Comparison of Otoliths and Scales in Estimating Age of Redbreast Sunfish and Green Sunfish in the Yellow River Watershed, Georgia

Peter C. Sakaris, *Georgia Gwinnett College, School of Science and Technology, 1000 University Center Lane, Lawrenceville, GA 30043*

Jesse J. Sunga, *Georgia Gwinnett College, School of Science and Technology, 1000 University Center Lane, Lawrenceville, GA 30043*

Jessica N. Coover, *Georgia Gwinnett College, School of Science and Technology, 1000 University Center Lane, Lawrenceville, GA 30043*

Abstract: Population-level studies often require age estimation of fish, but populations in small rivers and streams are generally smaller than those in large rivers or reservoirs. Therefore, non-lethal aging methods are generally recommended to minimize the potentially negative effects of sampling on population size. Accordingly, our main goal was to compare otoliths and scales as structures for estimating the age of redbreast sunfish (*Lepomis auritus*) and green sunfish (*L. cyanellus*) in an urban watershed. Reader agreement was greater for otoliths (88%–89%) than for scales (73%–79%), and precision (mean CV) in age estimates was better for otoliths (3.7%–4.0%) than scales (6.1%–9.4%) for both species. Readers were significantly more confident in their otolith-derived age estimates than scale-derived age estimates for both species. For redbreast sunfish and green sunfish, age estimation bias between readers was apparent for scales, but not for otoliths. Otolith-estimated ages of redbreast sunfish ranged from 0 to 7 years, while scale-estimated ages ranged from 1 to 6 years. For green sunfish, otolith-estimated ages ranged from 1 to 7 years, while scale-estimated ages ranged from 0 to 5 years. Mean estimated ages were significantly different between otolith-based and scale-based ages for both species. Scales generally overestimated otolith-assigned ages of younger redbreast sunfish and underestimated otolith-assigned ages of older fish. For green sunfish, scales generally underestimated otolith-assigned ages of older fish. Annual survival estimates derived from catch-curve analyses also differed between otoliths and scales for both species. We recommend that biologists avoid using scales to estimate the ages of redbreast sunfish and green sunfish. When a non-lethal aging method is required, biologists should use extreme caution in using scales, and explore other non-lethal methods for age estimation.

Key words: aging precision, age bias plots, *Lepomis cyanellus, Lepomis auritus*

Journal of the Southeastern Association of Fish and Wildlife Agencies 6:44–50

Studies of fish age, growth, and survival require a reliable method for estimating the ages of individuals sampled from a population. Published methods have used various hard parts for estimating fish age, including scales, spines, fin rays, vertebrae, cleithra, and otoliths, with varying levels of accuracy and precision (Maceina et al. 2007, Phelps et al. 2017). Otoliths are typically considered the superior structure for estimating fish age (Maceina et al. 2007, Phelps et al. 2017), but otolith extraction is a lethal method. Consequently, fish biologists often explore non-lethal methods that may provide age data that closely approximates otolith-age derived data.

An important factor to consider when pursuing life history studies of fishes in small rivers and streams is that fish populations are generally smaller than those found in large rivers or reservoirs. Therefore, sacrificing a large sample of fish, or removing any individuals, from a small creek or stream may not be possible or advisable, particularly for long-term studies that require routine sampling of populations. Scientists should consider using alternative, non-lethal measures to estimate the ages of fish in these smaller systems, but not all hard structures provide the reliably accurate age data that are required to conduct population assessments (Phelps et al. 2017). Thus, studies should initially explore the prospects of using non-lethal methods by collecting a smaller sub-sample of fish to evaluate and compare different structures for age estimation (e.g., otoliths versus scales).

Non-lethal aging methods for perciform fishes have attempted to use fin spines, fin rays, and scales to estimate ages with limited success (Welch et al. 1993, Secor et al. 1995, Besler 1999, Isermann et al. 2011, Klein et al. 2017). For example, Secor et al. (1995) reported that scale and otolith ages were not significantly different for striped bass (*Morone saxatilis*) aged 5 to 11 years, but ages of old fish (22–31 years) were severely underestimated with scales. Besler (1999) reported that scales were ineffective for estimating the ages of largemouth bass (*Micropterus salmoides*) in North Carolina, with much lower between-reader precision when compared to reads of whole otoliths and otolith sections. Klein et al. (2017) recently reported that otoliths were more accurate and precise than anal fin spines and dorsal fin spines in estimating ages of known-age largemouth bass. Moreover, otoliths were found to be more accurate and precise than scales in age estimation of white crappie (*Pomoxis annularis*) and black crappie (*P. nigromaculatus*)

