Notes

Precision and Bias of Cleithra and Sagittal Otoliths Used to Estimate Ages of Northern Pike

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Abstract

Cleithra are thought to accurately record age information and produce the most reliable age estimates relative to other calcified structures (e.g., scales) for long-lived species of Esocidae such as muskellunge *Esox masquinongy* and northern pike *E. lucius*. Sagittal otoliths provide the most accurate and precise age estimates for other fish species, yet sagittal otoliths have never been evaluated for age estimation of any species of Esocidae. Our objectives were to determine if: 1) sagittal otoliths provided more precise age estimates than cleithra for northern pike from two populations, and 2) sagittal otolith age estimates differed systematically from cleithrum age estimates for two populations of northern pike. Ages were estimated by three independent individuals with different experience levels from sagittal otoliths and cleithra collected from 66 northern pike (32–101 cm total length) from Devils Lake, North Dakota and 45 northern pike (27–52 cm total length) from Cable Lake, Wisconsin. Cleithrum age estimates were more precise than those from sagittal otoliths for northern pike from Devils Lake, and were similar to sagittal otolith age estimates for northern pike from Cable Lake. Sagittal otolith age estimates were similar to cleithrum age estimates for northern pike from Devils Lake, but were dissimilar for northern pike from Cable Lake. We recommend using cleithra for estimating age of northern pike given that no specialized equipment is required for processing and age estimation. However, other studies are needed to further investigate the use of sagittal otoliths to estimate age of northern pike.

Keywords: age estimation; bias; cleithra; northern pike; precision; sagittal otoliths

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Introduction

Age estimates are important for fisheries managers because age data are routinely used to assess recruitment, growth, and mortality of fish stocks (Maceina et al. 2007; Quist et al. 2012). The most common method of age estimation is interpretation of calcified structures (Casselman 1987; Quist et al. 2012). Many different calcified structures have been used to estimate fish age including cleithra (a bone in a fish's pectoral girdle), fin rays, fin spines, opercular bones, otoliths (ear bones), vertebrae, and scales (Casselman and Crossman 1986; Rien and Beamesderfer 1994; Burnham-Curtis and Bronte 1996; Hoxmeier et al. 2001; Buckmeier et al. 2002; Maceina et al. 2007). Furthermore, accuracy (i.e., proximity of an age estimate to true age; Campana et al. 1995) and precision

