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MANAGEMENT BRIEF

An Assessment of Calcified Structures for Estimating Northern Pike Ages

Brian G. Blackwell,* Todd M. Kaufman, and Tyrel S. Moos
South Dakota Department of Game, Fish and Parks, 603 East 8th Avenue, Webster, South Dakota 57274, USA

Abstract
An important component of effective fisheries management is estimating fish ages. Age estimates can be used to estimate recruitment, relative abundance of age-groups, total mortality, and growth. Because of difficulty in estimating Northern Pike *Esox lucius* ages using scales and whole cleithra, we compared precision and bias of age estimates from whole cleithra, sectioned cleithra, metapterygoid bones, otoliths, and scales. Metapterygoid bones and sectioned cleithra represent two novel structures for estimating ages in North America, and the assessment of otoliths is limited. Complete agreement and consensus agreement rates were greatest for otoliths, sectioned cleithra, and metapterygoid bones. Otoliths provided the most precise age estimates and whole cleithra were the least precise. Consensus age estimates for sectioned cleithra were lower than whole cleithrum estimates. Consensus age estimates from sectioned otoliths were lower than mean age estimates from scales, sectioned cleithra, and whole cleithra, and *y*-intercepts from the age-bias plots were significantly different than zero. We suggest that otoliths, sectioned cleithra, and metapterygoid bones can be used to estimate Northern Pike ages, but recommend the use of sectioned otoliths because they had the highest precision and otoliths have become a common means of estimating ages for many species.

As an apex predator, Northern Pike *Esox lucius* populations are important in regulating the ecology of aquatic systems through top–down control (He and Kitchell 1990; Venturelli and Tonn 2006). Crane et al. (2015) suggested that special attention is needed to reduce selective harvest of larger Northern Pike because of their role as keystone predators in maintaining stable and productive fish communities. In addition to their ecological role, Northern Pike populations often provide important recreational fisheries across their range (Margenau et al. 2003; Crane et al. 2015). Prior to the 1980s, most Northern Pike populations received no special management (Crane et al. 2015). However, since the 1980s increased efforts have been devoted to managing Northern Pike populations because of their importance in fish community dynamics and the recreational fisheries they provide (Diana and Smith 2008; Margenau et al. 2008; Pierce 2010; Crane et al. 2015).

An important component of effective fisheries management is estimating ages of sampled fish. Age estimates can be used to assess recruitment, relative abundance of age-groups, total mortality, and growth when age is combined with size information (Isely and Grabowski 2007). A variety of calcified body parts have been used to estimate ages of Northern Pike across their distribution, including opercular bones (Frost and Kipling 1959), cleithra (Diana 1983; Laine et al. 1991; Neumann et al. 1994), fin rays (Babaluk and Craig 1990; Oele et al. 2015), scales (Laine et al. 1991; Headrick and Carline 1993; Pierce and Tomcko 2003), metapterygoid bones (Sharma and Borgstrøm 2007), and otoliths (Rydell et al. 2008; Faust et al. 2013; Oele et al. 2015). A survey of state and provincial fisheries agencies found that cleithra, fin rays, scales and otoliths were used by agencies to estimate ages of esocids in North America (Maceina et al. 2007).

Scales and cleithra have been the most commonly used structures for estimating Northern Pike ages (Maceina et al. 2007). Unfortunately, obtaining reliable age estimates from scales has proven difficult (Mann and Beaumont 1990; Oele et al. 2015) and estimates from whole cleithra can be influenced by reader experience (Faust et al. 2013; Oele et al. 2015); this difficulty in estimating ages can result in low precision and high reader bias. Campana (2001) stated that precision is the reproducibility of individual measurements on a given structure and bias denotes a systematic difference in the proximity of age estimates to a true value. Metapterygoid bones, sectioned cleithra, and sagittal otoliths, which have

*Corresponding author: brian.blackwell@state.sd.us
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received little to no evaluation, provide potential alternatives to using scales and whole cleithra for estimating Northern Pike ages.

Metapterygoid bones are the recommended structure in Sweden for estimating Northern Pike ages (Appelberg 2000). At Lake Arugan, Norway, annuli were found to form on metapterygoid bones and back-calculated lengths from measured annuli were similar to fish lengths at corresponding ages (Sharma and Borgstrøm 2007). The use of metapterygoid bones appears to be minimal; i.e., we could find no published studies on the use of metapterygoid bones in North America and just a few from other regions.

Although whole cleithra are commonly used to estimate Northern Pike ages, to our knowledge there are no published studies on the use of sectioned cleithra for estimating Northern Pike ages. Sectioning has been used to aid in identifying annuli on otoliths and fish spines (Quist et al. 2012). The cleithrum of older or slow-growing fish may become thickened in the heel region or the inner rib may grow over the heel region. When this occurs, early annuli can become obscured making age estimation difficult. Annuli of old or slow-growing fish can crowd on the cleithrum edge making it difficult to recognize individual annuli (Laine et al. 1991). Sectioning the cleithrum may make it easier to identify annuli and improve precision of age estimates.

The use of sagittal otoliths (otoliths, hereafter) to estimate Northern Pike ages has been limited, only 4 of 28 state and provincial fisheries agencies reporting estimating the ages of esocids with otoliths (Maceina et al. 2007). Only two published studies have compared otolith age estimates to other commonly used age estimation structures (Faust et al. 2013; Oele et al. 2015). Otoliths may have an advantage for estimating Northern Pike ages because they continue to grow and record cyclic seasonal growth patterns even in populations that contain slow-growing or old fish (Casselman 1996). Otoliths have not been validated for Northern Pike but Gerdeaux and Dufour (2012) indicated that 1 year of otolith growth for Northern Pike comprises adjacent translucent and opaque zones. The limited use of otoliths to estimated ages of esocids may be related to the processing time and equipment required to prepare otoliths for viewing (i.e., Northern Pike otoliths are opaque and require sectioning to be able to view annuli).