(Boxrucker 1986, Hammers and Miranda 1991, Ross et al. 2005). Phelps et al. (2017) recently provided a thorough literature review of aging structures and reported that the lethal method (i.e., otoliths) has typically provided greater accuracy and precision than non-lethal methods (i.e., fin spines, fin rays, or scales) in estimating the ages of centrarchids, percids, and moronids. However, non-lethal methods are still recommended when sacrificing fish is not an option, with the caveat that non-lethal structures may underestimate the ages of old fish (Phelps et al. 2017). In addition, although the majority of studies favor the use of otoliths over other structures, non-lethal methods may provide reliable age data on a species- and system-specific basis (e.g., black crappie in South Dakota, Kruse et al. 1993) and should be tested.

Population studies of sunfishes (*Lepomis* spp.) have typically used scales to estimate age. Several studies have either attempted some form of validation for scales and/or have identified potential issues with using scales for age estimation. Regier (1962) used scales to estimate age of known-age bluegill sunfish (*L. macrochirus*) and noted several issues associated with using scales for aging (e.g., occurrence of accessory checks, difficulties in recognizing the first annulus, starving bluegill may not form annuli, and portions of scales may be resorbed, etc.). Despite these issues, numerous studies have still used scales to estimate age of sunfishes (Tharratt 1966, Bacon and Kilambi 1968, Osenberg et al. 1988, Fox 1994, Bertschy and Fox 1999, Klumb et al. 1999, Delp et al. 2000, Schindler et al. 2000, Quist and Guy 2001, Uzunova et al. 2008). For example, Osenberg et al. (1988) used scales to estimate ages of bluegill sunfish and pumpkinseed sunfish (*L. gibbosus*) and used scales to back-calculate lengths at age for both species. Validation of their technique involved comparing age estimates from scales to independent age estimates derived from age-class identification in length-frequency distributions. Osenberg et al. (1988) reported that previous reads of some of their scales included accessory checks, and that these scales had to be reread for their particular study.

In the current study, our primary goal was to assess and compare otoliths and scales as structures for estimating the age of redbreast sunfish (*L. auritus*) and green sunfish (*L. cyanellus*) from an urban stream. Previous studies have used otoliths or scales for estimating the ages of redbreast sunfish and green sunfish (Sandow et al. 1974, Quist and Guy 2001, Sammons and Maceina 2009, Gautreau and Curry 2012). In addition, microchemical analyses of green sunfish otoliths have been conducted to assess their exposure to selenium in mining-impacted streams (Arnold et al. 2015) and to identify their origin where they are non-native in the upper Colorado River (Whitledge et al. 2007). However, past studies comparing lethal and non-lethal aging structures for centrarchids have focused on sportfishes (Phelps et al. 2017), and, to our knowledge, no studies have compared otoliths and scales for estimating age of green sunfish and redbreast sunfish. Therefore, our main objectives were to: 1) compare reader agreement, bias, precision, and confidence between otoliths and scales in estimating age of redbreast sunfish and green sunfish, and 2) compare age distributions and survival estimates computed from otolith- and scale-derived age data for both species.

Methods

Fish Collection

From 22 October 2015 to 12 November 2016, we used backpack electrofishing to collect redbreast sunfish and green sunfish from three locations in the Yellow River watershed near the Georgia Gwinnett College campus (Lawrenceville, Georgia). Georgia Gwinnett College is located northeast of Atlanta, in the Atlanta-metro sub-region. Our research was conducted near the headwaters of the Yellow River, which is a tributary of the Ocmulgee River that flows through the lower Piedmont and the Atlantic coastal plain. Our three sampling locations were an upper and lower site on Tree Creek, which is a small tributary of the Yellow River, and a location near the confluence of Tree Creek with the Yellow River. Electrofishing was conducted once per month on a rotational basis through our sampling locations to minimize any negative effects of sampling on these stream fish populations, and to allow for future population comparisons (e.g., body condition) by season and species. During each month of sampling, representative samples of approximately 9–13 redbreast sunfish and 4–14 green sunfish were collected, euthanized in an ice-water bath, and then transported to lab for processing. Deviations from this sampling plan were: 1) only one green sunfish was collected in November 2015 and in April 2016, 2) sampling was not conducted in October 2016, as a sufficient sample was collected for both species in October 2015, and 3) redbreast sunfish were not collected in November 2016, as a sufficient sample of redbreast sunfish was collected in November 2015.