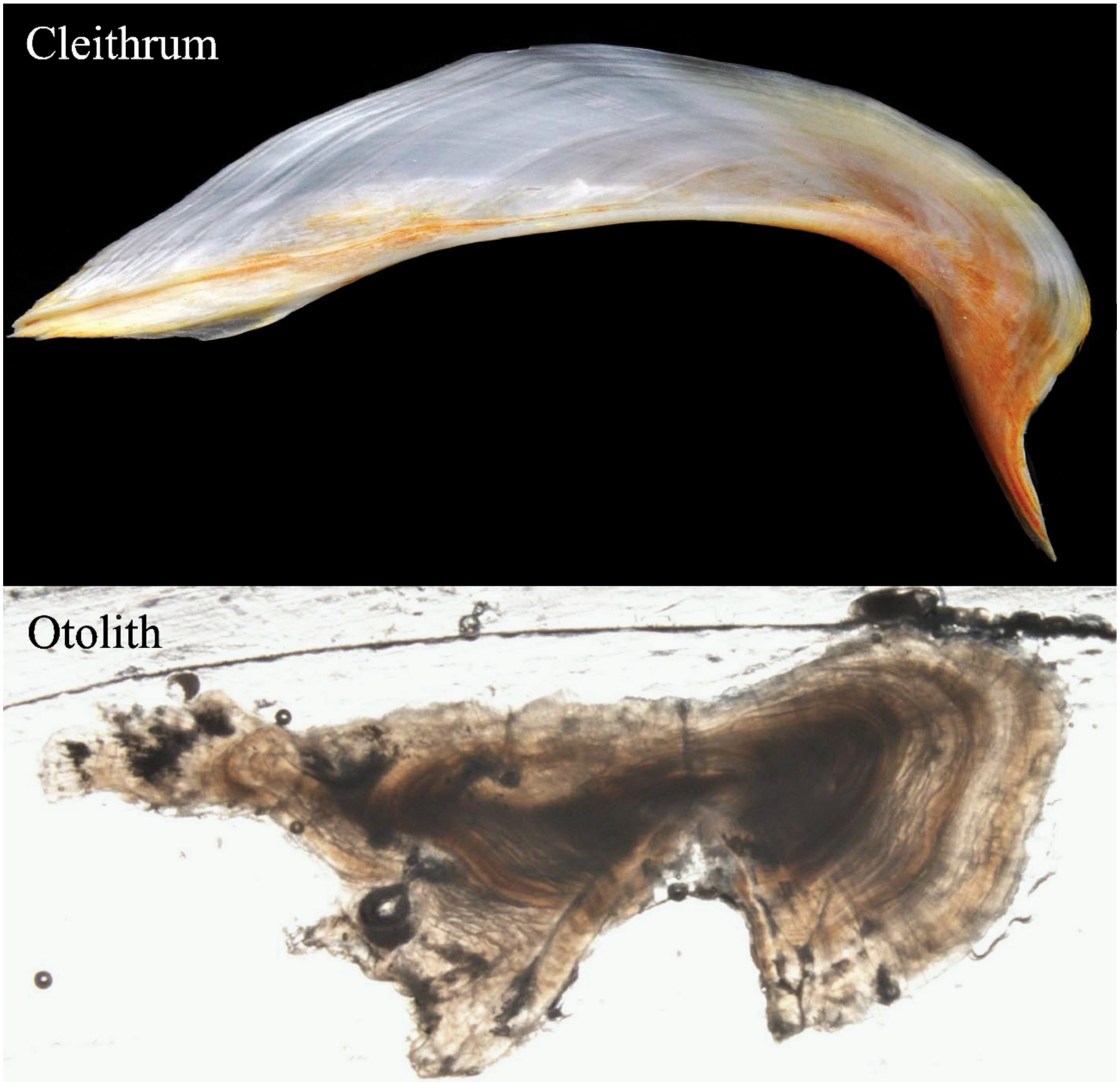

Figure 1. Photographs of cleithrum and thin-sectioned sagittal otolith used to estimate age of northern pike *Esox lucius* from Devils Lake, North Dakota and Cable Lake, Wisconsin.

(i.e., reproducibility of age estimates; Campana et al. 1995) of age estimates for an individual species or population depends on the calcified structure used (Johnson 1971; Isermann et al. 2003; Bruch et al. 2009).

Certain calcified structures are more commonly used to estimate ages of specific groups or species of fish (Maceina et al. 2007). For example, scales are commonly used to estimate ages of centrarchids (e.g., black bass and sunfishes) and moronids (i.e., true basses), whereas fin spines are the structure most commonly used to estimate ages of ictalurids (i.e., catfishes; Maceina et al. 2007). The decision to use one calcified structure rather than another may be related to ease of collection, accuracy and precision of age estimates, or the ability to sacrifice fish (Maceina et al. 2007; Quist et al. 2012). Additionally, the choice of which calcified structure to use for age estimation can influence estimates of recruitment, growth, or mortality of a fishery, and ultimately affect management decisions. For example, total annual mortality rates for yellow perch *Perca flavescens* in Lake Erie were estimated to be 4% and 20% higher when using ages estimated from fin spines and scales, respectively, compared with ages estimated from otoliths (Vandergoot et al. 2008).

Accurate and precise age estimates of esocids (e.g., muskellunge *Esox masquinongy* and northern pike *E. lucius*) have been difficult to obtain (Johnson 1971; Mann and Beaumont 1990; Fitzgerald et al. 1997). Scales are only accurate for muskellunge and northern pike younger than age 10 (Frost and Kipling 1959; Johnson 1971; Laine et al. 1991), although age estimates for muskellunge as young as age 3 were found to be inaccurate (Fitzgerald et al. 1997). Fin rays and opercular bones produce age estimates similar to scales, and were accurate for muskellunge and northern pike younger than age 10–12 (Frost and Kipling 1959; Johnson 1971; Brenden et al. 2006). Cleithra are thought to accurately record age information throughout an esocid's lifetime and produce the most reliable age estimates (Casselman 1979; Casselman 1990; Laine et al. 1991). Otoliths are rarely used in esocid age estimation (Maceina et al. 2007) despite generating accurate (Erickson 1983; Heidinger and Clodfelter 1987; Ross et al. 2005) and precise (Boxrucker 1986; Isermann et al. 2003; Vandergoot et al. 2008) age estimates for many species. Further, accuracy and precision of age estimates from otoliths have never been evaluated for northern pike.

Our objectives were to determine if: 1) sagittal otoliths (hereafter otoliths) provided more precise age estimates than cleithra for two populations of northern pike; and 2) otolith age estimates differed systematically from cleithrum age estimates for two populations of northern pike. Cleithra and otoliths were removed from northern pike collected from Devils Lake, North Dakota and Cable Lake, Wisconsin during June–August, 2011 and age estimates were compared to determine if otoliths provided more precise age estimates than cleithra, and if otolith age estimates systematically differed from cleithrum age estimates.