Scales and whole cleithra have commonly been used for Northern Pike age estimation, but with uncertainty. As a result, other structures have been evaluated for their utility in estimating Northern Pike ages. Metapterygoid bones and sectioned cleithra represent two novel options to estimate Northern Pike ages in North America, and sectioned otoliths have received only limited use. We therefore tested the precision and agreement of age estimates among readers for scales, whole cleithra, sectioned cleithra, sectioned otoliths, and metapterygoid bones. We also compared age estimates of whole cleithra and sectioned otoliths with estimates from all other structures. Our assessment of metapterygoid bones represents the first in North America, our assessment of sectioned cleithra is the first to evaluate for age estimation, and our otolith age estimates can be compared with previous studies to determine their usefulness.

METHODS

Northern Pike were collected from 19 lakes in northeast South Dakota during 2012 and 2013. Fish were collected using a variety of gears, including gill nets, modified fyke nets, electrofishing, and angling. Because we were only interested in the ability of the various structures to estimate ages, we were not concerned with potential bias associated with using multiple collection gears. Fish were measured for total length (mm) before removing the structures to be used in age estimation.

Scale samples were taken from above the lateral line just anterior to the dorsal fin (Laine et al. 1991) and stored in individually labelled coin envelopes. Otoliths were removed, wiped clean, and stored in individually labelled plastic vials. Cleithra were removed and placed for a short time in boiling water to aid in removing connected flesh. The skull of each fish was placed in boiling water for several minutes for easier removal of the metapterygoid bones; the dissected metapterygoid bones were cleaned of attached flesh. Both the cleithra and metapterygoid bones were allowed to air dry before placing in individually labelled coin envelopes.

The right otolith and a portion of the heel region of the right cleithrum were mounted in two-part epoxy (EpoxiCure, Buehler, Lake Bluff, Illinois) and upon hardening each structure was sectioned (0.6 mm) using an Isomet low-speed saw (Buehler, Lake Bluff, Illinois). Several sections of each structure were made and the sections were stored in individually labeled plastic vials. Otolith sections were made through the dorsoventral plane (i.e., transverse section) to ensure that that the nucleus was present and cleithra were sectioned to include the origin.

Scale samples were placed between two glass slides and otolith and cleithrum sections were mounted on glass slides to view. Otolith and cleithrum sections were sanded with 600-grit sandpaper and immersion oil was applied when necessary to improve clarity. Scales, otolith sections, and cleithrum sections were viewed with a stereo microscope (UNITRON Z850; Commack, New York) using transmitted light through a dark-field attachment, and a digital image of each structure from each fish was recorded with a Motic 5.0 MP camera (Richmond, British Columbia) mounted on the trinocular body of the stereo microscope. Whole cleithra were submersed in water over a black background and metapterygoid bones were placed on a black background to view; both structures were viewed with the aid of a magnifying lamp.

Annuli on scales were defined as those areas where circuli exhibit “cutting over” (Figure 1; Quist et al. 2012). The opaque bands present on otoliths were counted as annuli

FIGURE 1. Photographs of metapterygoid bone, scale, sectioned otolith, whole cleithrum, and sectioned cleithrum used to estimate ages of Northern Pike collected from 19 lakes in northeast South Dakota during 2012 and 2013.

(Quist et al. 2012). Readers identified annuli on both whole and sectioned cleithra using the description provided by Casselman (1996). Specifically, annuli are translucent zones usually discernable in the posterior and lateral regions of the cleithrum and pseudoannuli only appear in the anterior region. Dense white bands on metapterygoid bones were considered annuli as described by Sharma and Borgstrøm (2007).

Two readers independently estimated the age for each structure without knowledge of fish capture date, location, biological information (e.g., size or sex), and estimated age for other structures. If age estimates differed between the two readers, a third reader then also estimated the age. If two of the three readers agreed on an age estimate, then a consensus age was reached (Isermann et al. 2010). All three readers were experienced at estimating fish ages using scales and otoliths, but experience with estimating ages from cleithra and metapterygoid bones was limited to approximately 250 fish for each reader. Prior to estimating ages, all readers familiarized themselves and came to an agreement on what would be an annulus on each structure based on descriptions available in the literature, e.g., cleithrum (Casselman 1996), metapterygoid bone (Sharma and Borgstrøm 2007).

Coefficient of variation (Chang 1982), percent complete agreement (both reader 1 and 2 agree on age estimate; Isermann et al. 2010), percent agreement within 1 year for reader 1 and 2 (Stolarski and Sutton 2013), and consensus agreement (two of three readers agree on age estimate; Isermann et al. 2010) were used to evaluate the precision of age estimates for each structure. We used ANOVA to test whether CV estimates were significantly different across structures. When significant differences (α = 0.05) in ANOVA were identified, Tukey's honestly significant difference test was used to identify where differences occurred. Chi-square analysis was used to test whether the percent complete agreement, within 1 year, and consensus agreement were significantly different across structures. To maintain an experimentwise error rate of $\alpha = 0.05$ for chi-square tests, the significance level for each comparison was determined by dividing 0.05 by the number of comparisons (Neumann and Allen 2007).