Fish Processing

Redbreast sunfish and green sunfish were weighed (g) and measured (mm TL) in the laboratory, and sagittal otoliths were extracted from each fish. Approximately 8–10 scales were removed from the side of each fish, following the methods illustrated by McInerny (2017). Otoliths and scales were cleaned in distilled water, and stored dry for later analyses. Scales were cleaned carefully to remove all mucous and debris, which can reduce light transmission and readability of scales (McInerny 2017).

Otolith and Scale Preparation and Aging

Otoliths were embedded in a clear epoxy resin (WestSystem 105 epoxy resin and 206 slow hardener) in a rubber epoxy mold.

We used a high-precision sectioning saw to cut each otolith at the core ("nucleus") along a transverse plane (Preciso-CL sectioning saw, Model CL40, Top Tech Machines Co., Taichung City, Taiwan, equipped with an IsoMet Diamond Wafering Blade, (10.16 × 0.03 cm), Buehler Co., Lake Bluff, Illinois). Sectioned otoliths were then fixed to microscope slides with CrystalBond, positioned perpendicular to the plane of the slide.

Scales were initially examined under a dissecting microscope to identify approximately 4–6 scales with identifiable foci and annuli. Regenerated scales were eliminated and not used for age estimation (McInerny 2017). Selected fish scales were pressed tightly between two, clean microscope slides that were taped together and labeled for age estimation (DeVries and Frie 1996), similar to methods used by Hammers and Miranda (1991) for estimating the ages of white crappies. All mounted scales for a fish were aligned in a row and in the same anterior-posterior orientation.

Otolith sections were viewed on a stereomicroscope with incident light to illuminate annuli. Mineral oil was also applied to each otolith section to enhance the clarity of annuli. Scales were also examined on a stereomicroscope, but with transmitted light to visualize annuli. We used a mounted camera and image analysis system to display images of otolith sections and scales on a computer monitor to facilitate reading and age estimation. Each structure was read independently by two experienced readers, and any disagreements in age assignments were reconciled with concert reads (i.e., mutual examination between readers). Readers had no knowledge of the total length of each fish that was aged. If an agreement could not be reached, an experienced third reader intervened to provide a third age estimate for the sample under question. For each mounted otolith and scale that was aged, the two readers independently assessed their overall reading "confidence" on a rating scale from 1 to 3, where:

1. Annuli are not distinct, faint; false annuli are likely present
2. Annuli are somewhat distinct and can be read without much difficulty; false annuli may be present, and
3. Annuli are clear and easily readable; false annuli are typically not present, or easily distinguishable from true annuli

Ihde and Chittenden (2002) used a similar approach to assess reader confidence in estimating ages of spotted seatrout (*Cynoscion nebulosus*), but limited guidance was provided in assessing confidence. That study ranked readings of sectioned pectoral fin rays, sectioned dorsal fin spines, scales, and whole and sectioned otoliths from 1 (low confidence) to 5 (high confidence). Spiegel et al. (2010) applied a confidence ratings scale (0–3) to age estimation of three carpsucker species, providing a more guided and informative approach to ranking confidence. We adapted our approach after Spiegel et al. (2010), but we used three levels (1–3) and developed confidence criteria that were specific to our study species (see above).

Statistical Analyses

We calculated reader agreement (%) for otoliths and scales of both species. To assess precision between readers, CV in scale-derived and otolith-derived age estimates was computed for both species (Campana et al. 1995). Reader confidence was compared between structures for each species (paired *t*-tests) and for each structure between species (two-sample *t*-tests). To detect potential bias between readers for scales and otoliths of both species, age-bias plots were constructed according to Campana et al. (1995). Paired *t*-tests were used to compare mean estimated ages derived from scales and otoliths for each species. To test for overall agreement between structures in age estimation, mean scale age (with 95% CL's) was plotted against the given otolith-assigned age for each species. In addition, age structure histograms derived from otolith and scaled-based age data were constructed to further facilitate comparisons between aging structures. To assess implications of potential aging bias in population analyses, catch-curve analyses were conducted using otolith-based and scale-based data to estimate annual survival of each species. Statistical tests were considered significant at $P \leq 0.05$.

