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MANAGEMENT BRIEF

Precision and Bias in Aging Northern Pike: Comparisons among Four Calcified Structures

Daniel L. Oele*
Wisconsin Department of Natural Resources, 2801 Progress Road, Madison, Wisconsin 53716, USA

Zachary J. Lawson
Wisconsin Department of Natural Resources, 5291 North State House Circle, Mercer, Wisconsin 54547, USA

Peter B. McIntyre
Center for Limnology, University of Wisconsin–Madison, 680 North Park Street, Madison, Wisconsin 53706, USA

Abstract
Age estimates based on calcified structures are central to the analysis and management of fish populations. A variety of structures have been used in studies of Northern Pike *Esox lucius* despite limited data on whether the structures provide comparable results. We quantified precision and bias of ages estimated from cleithra, otoliths, anal fin rays, and scales of Northern Pike sampled in tributaries of Green Bay, Lake Michigan. For three independent readers, the precision (CV) of age estimates did not significantly differ among otoliths, cleithra, and anal fin rays but was significantly lower for scales than for the other structures. Similarly, partial agreement among readers was greater than 90% for ages estimated from otoliths, cleithra, and anal fin rays, whereas partial agreement was 76% for scale-based ages. We discuss the tradeoffs associated with precision and bias for each structure in the context of reader experience, fish age, and management goals. We recommend that when fish mortality is not a concern, otoliths or cleithra should be used to achieve high-precision aging of Northern Pike. For strictly nonlethal sampling scenarios, the anal fin rays of Northern Pike will yield more precise age estimates than scales.

Age estimation is a cornerstone of fisheries science, enabling analysis of both individual growth and population age structure. Many calcified structures include periodic growth intervals that can be used to estimate a fish's age (Robillard and Marsden 1996). Each type of structure has advantages and limitations, and many studies have investigated which structure offers the most accurate, precise, and unbiased age estimates for a particular species (Maceina et al. 2007). For instance, comparative studies favor sagittal otoliths in estimating the ages of Walleyes *Sander vitreus* (Isermann et al. 2003 and references therein), but fin rays are recommended for aging Bluehead Suckers *Catostomus discobolus* and Flannelmouth Suckers *C. latipinnis* (Quist et al. 2007). In contrast, opercular bones are recommended for aging Largemouth Bass *Micropterus salmoides* and Smallmouth Bass *M. dolomieu* (Sotola et al. 2014). Collectively, these studies indicate a need for species-specific assessment of which structure provides the most robust age data for purposes of research and management.

The Northern Pike *Esox lucius* is a popular sport fish in much of the northern hemisphere, and ages of Northern Pike have been estimated from at least four separate calcified structures: otoliths, anal fin rays, cleithra, and scales (Frost and Kipling 1959; Casselman 1974; Babaluk and Craig 1990; Faust et al. 2013). Each of the aforementioned studies has compared multiple structures from the same individual fish, but systematic comparisons of precision and bias among all four structures have not been conducted. Simultaneous comparisons within a single study are important because they control for potential differences arising from observer biases or from variation in growth trajectories among study populations.

*Corresponding author: daniel.oele@wisconsin.gov
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In the present study, we tested whether among-reader precision varied among structures that are used to estimate the age of Northern Pike. We directly compared all four structures from the same individual fish collected in tributaries of Green Bay (Lake Michigan), and we used independent data from three observers to quantify precision and bias for each structure. In addition, we evaluated whether reader experience influenced the degree of consistency in age estimation among structures. The results of these comparisons assist in resolving which structures provide the most consistent age estimates for situations requiring lethal versus nonlethal sampling of Northern Pike.