Age-bias plots (Campana et al. 1995) were constructed for each structure to explore whether bias existed between readers 1 and 2. Means of estimated ages ($n \geq 3$) with 95% confidence intervals (CIs) for reader 2 were plotted against reader 1 estimated ages for each structure. The 95% CIs were used to determine whether the estimated mean ages included the 1:1 equivalency line between readers. To examine bias between readers, we tested whether the y-intercept differed from 0 or the slope differed from 1 in the age-bias plots (Campana et al. 1995). We used linear regression to determine whether the intercepts of age-bias plot best-fit lines were significantly different from 0 or the slopes of the age-bias plot lines were significantly different from 1 because a slope other than 1 or an intercept different from 0 may indicate a bias between readers (Campana et al. 1995). Age-frequency histograms based on consensus (i.e., two readers agree) age estimates were created for each structure. The Kolmogorov–Smirnov two-sample test was used to test for differences in the estimated age distribution across the five structures.

Age-bias plots were constructed by plotting the means of age estimates ($n \geq 3$) with 95% CIs for sectioned otoliths, metapterygoid bones, sectioned cleithra, and scales against whole cleithrum age estimates because whole cleithrum annuli have been validated (Babaluk and Craig 1990; Laine et al. 1991). We also compared consensus ages of sectioned otoliths with consensus ages for other structures by constructing age-bias plots of mean age estimates ($n \geq 3$) with 95% CIs for whole cleithra, metapterygoid bones, sectioned cleithra, and scales against sectioned otolith age estimates. The slope of each relationship was tested to determine if it significantly varied from 1, and the intercept of each age-bias plot linear regression was tested to determine if it was significantly different from 0. All statistical tests were completed with Systat 13 (Systat Software, Inc., Richmond, California) with a significance level of $\alpha = 0.05$.

RESULTS

We collected 243 Northern Pike ranging from 265 to 920 mm TL. Consensus age estimates from whole cleithra and metapterygoid bones ranged from 0 to 10, estimates from sectioned cleithra and scales ranged from 0 to 11, and sectioned otolith estimates ranged from 0 to 12 (Figure 2). Most (>82%) consensus age estimates were age 5 or less for all structures. Consensus age estimate distribution for scales differed significantly from metapterygoids ($P = 0.001$), sectioned otoliths ($P < 0.001$), sectioned cleithra ($P = 0.032$), and whole cleithra ($P = 0.021$); age distribution for sectioned cleithra differed significantly from that of metapterygoids ($P = 0.042$) and sectioned otoliths ($P = 0.004$).

Reader Agreement

Significant differences in CV ($F_{4,\ 1210} = 29.27$, $P < 0.001$) were identified across structures (Table 1). Sectioned otoliths had the lowest mean CV (4.0, SE = 0.7) and whole cleithra had the highest (17.8, SE = 1.2). The CV for whole cleithra was significantly higher than that of other structures. Scales had the next highest mean CV, which was significantly different from the other structures. Otolith CV values were significantly lower than metapterygoids but similar to sectioned cleithra.

Agreement rates varied (Table 1). Percent complete agreement between readers 1 and 2 varied significantly across structures ($\chi^2 = 133.3$, df = 4, $P < 0.001$). Otoliths had the highest complete agreement (81.9%), and whole cleithra had the lowest (38.7%). Sectioned cleithra and metapterygoids had lower complete agreement than otoliths, but their percent agreement was significantly higher than that of scales or whole cleithra. Similar to complete agreement, agreement rates within 1 year differed significantly across structures ($\chi^2 = 117.32$, df = 4, $P < 0.001$). Age estimates for readers 1 and 2 were within 1 year of each other for 97.9% of the sectioned otoliths and 95.5% of sectioned cleithra but fell to only 68.7% of whole cleithra. Consensus agreement (i.e., two of three readers agree) values differed significantly across structures ($\chi^2 = 103.97$, df = 4, $P < 0.001$). Otoliths and sectioned cleithra had significantly higher consensus agreement rates than other structures. Scales, whole cleithra, and metapterygoid consensus agreement rates were similar, although the rate for metapterygoids was substantially higher.

Estimated ages for sectioned otoliths were similar between readers 1 and 2 (Figure 3). For other structures reader 1 tended to estimate ages older than reader 2; however, 95% confidence intervals generally included the 1:1 line. Slopes of age-bias plots between reader 1 and 2 were significantly different from 1 for sectioned cleithra ($F_{1,\ 9} = 38.35$, $P < 0.001$), whole cleithra ($F_{1,\ 9} = 67.72$, $P < 0.001$), metapterygoids ($F_{1,\ 9} = 16.17$, $P = 0.003$), and scales ($F_{1,\ 10} = 76.28$, $P < 0.001$). The identified bias between readers increased as fish age increased, and there was substantial variability in age estimates at ages ≥ 6 years (i.e., 95% CIs were broad and often overlapped the 1:1 line). Although slopes of age-bias plots indicated bias, only the intercept for the scale age-bias plot differed from 0 ($t = 4.30$, $P = 0.002$).