Results

Redbreast Sunfish

A total of 126 redbreast sunfish were collected, varying from 53–177 mm TL and 2.2–83 g. Otolith-estimated ages of redbreast sunfish ranged from 0 to 7 years, while scale-estimated ages ranged from 1 to 6 years. Reader agreement for otoliths was 89%, while reader agreement was only 73% for scales. Mean CV in otolith age estimates was 4.0%; whereas, mean CV was 9.4% in scale age estimates. Reader confidence was significantly greater for otoliths (mean ± S.E. = 2.42 ± 0.05) than for scales (1.88 ± 0.05, $t = 7.34$, $P < 0.01$). Age estimation bias between readers was not present for otoliths (Figure 1a). In contrast, age estimation bias was apparent for scales, with reader 2 overestimating age at age-1 and underestimating the ages assigned by reader 1 at ages 4 and 5 (Figure 1b).

Mean estimated ages significantly differed between otoliths (2.14 ± 0.13 years) and scales (2.46 ± 0.10, $t = -3.01$, $P < 0.01$). Scales generally overestimated otolith-assigned ages at ages 0, 1, and 2, and underestimated otolith-assigned ages at ages 3+ (Figure 2a). These differences in age assignments between structures were observed in age structure histograms, with fewer fish being assigned age-1 and more fish being assigned ages 2 and 3 with scales (Figure 3a). Annual survival rates were estimated at 55% with otolith-derived age data and 44% with scale-derived age data (Figure 4a).

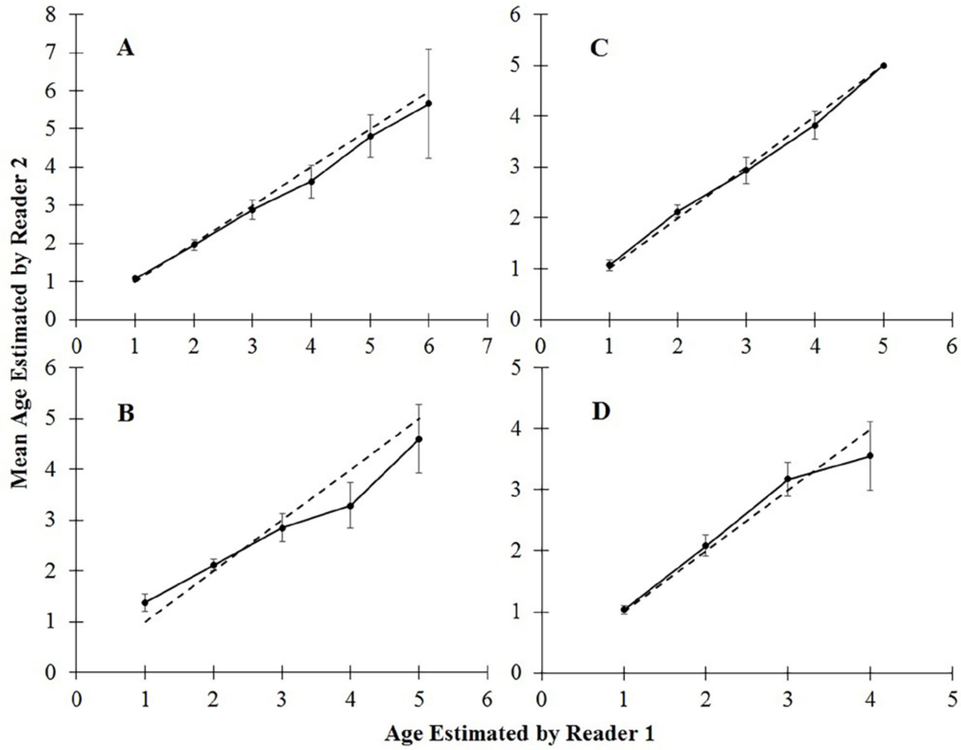

Figure 1. Age-bias plots for otolith-based and scale-based age estimates for redbreast sunfish (A–Otolith, B –Scale) and green sunfish (C–Otolith, D–Scale, Bars = 95% CLs).

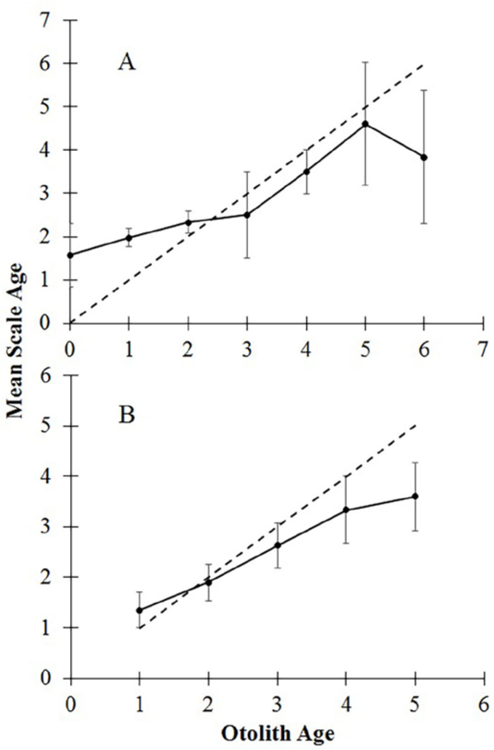

Figure 2. Mean scale age plotted against the given otolith age for redbreast sunfish (A) and green sunfish (B) (Bars = 95% CLs).