METHODS

Sample collection and preparation.—Postspawn adult Northern Pike were collected from tributaries along the west shore of Green Bay during spring 2011 and 2012 by using a variety of sampling gears (fyke nets, dip nets, and angler donations). Sagittal otoliths, scales, anal fin rays, and cleithra were extracted in the field. In the laboratory, otoliths were mounted in EpoxiCure resin (Buehler, Lake Bluff, Illinois), sectioned along the transverse axis, affixed to glass microscope slides with CrystalBond adhesive, and polished by using increasingly fine aluminum oxide powder until the core and annuli were clearly visible under a dissecting microscope. Anal fin rays were extracted after cutting off the first several fin rays and associated tissue along the base of the fin by using a diagonal cutter. The first ray (i.e., the anteriormost ray, nearest the vent) was isolated and prepared in the same manner as the otoliths. Cleithra were prepared in accordance with Faust et al. (2013); whole cleithra were immersed in nearly boiling water, scrubbed until all soft tissue was removed, and dried. A minimum of four scales were removed above the lateral line near the dorsal fin of each fish. All scales were pressed (Ann Arbor roller) onto sheets of clear cellulose acetate plastic. The single clearest, most complete scale impression for each fish was provided to all readers for aging.

Three independent readers estimated each fish's age based on examination of each structure. Readers 1 and 2 had experience in estimating fish ages by use of otoliths and scales, but they had little experience with cleithra or fin rays. Reader 3 had no previous experience in estimating fish age, but that reader received one training session and then practiced with each structure before recording data for the present study. Each reader examined each aging structure independently and without knowledge of fish sex or length. Pressed scales, sectioned otoliths, and sectioned fin rays were read by using a dissecting microscope; cleithra were read without magnification, either in natural light or via illumination from below using a fiber optic light source within a darkened box.

Analyses.—We constructed age–bias plots to test for differences in age estimates among readers and among structures. To assess the precision of age estimates among structures, we computed CVs across all individual fish (Chang 1982; Campana et al. 1995), and we tabulated the degree of agreement among readers (complete or partial agreement across the three independent readers) for each fish and each structure (Isermann et al. 2010; Faust et al. 2013). To visualize reader agreement, the cumulative frequency distribution of age estimates for each structure was compared between pairs of readers. To explicitly determine which factors influenced precision (i.e., CV), we used multiple linear regression of fish length (TL, mm), structure, and sex as predictors of the CV in age estimates. To quantitatively examine differences in CV, we compared the mean CV among structures by using ANOVA, with all pairwise comparisons based on Tukey's honestly significant difference test.

Cleithra are believed to produce the most the reliable age estimates for Northern Pike (Casselman 1979; Babaluk and Craig 1990; Laine et al. 1991; Faust et al. 2013). Therefore, by using only individual fish with partial agreement across observers for cleithrum-based ages, we constructed age–bias plots comparing the results from cleithra with the results from the remaining structures.

RESULTS

Northern Pike used in the study ranged in size from 290 to 1,010 mm TL (mean = 570 mm TL; Figure 1); the sample consisted of 96 males, 91 females, and 3 fish for which sex was indeterminate. Across all readers, age estimates ranged from 2 to 10 years (Figure 2). Multiple linear regression showed that CV increased (precision decreased) with scale-based age estimates ($P < 0.001$) and decreased with fish TL ($P = 0.02$). There was no evidence of differences in precision between males and females or among structures other than scales ($P > 0.20$).

Across ages and structures, readers 1 and 2 (experienced readers) had greater agreement with each other than with reader 3 (the least experienced reader; Figure 3). Reader 3 deviated from the other readers more often for age estimates from fin rays, cleithra, and scales than for otolith-based estimates. The largest differences among readers were for scales (Figures 2, 3). Precision of age estimates was similar between otoliths and cleithra (lowest CVs), slightly lower for anal fin rays, and lowest for scales (highest CVs; Table 1). Otoliths, cleithra, and fin rays had similar frequencies of partial agreement (92, 94, and 93%, respectively), whereas scales had a substantially lower partial agreement frequency (76%). Likewise, frequencies of complete agreement were similar among otoliths, cleithra, and anal fin rays (36, 39, and 33%, respectively) and were greater for those structures than for scales (17%). The ANOVA indicated differences among structures in the mean CV of age estimates across readers; the CV for scale ages was higher than the CVs for ages from the other three structures (Tukey's honestly significant difference test: $P < 0.05$). Thus, scales exhibited lower precision of age