Agreement Between Structures

The maximum consensus age included in age-bias plots ranged from 5 to 7 years. Older consensus ages were not included in age-bias plots because the number of fish at each older age was less than three. Estimated consensus ages were similar between whole cleithra and scales and between whole cleithra and metapterygoids (Figure 4). Whole cleithra age estimates were higher than estimates for sectioned otoliths and sectioned cleithra. Age-bias plot slopes differed from 1 for the relationship between age estimates for whole cleithra

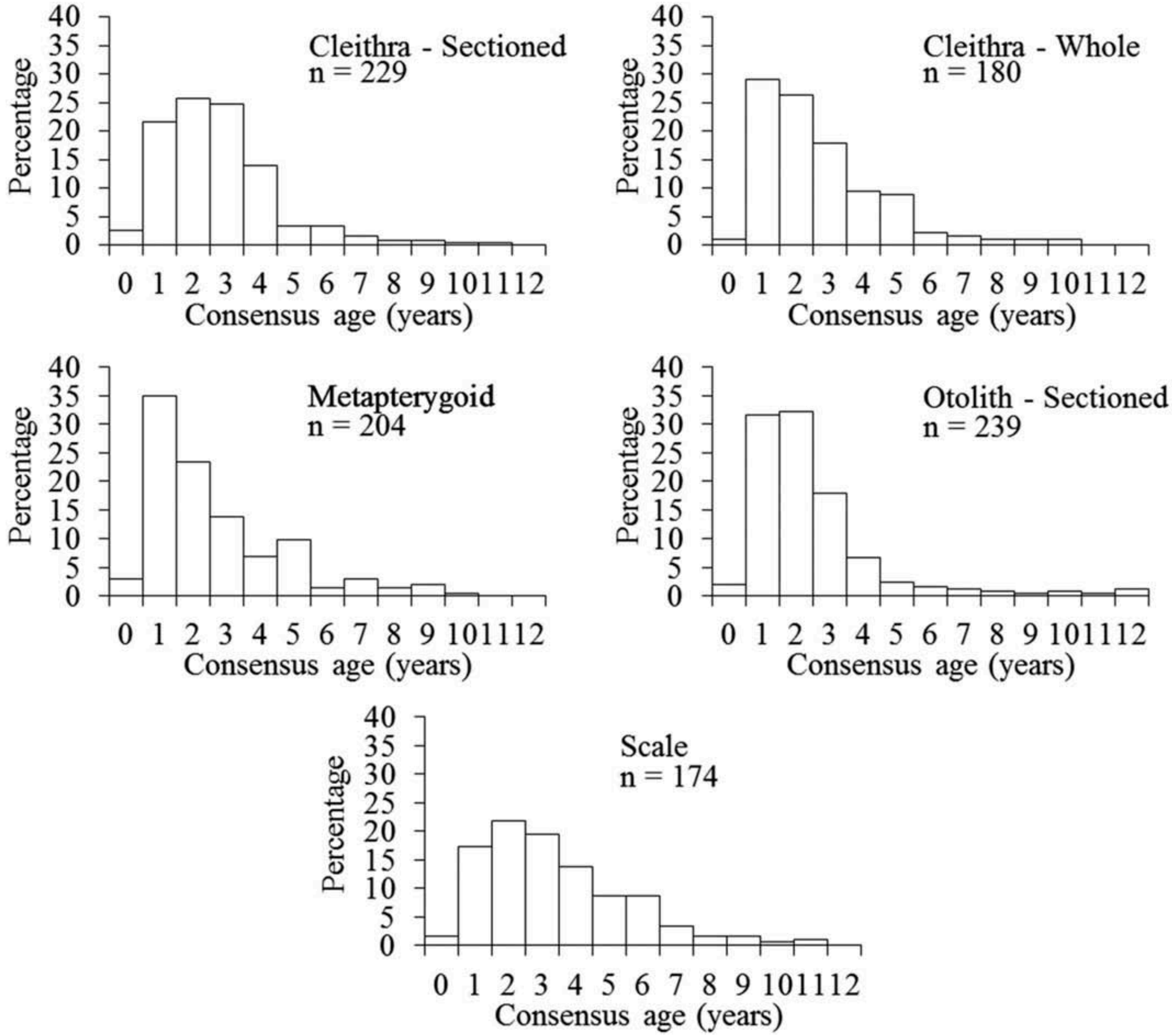

FIGURE 2. Age frequency histograms based on consensus age (two of three readers agree on age) estimates from sectioned cleithra, whole cleithra, metapterygoid bones, sectioned otoliths, and scales for Northern Pike collected from 19 northeast South Dakota lakes in 2012 and 2013.

and sectioned cleithra ($F_{1,\ 5}$ = 87.50, P < 0.001) and whole cleithra and sectioned otoliths ($F_{1,\ 5}$ = 63.33, P < 0.001), and the intercept was significantly different from 0 for both age-bias plots (whole cleithra – section cleithra, t = 4.43, P = 0.007; whole cleithra – sectioned otoliths, t = 2.73, P = 0.041). Variability was generally greatest at ages ≥ 6 years and 95% confidence intervals often included the 1:1 line.

Sectioned otolith consensus ages were slightly lower than estimated ages for the other structures, but slopes of the age-bias plots comparing the relationship between sectioned otoliths and the other four structures did not significantly differ from 1 (Figure 5). However, intercepts were significantly different from 0 for three of the four comparisons: otoliths and sectioned cleithra (t = 3.46, P = 0.026), otoliths and whole cleithra (t = 5.67, P = 0.005), and otoliths and scale (t = 2.90, P = 0.027). Consensus age estimates between metapterygoid bones and otoliths were not statistically different. Confidence intervals for the age bias plots included the 1:1 line with the greatest variability in age estimates occurring at ages ≥ 5.

DISCUSSION

Fisheries personnel have commonly used scales and whole cleithra to estimate Northern Pike ages (Maceina et al. 2007).

TABLE 1. Mean coefficient of variation (SE in parentheses), percent complete agreement (agree), percent agreement within 1 year (within 1) for two readers, and two of three readers agree (consensus) on estimated ages (n = 243) for five age structures of Northern Pike collected from 19 lakes in northeast South Dakota during 2012 and 2013. Within a column means with different letters are significantly different (P < 0.05).

