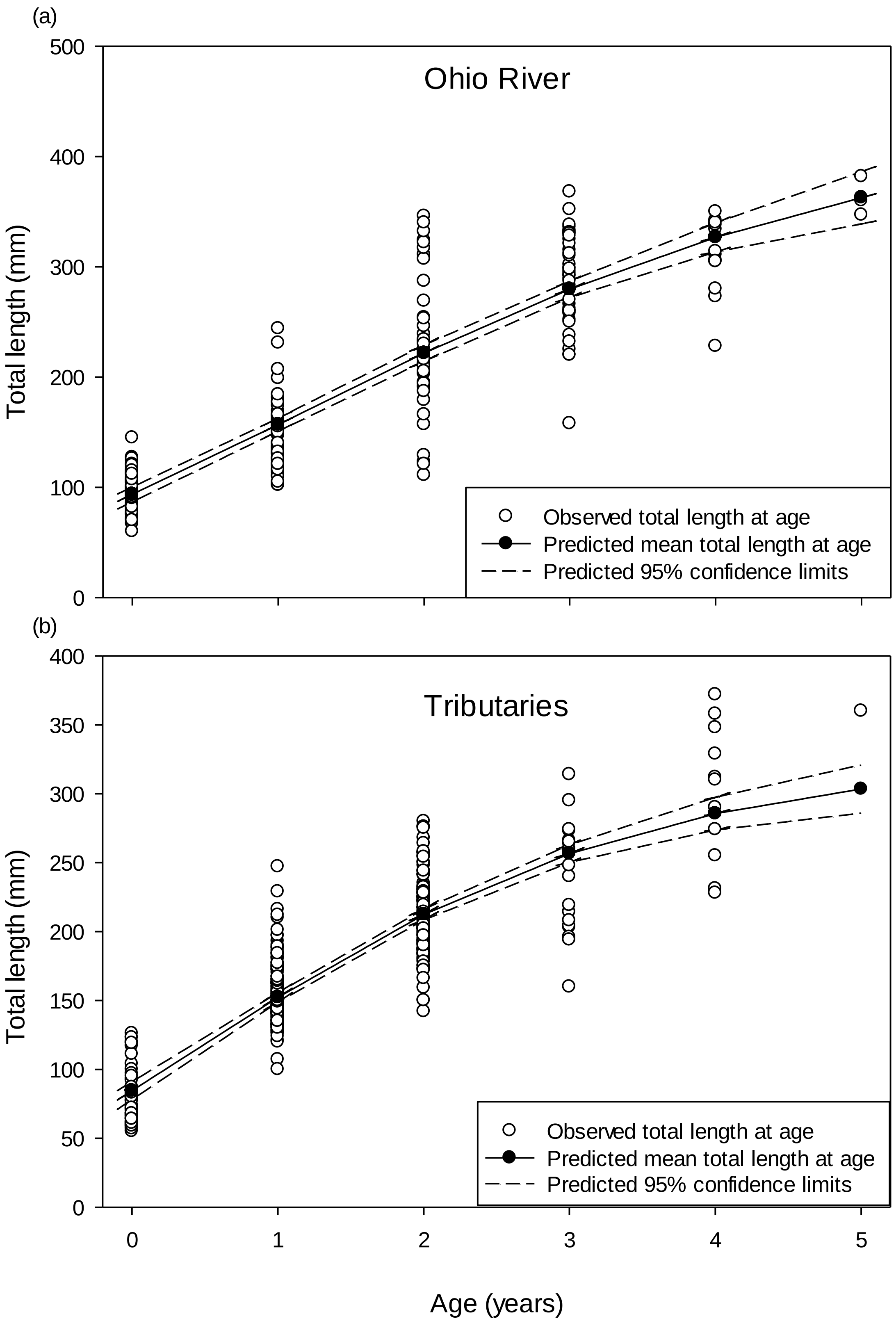
Figure 6. Catch curves for spotted bass collected from the Ohio River and tributaries. Solid and dashed lines are weighted linear regressions fit to catch-at-age data.