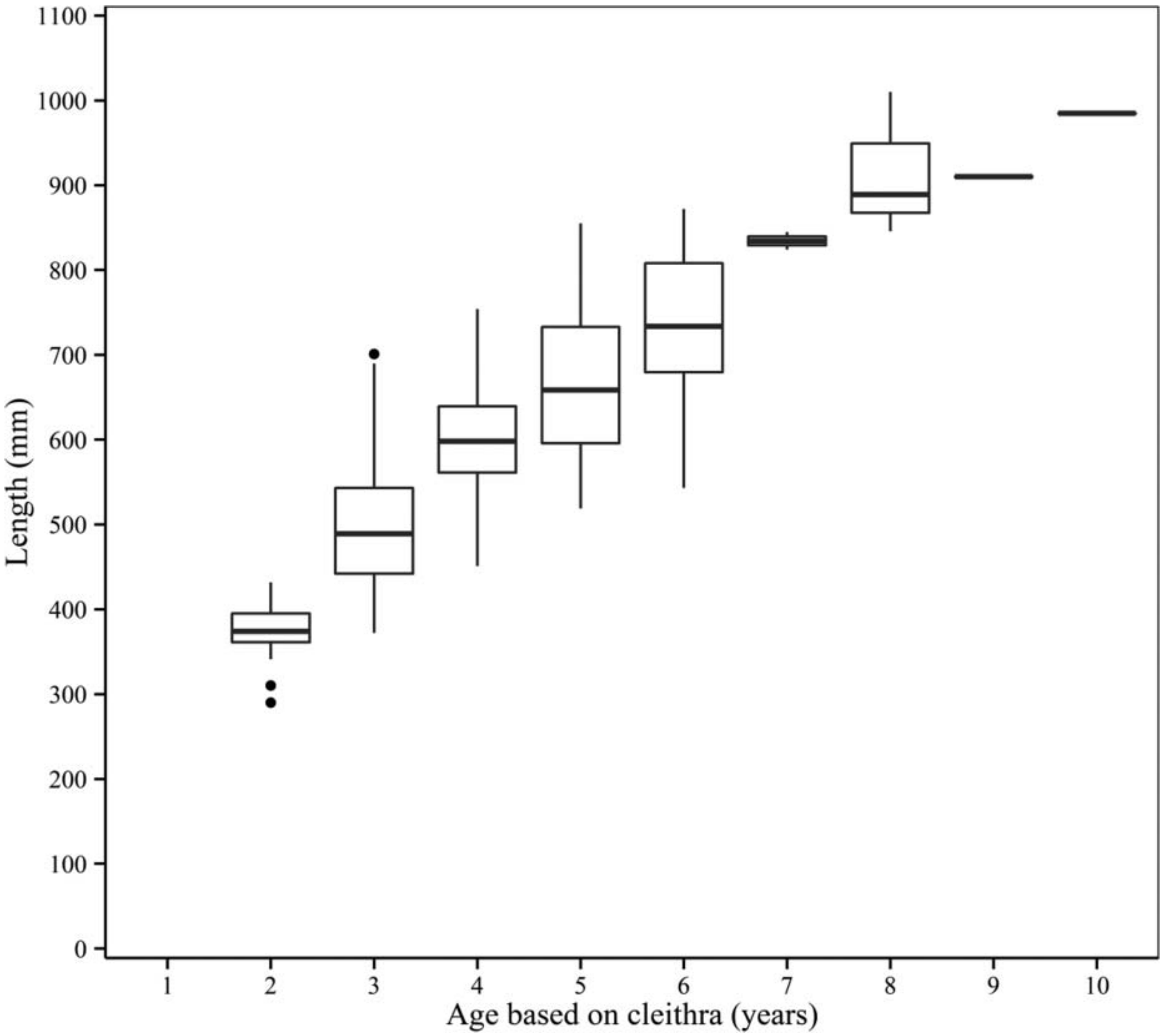

FIGURE 1. Box plot of TL at age (years; cleithrum-based estimates) for Northern Pike (n = 190) sampled from Green Bay (Lake Michigan) tributaries in 2011–2012. The lower and upper boundaries of the boxes indicate the 25th and 75th quantiles, respectively. Lines within the boxes indicate the medians. Whiskers indicate 1.5× the interquartile range, while black dots represent outliers.

estimates and lower reader agreement than otoliths, cleithra, or anal fin rays (Table 1).