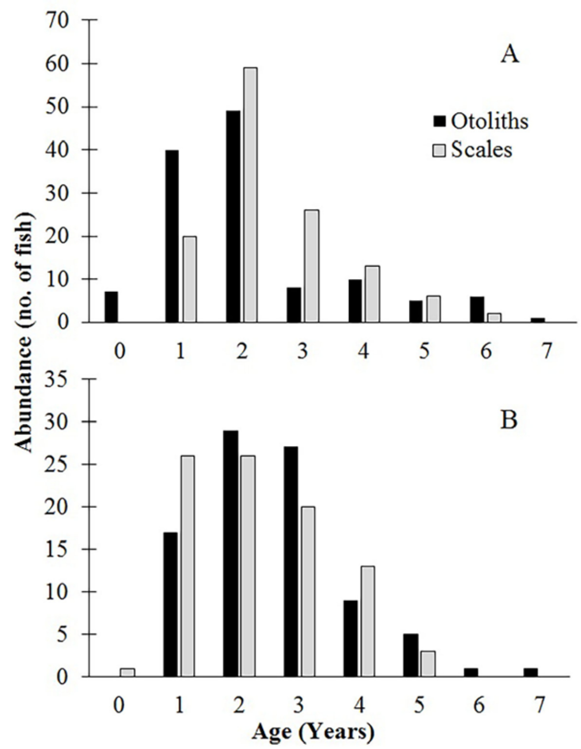

Figure 3. Age-structure histograms for redbreast sunfish (A) and green sunfish (B).

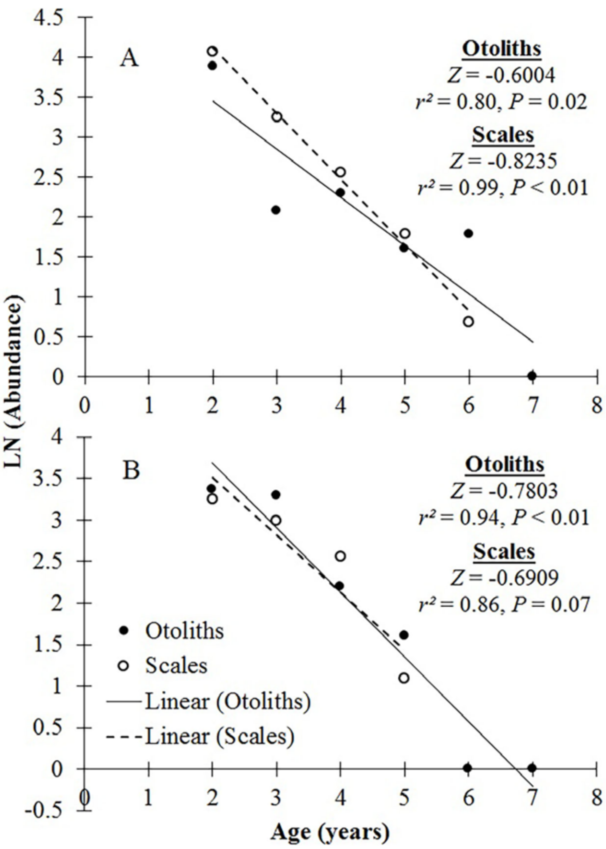

Figure 4. Comparison of catch-curve regressions for both redbreast sunfish (A) and green sunfish (B), with otolith-derived data (solid black symbol, solid trendline) and scale-derived data (open symbol, dashed trendline).

Green Sunfish

A total of 89 green sunfish were collected, varying from 59–145 mm TL and 2.7–56.7 g. Otolith-estimated ages of green sunfish ranged from 1 to 7 years; whereas, scale-estimated ages ranged from 0 to 5 years. Reader agreement for otoliths was 88%, while reader agreement was 79% for scales. Mean CV in otolith age estimates was 3.7%; whereas, mean CV was 6.1% in scale age estimates. Reader confidence was significantly greater for otoliths (mean ± S.E. = 2.32 ± 0.05) than for scales (2.12 ± 0.06, $t = 2.22$, $P = 0.01$). Age estimation bias between readers was not present for otoliths (Figure 1c). However, with scales, reader 2 underestimated fish ages that were assigned an age of 4 by reader 1 (Figure 1d).