		Two readers		
Structure	CV (%)	Agree (%)	Within 1 (%)	Consensus (%)
Whole cleithra	17.8 (1.2) z	38.7 z	68.7 x	74.1 yx
Sectioned cleithra	6.9 (1.0) xw	70.4 y	95.5 z	94.2 z
Metapterygoid bones	7.8 (0.9) w	64.6 y	88.1 y	84.0 x
Sectioned otoliths	4.0 (0.7) x	81.9 x	97.9 z	98.4 z
Scales	11.7 (1.0) y	44.3 z	78.6 x	71.6 x

We found these two structures to have the poorest reader agreement across the structures examined in this study. Our results showed that otoliths, metapterygoid bones and sectioned cleithra provided age estimates that were precise and suggest that these structures can be used as alternatives to scales and whole cleithra for estimating Northern Pike ages. Campana (2001) recommended that a CV of 5% could serve as a reference for many fishes of moderate longevity and Laine et al. (1991) recommended a precision of 5% for CV when assessing age estimates for Northern Pike populations. Precision across readers was good for otoliths because our mean CV value was below 5%. Our precision for sectioned cleithra and metapterygoid bones was higher than 5% but similar to that reported by Oele et al. (2015) and Faust et al. (2013) when recommending structures for Northern Pike age estimation. Mean CV values ranged from 12% to 14% for Northern Pike ages estimated using otoliths, cleithra, and anal fin rays for fish collected from tributaries to Green Bay in Lake Michigan (Oele et al. 2015). Age estimates of Northern Pike collected from Devils Lake, North Dakota, had a mean CV of 17% for otoliths and 10% for cleithra, and the CV of age estimates for fish collected from Cable Lake, Wisconsin, were 10% for otoliths and 11% for cleithra (Faust et al. 2013).

We found annuli difficult to recognize on scales, resulting in poor precision. Scales can have false annuli or summer checks that often resemble true annuli (Frost and Kipling 1959; Mann and Beaumont 1990; Neumann et al. 1994). In addition, Mann and Beaumont (1990) noted that the first annulus on Northern Pike scales could be difficult to identify when growth of age-0 pike was slow. Scales may also be subject to resorption or erosion in populations containing slow-growing or older fish, resulting in underestimation of ages (Casselman 1990). Our findings and those of Oele et al. (2015) contrast those of Laine et al. (1991), who indicated that scales were suitable for estimating Northern Pike ages through age 10 for fish collected from an oligotrophic lake in Ontario. Northern Pike age estimates from scales were found to have low precision and high reader bias for fish collected from tributaries to Green Bay, Lake Michigan (Oele et al. 2015). We agree with Oele et al. (2015) in discouraging the use of scales for estimating Northern Pike ages. If sacrificing fish is not permissible, anal fin rays were found to provide more precise age estimates than scales (Oele et al. 2015).

We believe that sectioning improved our ability to identify annuli on cleithra. We had poor reader agreement for whole cleithra, the mean CV exceeding 17%. Lower age estimates were obtained for sectioned cleithra than whole cleithra, suggesting that pseudoannuli may have been present and included in whole cleithrum age estimates. Annuli crowding and the presence of pseudoannuli can make recognition of annuli on whole cleithra difficult for both skilled and novice age estimators. Laine et al. (1991) reported that the annuli of older and slow-growing fish may crowd at the edge of the cleithrum; additionally, the presence of pseudoannuli may complicate annuli identification. However, it is also possible that cleithrum sections may not include the origin and early annuli, which may cause early annuli to be missed. Precision of sectioned cleithrum age estimates was higher than the recommended 5% (Laine et al. 1991; Campana 2001); however, the mean CV value for sectioned cleithra approximated the mean CV value for otoliths and was lower than that for whole cleithrum, anal fin ray. and scale age estimates for Northern Pike collected from tributaries to Green Bay in Lake Michigan (Oele et al. 2015). We found sectioned cleithra to have greater precision than whole cleithra making them preferred to whole cleithra.

Precision of metapterygoid bones was good across readers. We found a portion of the metapterygoids to be difficult to read because they did not have dense white checks (Sharma and Borgstrøm 2007) but had rather diffuse endpoints, making it difficult to identify annuli. On those that had well-defined checks, annuli were easy to identify. The presence of spongy bone on metapterygoids of old fish was identified as making it difficult to define annuli on metapterygoids (Sharma and Borgstrøm 2007), and this probably reduced precision of metapterygoid age estimates. Sharma and Borgstrøm (2007) suggested that Northern Pike metapterygoids from different geographic regions should be studied to verify their applicability for age determination. Our study is the first we are aware of to evaluate metapterygoids in North America. We