The comparison of age estimates derived from otoliths, anal fin rays, and scales to age estimates from cleithra elucidated patterns of differences among structures and among readers. Fish age appeared to influence the consistency of age estimates across structures, and that pattern was consistent across readers. For fish with age estimates of 2–5 years based on cleithra, all other structures indicated equivalent ages. In contrast, fish with cleithrum-derived ages of 6 years or more deviated from the 1:1 equivalency line (Figure 4). Scales consistently underestimated cleithrum-based ages, as the mean deviation from the equivalency line was 2.20 years and individual deviations were as extreme as 4 years. Anal fin ray estimates exhibited closer alignment (mean deviation = 1.39 years) with cleithrum-based ages than did scales; otoliths were the most precise structure when compared with ages estimated from cleithra (mean deviation = 0.78 years; Figure 4).

DISCUSSION

Comparisons of age estimates obtained using four calcified structures from Northern Pike indicated that the choice of aging structure can strongly affect data quality, similar to findings from studies of other fish species. Cleithra, otoliths, and anal fin rays of Northern Pike each provided age estimates that were precise and likely to be highly accurate, whereas scales did not. Moreover, the precision and bias of age estimates from any given structure depended upon both the reader experience level and fish age.

Several previous studies have examined the precision and accuracy of Northern Pike ages derived from a subset of