Mean estimated ages significantly differed between otoliths (2.58 ± 0.13 years) and scales (2.30 ± 0.12, $t = 2.51$, $P = 0.01$). Scales generally underestimated otolith-assigned ages at ages 3+ (Figure 2b). Differences in age assignments between structures were observed in age structure histograms, with fewer age-5 estimates and no age-6 or 7 estimates being assigned with scales (Figure 3b). The annual survival estimate was 46% with otolith-derived age data, and 50% with scale-derived data (Figure 4b).

Redbreast Sunfish vs. Green Sunfish

Readers were equally confident in estimating age with otoliths for redbreast sunfish (2.42 ± 0.05) and green sunfish (2.32 ± 0.05, $t = -1.31$, $P = 0.19$). However, readers were significantly more confident when estimating age from green sunfish scales (2.12 ± 0.06) than redbreast sunfish scales (1.88 ± 0.05; $t = -3.25$, $P < 0.01$). Age estimation bias was more apparent when aging redbreast sunfish scales than the scales of green sunfish (Figures 1b and 1d).

Discussion

As observed in other studies with perciform fishes, reader agreement was better for otoliths than scales for both redbreast sunfish and green sunfish, and precision was better in otolith age estimates than in scales age estimates. Our results were consistent with those reported by Hoxmeier et al. (2001) for the closely related bluegill sunfish (percent agreement: 90.5% for otoliths, 73% for scales; mean CV: 2.62% for otoliths, 7.14% for scales). Hammers and Miranda (1991) also reported very similar results for the white crappie with reader agreement being 91% for otoliths and 79% for scales. Similar results were also reported for largemouth bass, smallmouth bass (*M. dolomieu*), and yellow perch (*Perca flavescens*) (Maceina and Sammons 2006).

Readers were generally more confident in their estimation of age with otoliths than with scales for both species. Ihde and Chittenden (2002) were also more significantly confident in their reads of otolith sections than reads of scales and dorsal spine sections of the spotted seatrout. In our study, annuli along otolith sections were typically more distinct than the annuli on scales, and false annuli were typically more easily identifiable as accessory checks in otoliths than in scales. Furthermore, the resorption of scales in older individuals may have resulted in the underestimation of ages of older fish (McInerny 2017). For young fish, readers may have misidentified accessory checks for true annuli on scales, leading to the overestimation of age. Coble (1970) suggested that a lack of food, low temperature, disease, low concentrations of dissolved oxygen, and photoperiod may all play a role in the formation of false annuli in sunfish scales. Liao et al. (2013) also reported that scales overestimated the age of young, known-age striped bass and underestimated the age of old fish.

For redbreast sunfish, age estimation bias between readers was very apparent for scales, but little bias was observed for our sectioned otoliths. For the green sunfish, bias between readers was also present for scales, and did not occur for otoliths. Similar findings were reported for largemouth bass, smallmouth bass, and spotted bass, *M. punctulatus* (Long and Fisher 2001) and for striped bass (Liao et al. 2013).

Our study revealed several potential implications of using scales to estimate ages of green sunfish and redbreast sunfish in life history studies and population assessments. First, when using scales to estimate age, longevity was underestimated by one year for redbreast sunfish and by two years for green sunfish. This issue is even more problematic for longer-lived species, like the striped bass (Secor et al. 1995). For both species, mean estimated ages differed between structures for both species. Second, if we assume that the otolith is the more accurate structure for estimating age (as supported by the literature; see Phelps et al. 2017), the use of scales may result in the misidentification of strong and weak year classes, which may confound analyses of environmental drivers of recruitment dynamics. Finally, estimates of annual survival computed from otolith-derived and scale-derived age data may differ, which may affect predictions of population growth and dynamics.

Based on our findings, we recommend that fish managers and conservation biologists avoid using scales to estimate ages of redbreast sunfish and green sunfish. In situations where conditions preclude the sacrificing of fish, efforts should be made to improve overall accuracy and precision when using scales to estimate ages of fish. First, scales are typically more difficult to age than otoliths (McInerny 2017), so extensive training is required for scientists that are new to scale aging. Scales from known-age fish would be useful and are recommended in Quality Assurance-Quality Control (QA-AC) training programs (Buckmeier et al. 2017). Only scientists who are highly experienced and well trained in using scales to estimate fish age should be assigned to such studies. Second, special care should be taken when preparing scales for reading and interpretation. Scales should be thoroughly cleaned of mucous and debris and mounted between clean, unused microscope slides. Finally, scientists are strongly encouraged explore alternative non-lethal methods other than scales (e.g., use of spines or fin rays as aging structures), and compare structures to find the best approach.