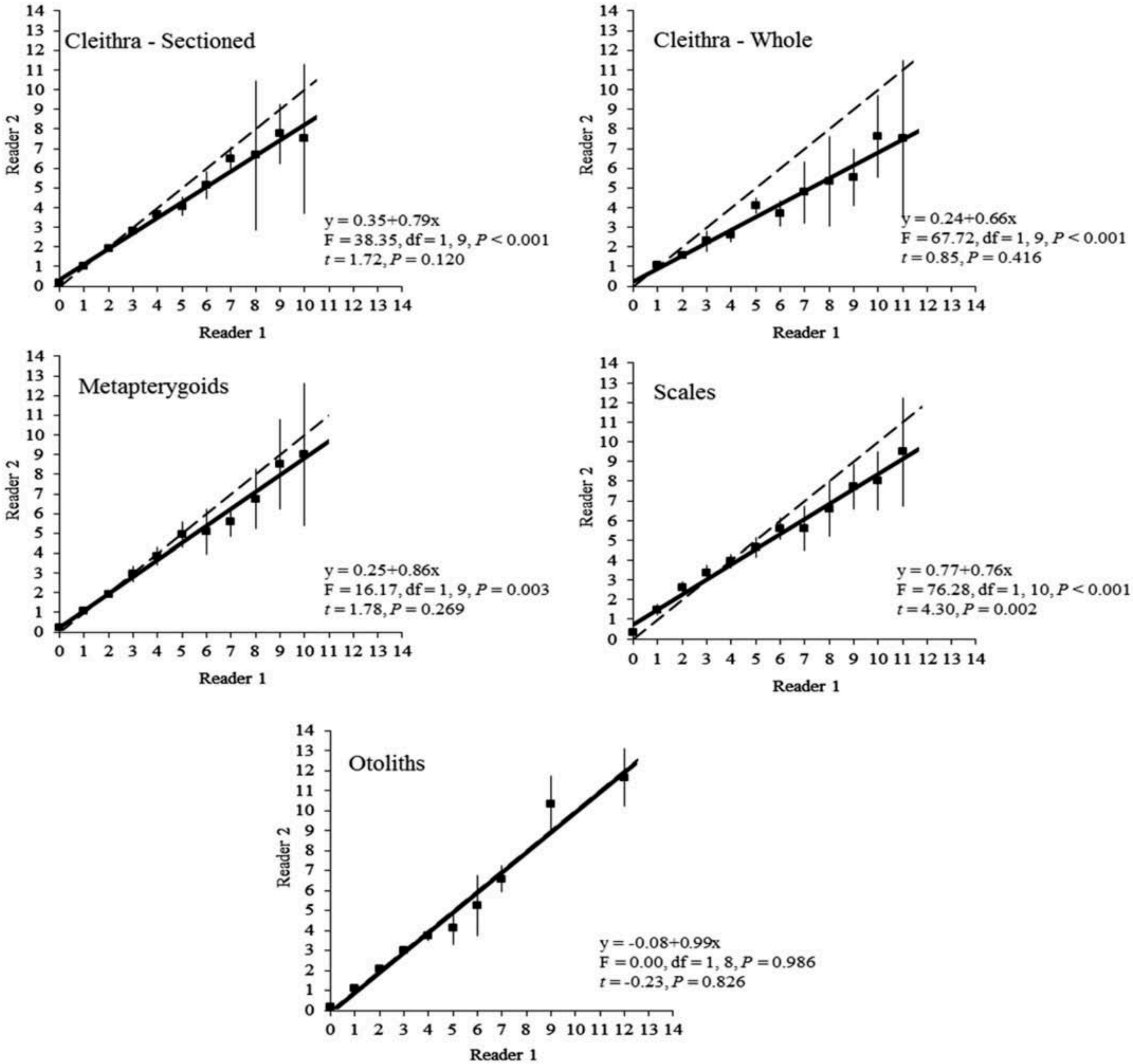

FIGURE 3. Between reader age-bias plots for sectioned cleithra, whole cleithra, metapterygoids, scales, and sectioned otoliths age estimates of Northern Pike collected from 19 northeast South Dakota lakes in 2012 and 2013. Mean ages for the dependent variable are the average of the age estimates ($n \geq 3$). In each panel, the error bars represent 95% confidence intervals and the dashed line indicates a 1:1 agreement line between readers. Linear regression equations and tests of whether the slopes differ from 1 (F-value) and y-intercepts differ from 0 (t-value) are provided.

believe that metapterygoids may have potential for estimating Northern Pike ages; an advantage of using metapterygoid bones is that no specialized equipment is needed.

Because of the increased precision often exhibited with otoliths, they have been recommended for estimating the ages of many species, e.g., Yellow Perch *Perca flavescens* (Vandergoot et al. 2008) and Walleye *Sander vitreus* (Isermann et al. 2003). Otoliths had the highest reader agreement of the structures we examined. Agreement rates of estimated ages from otoliths have been variable depending on the species examined. For example, the percent agreement between three readers using whole otoliths to estimate ages of Lake Michigan Alewives *Alosa pseudoharengus* was only 55% (LaBay and Lauer 2006), while that for Black Crappie