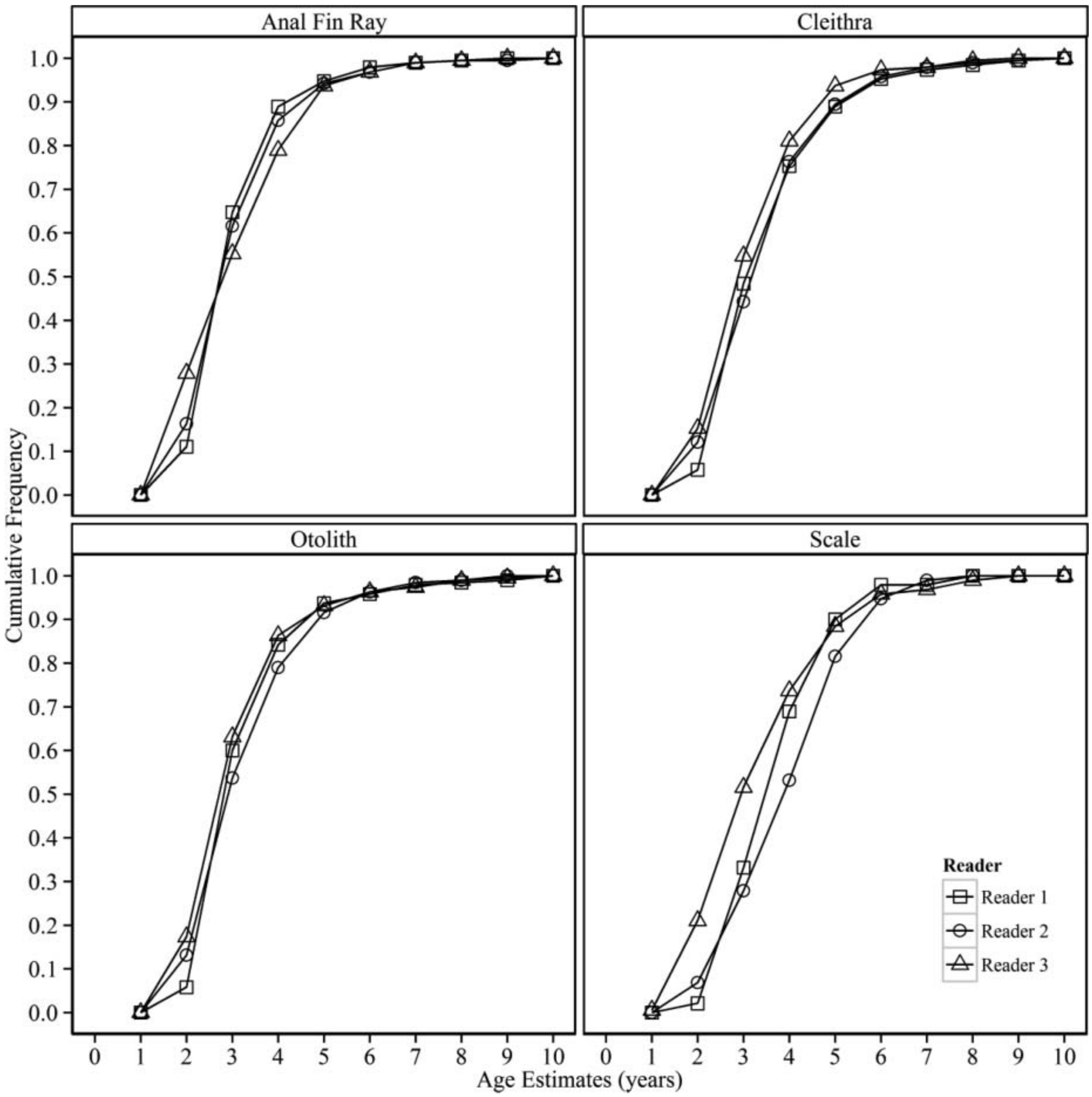

FIGURE 2. Cumulative frequency diagrams depicting Northern Pike age estimates based on four calcified structures examined by three independent readers. Fish (n = 190) were sampled from Green Bay (Lake Michigan) tributaries in 2011–2012; all four structures from each individual were used for age determinations.

calcified structures. Casselman (1974) demonstrated that cleithra yielded accurate ages, and subsequent studies focused on the precision of ages from other individual structures in comparison with cleithrum-based ages. Babaluk and Craig (1990) determined that the reliability of anal fin rays was comparable to that of cleithra. Laine et al. (1991) found that ages estimated from scales and cleithra were comparable for fish up to age 10. Faust et al. (2013) demonstrated similar ages from otoliths and cleithra. Our study is the first to compare the precision of Northern Pike age estimates obtained from all four structures; the results clearly show that cleithra, otoliths, and anal fin rays provide precise age estimates, whereas scales suffer from both low precision and high reader bias in age estimation.

Reader experience emerged as a key issue in estimating the ages of Northern Pike. Agreement in age estimates from the two experienced readers (readers 1 and 2) was strong for all structures except scales. Even the inexperienced reader with minimal training (reader 3) exhibited comparable agreement with readers 1 and 2 for otolith- and cleithrum-derived ages, whereas reader 3 deviated more when estimating ages from anal fin rays and scales. Using otoliths and cleithra, experienced readers can produce consistent and precise estimates for Northern Pike of all ages; the use of anal fin rays is a viable nonlethal option that is less influenced by reader experience than the use of scales.