Acknowledgments

We thank the School of Science and Technology at Georgia Gwinnett College (GGC) for providing the lab space and resources that supported this research. We thank Dr. Joseph Ametepe for supporting the STEC 4500 (Undergraduate Research) program at GGC.

Literature Cited

Arnold, M. C., L. A. Friedrich, T. T. Lindberg, M. Ross, N. M. Halden, E. Bernhardt, V. P. Palace, and R. T. Di Giulio. 2015. Microchemical analysis of selenium in otoliths of two West Virginia fishes captured near mountaintop removal coal mining operations. Environmental Toxicology and Chemistry 34:1039–1044.

Bacon, E. J. and R. V. Kilambi. 1968. Some aspects of the age and growth of the longear sunfish, *Lepomis megalotis*, in Arkansas waters. Arkansas Academy of Science Proceedings 22:44–56.

Bertschy, K. A. and M. G. Fox. 1999. The influence of age-specific survivorship on pumpkinseed sunfish life histories. Ecology 80:2299–2313.

Besler, D. A. 1999. Utility of scales and whole otoliths for aging largemouth bass in North Carolina. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 53:119–129.

Boxrucker, J. 1986. A comparison of the otolith and scale methods for aging white crappies in Oklahoma. North American Journal of Fisheries Management 6:122–125.

Buckmeier, D. L., P. C. Sakaris, and D. J. Schill. 2017. Validation of annual and daily increments in calcified structures and verification of age estimates. Pages 33–79 *in* M. C. Quist and D. A. Isermann, editors. Age and growth of fishes: principles and techniques. American Fisheries Society, Bethesda, Maryland.

Campana, S. E., M. C. Annand, and J. I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society 24:131–138.

Coble, D. W. 1970. False annulus formation in bluegill scales. Transactions of the American Fisheries Society 99:363–368.

Delp, J. G., J. S. Tillma, M. C. Quist, and C. S. Guy. 2000. Age and growth of four centrarchid species in southeastern Kansas streams. Journal of Freshwater Ecology 15: 475–478.

DeVries, D. R. and R. V. Frie. 1996. Determination of age and growth. Pages 483–512 *in* B. R. Murphy and D. W. Willis, editors. Fisheries Techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Fox, M. G. 1994. Growth, density, and interspecific influences on pumpkinseed sunfish life histories. Ecology 75:1157–1171.

Gautreau, M. D. and R. A. Curry. 2012. Ecology and status of the redbreast sunfish, *Lepomis auritus*, in Yoho Lake, New Brunswick. Northeastern Naturalist 19:653–664.

Hammers, B. E. and L. E. Miranda. 1991. Comparison of methods for estimating age, growth, and related population characteristics of white crappies. North American Journal of Fisheries Management 11:492–498.

Hoxmeier, R. J. H., D. D. Aday, and D. H. Wahl. 2001. Factors influencing precision of age estimation from scales and otoliths of bluegills in Illinois reservoirs. North American Journal of Fisheries Management 21:374–380.

Ihde, T. F. and M. E. Chittenden, Jr. 2002. Comparison of calcified structures for aging spotted seatrout. Transactions of the American Fisheries Society 131:634–642.

Isermann, D. A., M. H. Wolter, and J. J. Breeggemann. 2011. Estimating black crappie age: an assessment of dorsal spines and scales as nonlethal alternatives to otoliths. North American Journal of Fisheries Management 30: 1591–1598.

Klein, Z. B., T. F. Bonvechio, B. R. Bowen, and M. C. Quist. 2017. Precision and accuracy of age estimates obtained from anal fin spines, dorsal fin spines, and sagittal otoliths for known-age largemouth bass. Southeastern Naturalist 16:225–234.

Klumb, R. A., M. A. Bozek, and R. V. Frie. 1999. Validation of the Dahl-Lea and Fraser-Lee back-calculation models by using oxytetracycline-marked bluegills and bluegill × green sunfish hybrids. North American Journal of Fisheries Management 19:504–514.

Kruse, C. G., C. S. Guy, and D. W. Willis. 1993. Comparison of otolith and scale age characteristics for black crappies collected from South Dakota waters. North American Journal of Fisheries Management 13:856–858.