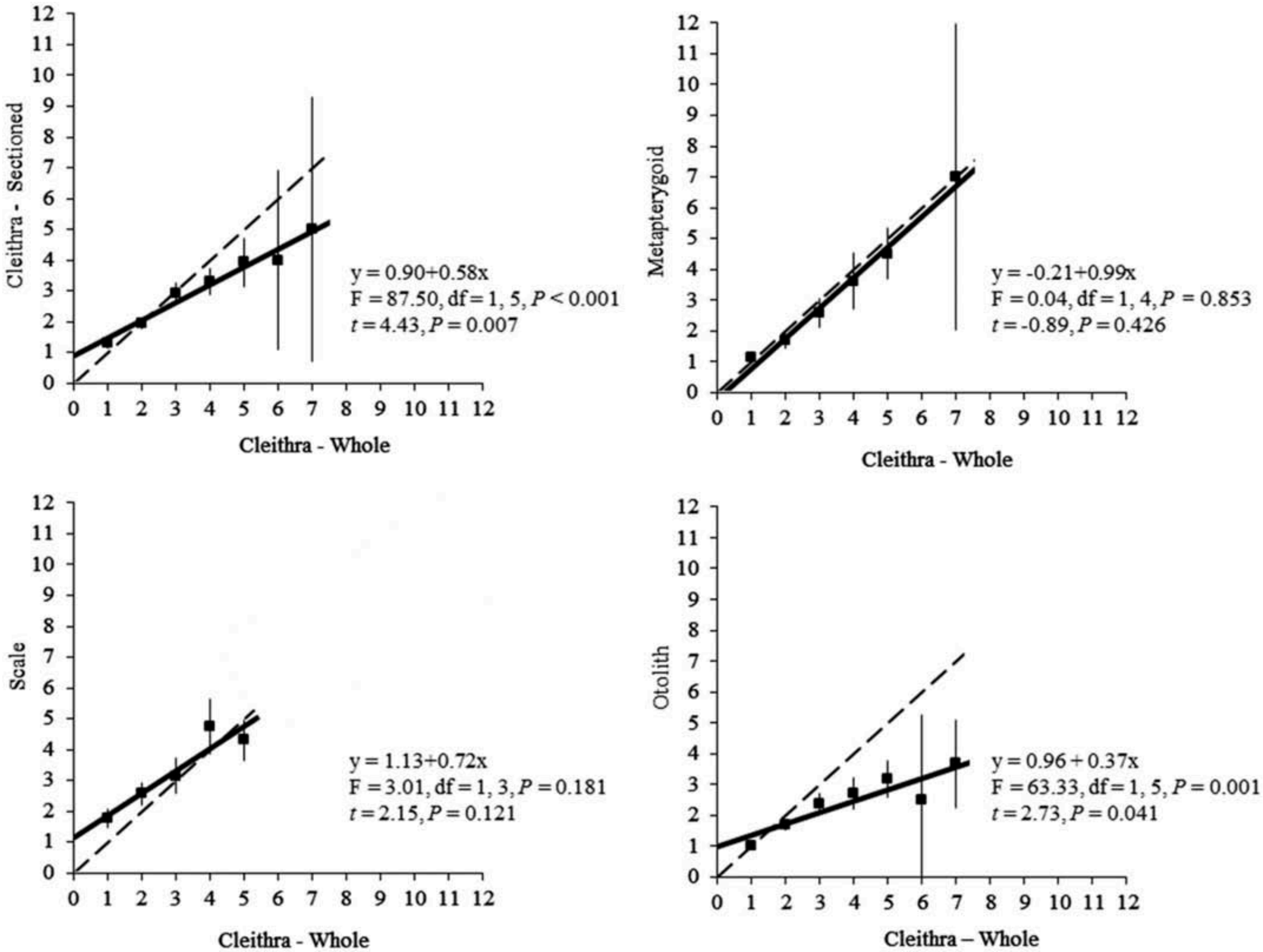

FIGURE 4. Age-bias plots of mean ages estimated from sectioned cleithra, metapterygoids, sectioned otoliths, scales, and scales in relation to whole cleithra age estimates for consensus age (2 of 3 readers agree on age) estimates for Northern Pike collected from 19 northeast South Dakota lakes during 2012 and 2013. Mean ages for metapterygoids, sectioned otoliths, scales, and sectioned cleithra are the average of the age estimates ($n \geq 3$). In each panel, the error bars represent 95% confidence intervals and the dashed line indicates a 1:1 agreement line between readers. Linear regression equations and tests of whether the slopes differ from 1 (*F*-value) and *y*-intercepts differ from 0 (*t*-value) are provided.

Pomoxis nigromaculatus in Upper Mission Lake, Minnesota, was 98% (Isermann et al. 2010). Otoliths may have an advantage for estimating Northern Pike age over other structures because they continue to grow and record cyclic seasonal growth patterns even in populations that contain slow growing or old fish (Casselman 1996). Most of our disagreements for otolith age estimates between the two readers were within 1 year and probably could have been resolved if consultation between readers had occurred. Our consensus agreement rate (98%) for otoliths was similar to that found for Northern Pike partial agreement (two of three readers agree) rates from Devils Lake, North Dakota, (92%; Faust et al. 2013); Cable Lake, Wisconsin (100%; Faust et al. 2013); and for tributaries to Green Bay in Lake Michigan (92%; Oele et al. 2015).

Our poor results for whole cleithra may have been influenced by our limited experience with estimating ages from whole cleithra. Our lack of experience in estimating ages with metapterygoid bones or sectioned cleithra may have hindered us somewhat because agreement was somewhat lower than that of otoliths. Both Faust et al. (2013) and Oele et al. (2015) demonstrated that reader experience influenced age estimates for Northern Pike. However, our extensive experience with scales did not improve our precision in estimating ages with scales. It is possible that individuals with more experience aging whole cleithra would have had better agreement rates. However, similar results (52% agreement) were found for Northern Pike >450 mm FL collected from the Artic–Yukon–Kuskokwim region of Alaska (Pearse and Hansen 1992).