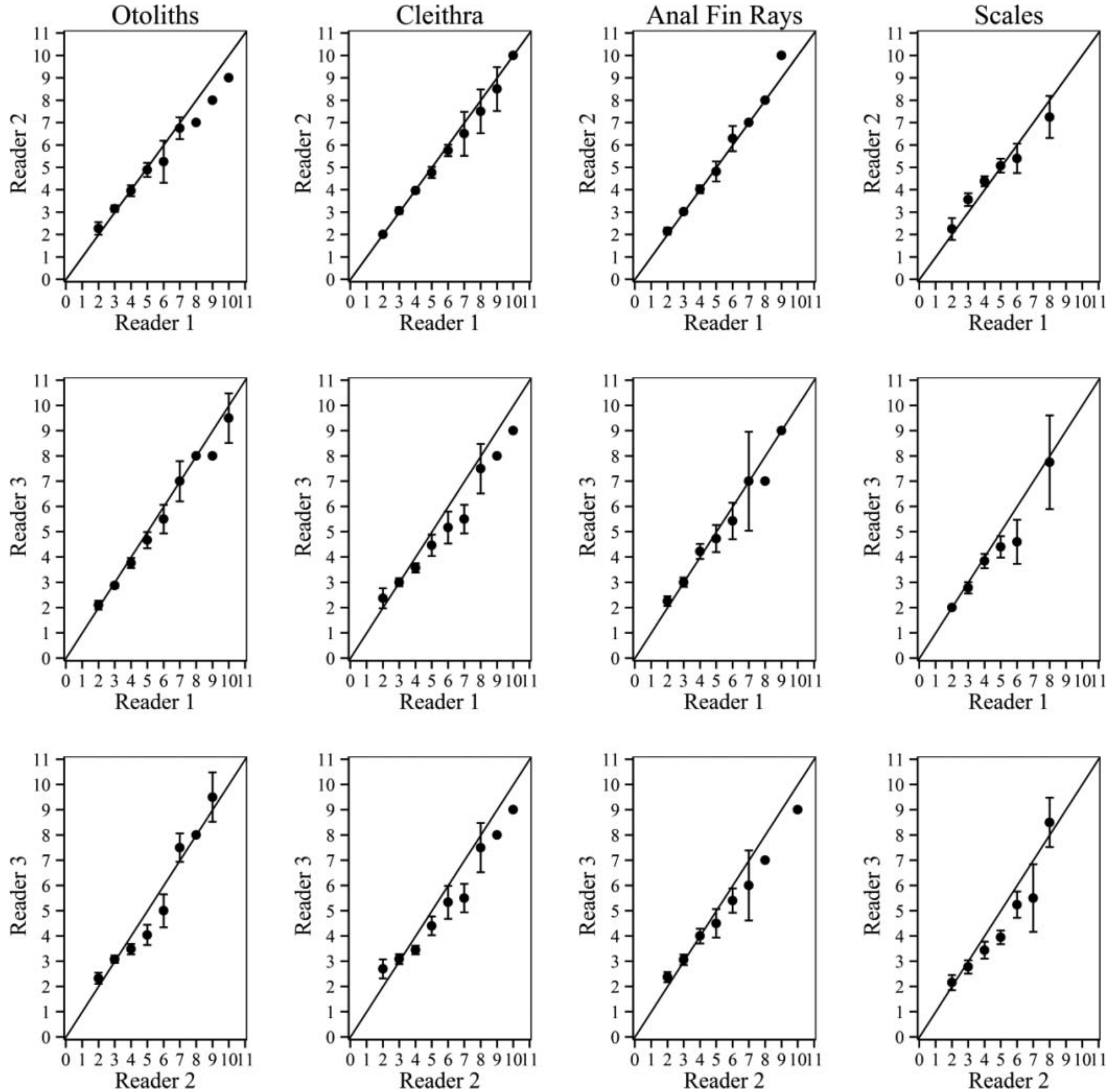

FIGURE 3. Age–bias plots comparing Northern Pike age estimates (years) obtained by the three independent readers based on their examination of four calcified structures. Fish ($n = 190$) were sampled from Green Bay (Lake Michigan) tributaries in 2011–2012; all four structures from each individual were used for age determinations. Error bars represent 95% confidence intervals; age estimates for $n = 1$ have no error bars.

In addition to reader experience, fish age influenced age estimates in predictable ways for each structure. Thus, the level of reader experience and the choice of structure should be carefully considered when estimating ages for Northern Pike populations with a high proportion of age-6 and older individuals. Otoliths provided the least bias and most precise estimates for this demographic, and anal fin rays were also adequately precise for most applications. Our results suggest that when scales are examined, even experienced readers exhibit strong bias in age estimates for fish older than 6 years, indicating that low accuracy is unavoidable in studies where only scales are available for analysis. For Northern Pike that

TABLE 1. Summary statistics for reader agreement in Northern Pike age estimates based on four calcified structures examined by three independent readers (complete agreement = all three readers agreed on the age estimate; partial agreement = at least two of the three readers agreed). Fish (n = 190) were sampled from Green Bay (Lake Michigan) tributaries in 2011–2012; all four structures from each individual were used for age determinations (i.e., n = 190 for each structure). The mean CV for a given structure was calculated based on all individual fish (mean SE is shown in parentheses).