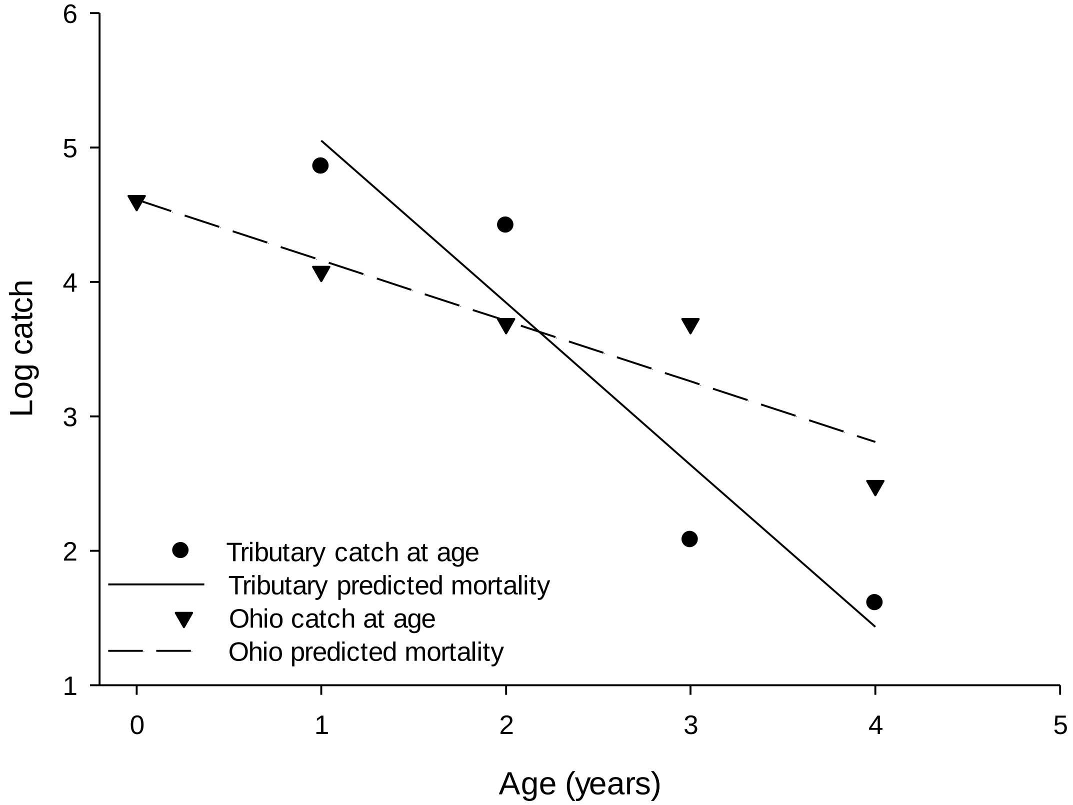

Indiana
Illinois
N
JT Myers Dam
Big Grand Pierre Creek
Big Creek
Lusk Creek
Ohio River
Smithland Dam
Kentucky
0
10
20
40 Kilometers


(a)
3.2
2.8
2.4
2
1.6
1.2
Sr:Ca (mmol/mol)
C, n=20
C, n=13
B, n=5
A, n=11
(b)
1.6
1.2
0.8
0.4
0
Ba:Ca (mmol/mol)
A, n=7
B, n=5
C, n=15
C, n=8
OHIO
LUSK
BGP
BIG
Site


(a)
y = 0.3271x - 0.1902
$F_{1,67}$ = 159.18, r^2 = 0.70, P < 0.001
Fin ray Sr:Ca (mmol/mol)
1.0
0.8
0.6
0.4
0.2
0.0
1.4
1.6
1.8
2.0
2.2
2.4
2.6
Water Sr:Ca (mmol/mol)
Fit
95% confidence intervals
95% prediction limits
(b)
y = 0.0285x + 0.0066
$F_{1,67}$ = 97.07, r^2 = 0.59, P < 0.001
Fin ray Ba:Ca (mmol/mol)
0.08
0.06
0.04
0.02
0.00
-0.02
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Water Ba:Ca (mmol/mol)
Fit
95% confidence intervals
95% prediction limits


Ba:Ca (mmol/mol)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Sr:Ca (mmol/mol)
0.2
0.4
0.6
0.8
1.0
○ Big Grand Pierre Creek
□ Big Creek
▽ Lusk Creek
▲ Ohio River


(a)
Ohio River
Total length (mm)
0
100
200
300
400
500
Observed total length at age
Predicted mean total length at age
Predicted 95% confidence limits
(b)
Tributaries
Total length (mm)
0
50
100
150
200
250
300
350
400
Observed total length at age
Predicted mean total length at age
Predicted 95% confidence limits
0
1
2
3
4
5
Age (years)


6
5
4
3
2
1
Log catch
0
1
2
3
4
5
Age (years)
Tributary catch at age
Tributary predicted mortality
Ohio catch at age
Ohio predicted mortality