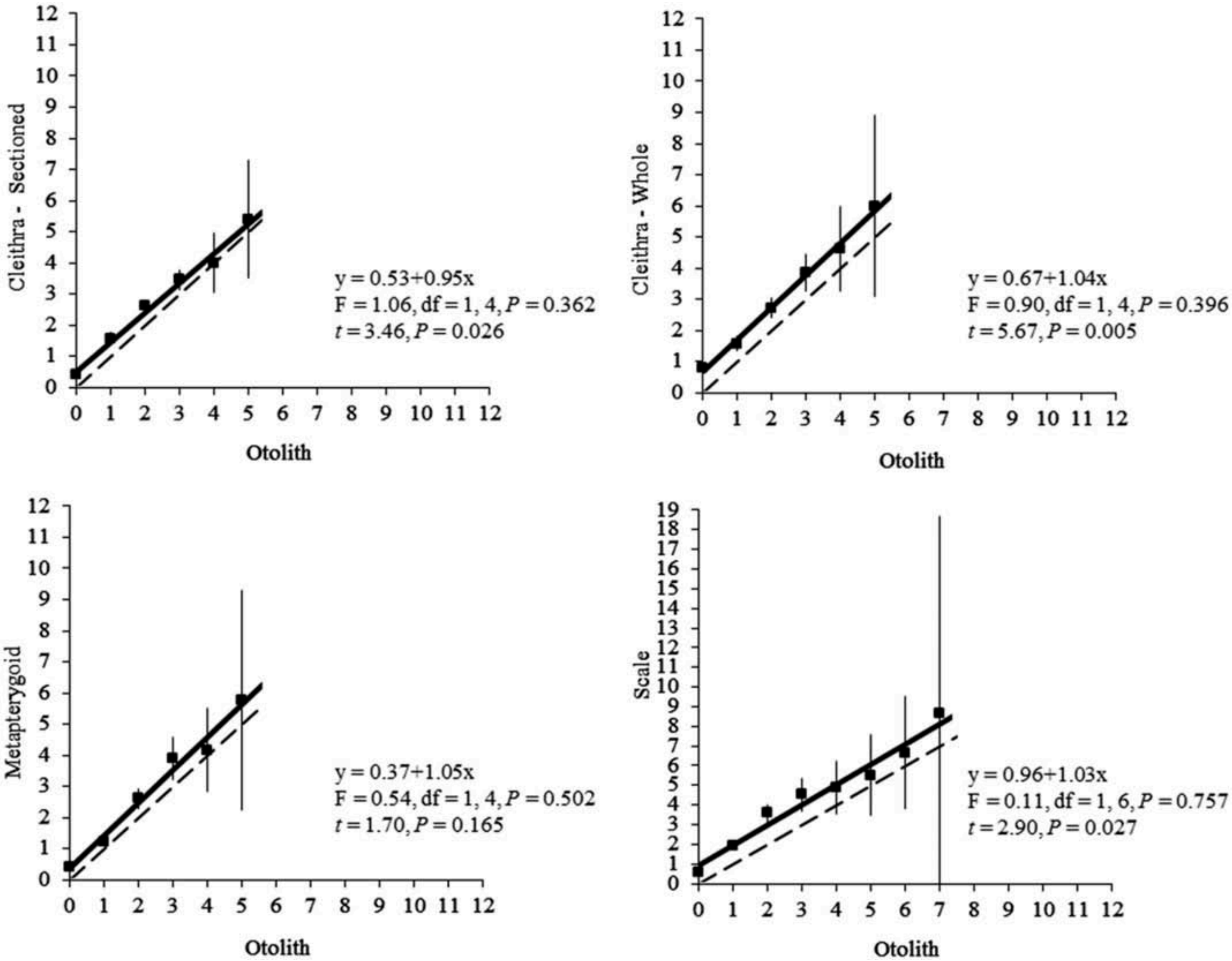

FIGURE 5. Age-bias plots of mean ages estimated from sectioned cleithra, whole cleithra, sectioned otoliths, and scales in relation to sectioned otolith age estimates for consensus age (2 of 3 readers agree on age) estimates for Northern Pike collected from 19 northeast South Dakota lakes during 2012 and 2013. Mean ages for sectioned cleithra, whole cleithra, metapterygoids, and scales are the average of the age estimates ($n \geq 3$). Linear regression equations and tests of whether the slopes differ from 1 (F-value) and y-intercepts differ from 0 (t-value) are provided.

Known age fish were not available for validating estimated ages in this study; however, cleithra have previously been validated (Babaluk and Craig 1990; Laine et al. 1991) and otoliths have been validated for many other species, e.g., Largemouth Bass *Micropterus salmoides* (Taubert and Tranquilli 1982) and Black Crappie and White Crappie *P. annularis*; (Ross et al. 2005). Northern Pike otoliths were found to be characterized by well-marked season variations in temperature through regular cyclic seasonal variations in the otolith oxygen isotope (Gerdeaux and Dufour 2012). The authors indicated that 1 year of otolith growth for Northern Pike is composed of an adjacent translucent zone and opaque zone. Because we had relatively poor agreement with whole cleithrum age estimates our age bias plots may not represent the actual relationship between whole cleithra and the other structures. When sectioned otoliths were the independent variable in the age-bias plots the relationship better approached a 1:1 relationship. Future studies should include known age fish to evaluate the accuracy of various structures for estimating Northern Pike ages.

Our findings suggest that otoliths, sectioned cleithra, and metapterygoid bones can be used as alternatives to whole cleithra for estimating ages of Northern Pike. A disadvantage of otoliths, metapterygoid bones, and sectioned cleithra is that fish need to be sacrificed. Additional disadvantages of otoliths and sectioned cleithra are the preparation time and specialized equipment is needed to complete age estimations, whereas an advantage of metapterygoid bones is that they can be viewed whole.

Any structure that is to be used for age estimation should be easy to interpret without substantial training. To that end, otoliths are becoming the common means for estimating fish ages for many species. Otoliths may be preferred for estimating Northern Pike age because they continue to grow and record cyclic seasonal growth patterns, even in populations that contain slow-growing or old fish (Casselman 1996), whereas nutritional status directly affects the size of cleithra (Casselman 1990; Casselman 1996). Being able to use whole or sectioned otoliths to estimate ages for all fish species collected for age estimation will provide consistency in procedures and make training of new personnel easier. An additional advantage to using otoliths is that they are not subject to resorption, and they continue to form annuli during periods of slow growth (Campana and Thorrold 2001). Future research should use known-age fish to directly validate annuli on Northern Pike otoliths. Along with validation, the utility of otoliths, sectioned cleithra, and metapterygoid bones for back-calculating length at previous ages should be explored because this would provide fisheries personnel an additional tool in the management of Northern Pike fisheries.

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