Structure	Lethality	Mean CV × 100 (SE)	Complete agreement (%)	Partial agreement (%)
Otolith	Lethal	11.58 (0.002)	0.36	0.92
Cleithrum	Lethal	12.37 (0.089)	0.39	0.94
Anal fin ray	Nonlethal	14.12 (0.006)	0.33	0.93
Scale	Nonlethal	18.63 (0.006)	0.17	0.76

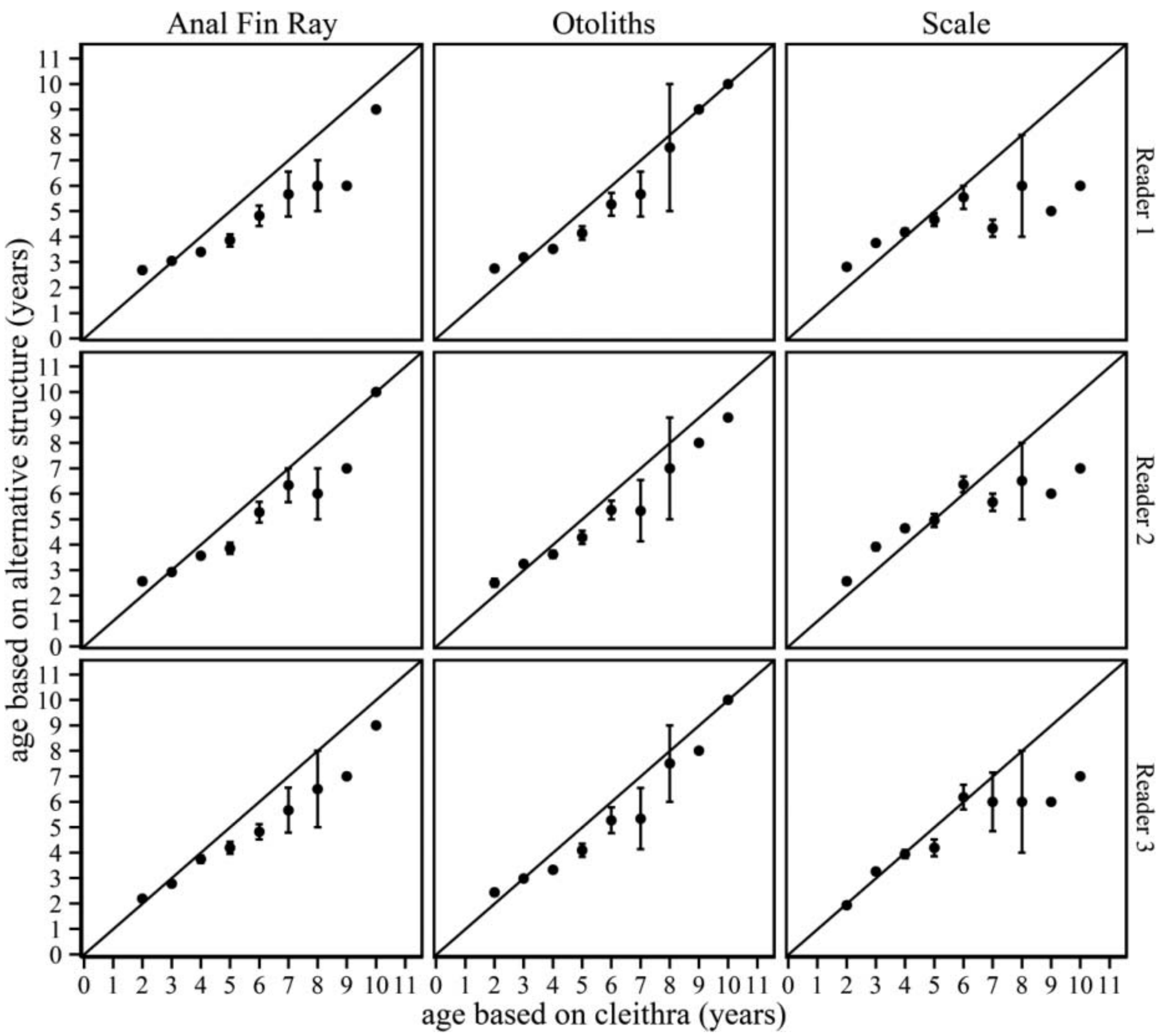

FIGURE 4. Age–bias plots comparing Northern Pike age estimates based on otoliths, anal fin rays, or scales with cleithrum-based ages produced by each reader (for samples with partial reader agreement: n = 179). Fish were sampled from Green Bay (Lake Michigan) tributaries in 2011–2012; all four structures from each individual were used for age determinations. Error bars represent 95% confidence intervals; age estimates for n = 1 have no error bars.

were younger than age 5, scales and anal fin rays performed similarly across readers and in comparison with cleithra and otoliths.