Liao, H., A. F. Sharov, C. M. Jones, and G. A. Nelson. 2013. Quantifying the effects of aging bias in Atlantic striped bass stock assessment. Transactions of the American Fisheries Society 142:193–207.

Long, J.M. and W.L. Fisher. 2001. Precision and bias of largemouth, smallmouth, and spotted bass ages estimated from scales, whole otoliths, and sectioned otoliths. North American Journal of Fisheries Management 21:636–645.

Maceina, M.J., J. Boxrucker, D.L. Buckmeier, R.S. Gangl, D.O. Lucchesi, D.A. Isermann, J.R. Jackson, and P.J. Martinez. 2007. Current status and review of freshwater fish aging procedures used by state and provincial fisheries agencies with recommendations for future directions. Fisheries 32:329–340.

——— and S.M. Sammons. 2006. An evaluation of different structures to age freshwater fish from a northeastern US river. Fisheries Management and Ecology 13:237–242.

McInerny, M.C. 2017. Scales. Pages 127–158 *in* M.C. Quist and D.A. Isermann, editors. Age and growth of fishes: principles and techniques. American Fisheries Society, Bethesda, Maryland.

Osenberg, C.W., E.E. Werner, G.G. Mittelbach, and D.J. Hall. 1988. Growth patterns in bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish: environmental variation and the importance of ontogenetic niche shifts. Canadian Journal of Fisheries and Aquatic Sciences 45:17–26.

Phelps, Q.E., S.J. Tripp, M.J. Hamel, R.P. Koenigs, and Z.J. Jackson. 2017. Choice of structure for estimating fish age and growth. Pages 81–105 *in* M.C. Quist and D.A. Isermann, editors. Age and growth of fishes: principles and techniques. American Fisheries Society, Bethesda, Maryland.

Quist, M.C. and C.S. Guy. 2001. Growth and mortality of prairie stream fishes: relations with fish community and instream habitat characteristics. Ecology of Freshwater Fish 10:88–96.

Regier, H.A. 1962. Validation of the scale method for estimating age and growth of bluegills. Transactions of the American Fisheries Society 91:362–374.

Ross, J.R., J.D. Crosby, and J.T. Kosa. 2005. Accuracy and precision of age estimation of crappies. North American Journal of Fisheries Management 25:423–428.

Sammons, S.M. and M.J. Maceina. 2009. Variation in growth and survival of bluegills and redbreast sunfish in Georgia rivers. North American Journal of Fisheries Management 29:101–108.

Sandow, J.T., D.R. Holder, and L.E. McSwain. 1974. Life history of the redbreast sunfish in the Satilla River, Georgia. Proceedings of the Annual Conference of the Southeastern Association of Game and Fish Commissioners 28:279–295.

Schindler, D.E., S.I. Geib, and M.R. Williams. 2000. Patterns of fish growth along a residential development gradient in north temperate lakes. Ecosystems 3:229–237.

Secor, D.H., T.M. Trice, and H.T. Hornick. 1995. Validation of otolith-based ageing and a comparison of otolith and scale-based ageing in mark-recaptured Chesapeake Bay striped bass, *Morone saxatilis*. Fishery Bulletin 93:186–190.

Spiegel, J.R., M.C. Quist, and J.E. Morris. 2010. Precision of scales and pectoral fin rays for estimating age of highfin carpsucker, quillback carpsucker, and river carpsucker. Journal of Freshwater Ecology 25:271–278.

Tharratt, R.C. 1966. The age and growth of centrarchid fishes in Folsom Lake, California. California Fish and Game 52(1):4–16.

Uzunova, E., B. Velkov, S. Studenkov, M. Georgieva, M. Nikolova, L. Pehlivanov, and D. Parvanov. 2008. Growth, age and size structure of the introduced pumpkinseed (Lepomis gibbosus l.) population from small ponds along the Vit River (Bulgaria). Bulgarian Journal of Agricultural Science 14(2):227–234.

Welch, T.J., M.J. Van Den Avyle, R.K. Betsill, and E.M. Driebe. 1993. Precision and relative accuracy of striped bass age estimates from otoliths, scales, and anal fin rays and spines. North American Journal of Fisheries Management 13:616–620.

Whitledge, G.W., B.M. Johnson, P.J. Martinez, and A.M. Martinez. 2007. Sources of nonnative centrarchids in the upper Colorado River revealed by stable isotope and microchemical analyses of otoliths. Transactions of the American Fisheries Society 136:1263–1275.