Currently, most management agencies rely on scales to estimate age for Northern Pike and other esocids (Maceina et al. 2007). Scales are often used because they are nonlethal and relatively easy to process (Zymonas and McMahon 2009). Although we found that scales provided low among-reader precision, we recognize that lethal sampling for cleithra and otoliths is not always compatible with research goals and management constraints. Thus, when only nonlethal methods are acceptable, we recommend the use of anal fin rays rather than scales. It is also important to consider differences in field and laboratory processing times among the various structures; the efficiency of obtaining age estimates depends on differences in the time required to extract, mount, cut, polish, and otherwise handle each structure. The pace of these procedures varies widely with experience but is also inherently different among the structures (Isermann et al. 2003). In our experience, the rank order of preparation times is as follows: sectioned otoliths $\geq$ sectioned anal fin rays $\gg$ cleithra $>$ scales. Therefore, fisheries professionals must balance a diverse suite of tradeoffs associated with lethal versus nonlethal methods, sample processing time, and the reliability of age estimates.

We were unable to assess the absolute accuracy of age estimates in the present study because we did not have access to known-age Northern Pike. Future studies should prioritize the inclusion of known-age samples to assess accuracy across all available calcified structures; even having access to samples from a few known-age individuals could lend great insight. It also remains to be seen whether the accuracy and precision of age estimates differ much among conspecific fish populations from different regions. Both the accuracy and the precision of ages are expected to be greater for fast-growing fish than for slow-growing conspecifics (Hoxmeier et al. 2001); therefore, comparisons of populations from across a species' latitudinal range would be informative.

Our results indicated that precision (CV) improved with fish TL. This result is less common than reports that precision decreases in relation to fish length (e.g., Sharp and Bernard 1988; Hoxmeier et al. 2001); our result could be attributable to the relatively young ages of Northern Pike examined in this study. Sources of reader disagreement include variation in identifying the first annulus (Isermann et al. 2003) and discarding false annuli, both of which reduce the reliability of age estimates (Frost and Kipling 1959; Campana 2001). In our study, these distinctions were most difficult with samples from the youngest Northern Pike, which were undergoing ontogenetic diet shifts combined with the rapid growth that is characteristic of early life, but such problems were rare for samples from larger fish. Although our sample included Northern Pike larger than 890 mm TL (via angler donations), collection permit restrictions prevented us from systematically sampling the largest and oldest portion of the population. However, in our 2 years of field work conducted during spring migrations in six watersheds, we encountered only 16 fish (out of 252 fish sampled) that were larger than 890 mm TL. It is possible that the Northern Pike population in Green Bay sustains sufficient growth rates to allow detection of clear annuli throughout much of their life span; we did not observe the substantial annulus crowding that is typical for the outer annuli of the oldest individuals in a population due to decreases in growth rate with age (e.g., Welch et al. 1993; Koenigs et al. 2015). We suspect that the observed patterns of among-reader precision and bias would persist into the oldest segments of the population, but to confirm this, it would be ideal to include older individuals.

Our study provides the most comprehensive guidance to date for the selection of calcified structures to obtain consistent age estimates for Northern Pike. In general, we recommend the use of anal fin rays, otoliths, or cleithra. Since cleithra are highly accurate (Casselman 1974) and since otoliths and fin rays are roughly equivalent in precision and bias, any of these three structures can be used to provide robust age estimates. Under circumstances in which fish mortality is not a concern, we recommend the use of otoliths or cleithra to maximize aging precision. However, for strictly nonlethal sampling scenarios, we strongly suggest the examination of anal fin rays rather than scales. Whenever possible, the expected demographics of the fish population and the experience level of readers should also be considered when selecting a calcified structure for age estimation.

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