Methods

Devils Lake is a large, saline, hypereutrophic natural lake located in east-central North Dakota. Devils Lake does not have a natural outlet until lake levels reach a high level. Since 1993, Devils Lake has been experiencing unprecedented flooding, with lake levels rising nearly 9.5 m. Devils Lake supports a simple fish assemblage dominated by walleye *Sander vitreus*, yellow perch, white bass *Morone crysops,* and northern pike; white sucker *Catostomus comersonii* and black crappie *Pomoxis nigromaculatus* are also present.

Cable Lake is a 67-ha drainage lake located in north-central Wisconsin. Cable Lake has a mean depth of 3.1 m and a maximum depth of 13.1 m, and is connected to Wiley Lake, which is a 24-ha drainage lake. The most common fish species in Cable Lake are bluegill *Lepomis macrochirus*, black crappie, rock bass *Ambloplites rupestris*, yellow perch, largemouth bass *Micropterus salmoides*, and northern pike.

Northern pike were collected during June–August, 2011 from Devils Lake, North Dakota and Cable Lake, Wisconsin. Northern pike from Devils Lake were caught using five-panel 38.1-m monofilament-experiment gill nets with bar mesh sizes ranging from 1.91 to 6.35 cm, whereas northern pike from Cable Lake were collected using standard angling methods and gear (e.g., 4.5–9.1-kg monofilament line, 1.8–2.4-m medium-action rods, and artificial lures). The goal was to collect 10 northern pike per 5-cm-length bin ranging in size from 25 to 100 cm (e.g., first 10 fish encountered within each length bin). Total length was recorded for all northern pike upon collection. Otoliths were removed, dried, and stored in plastic vials until preparation. Cleithra were removed,

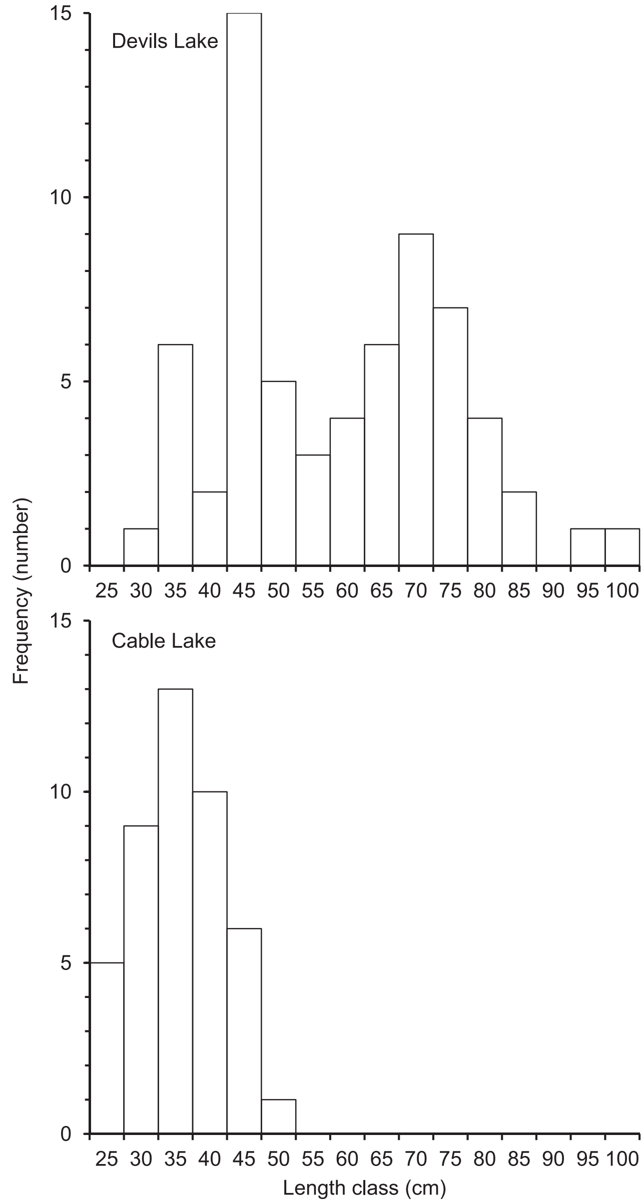

Figure 2. Length frequency (cm) for northern pike *Esox lucius* caught from Devils Lake, North Dakota and Cable Lake, Wisconsin during June–August, 2011.

cleaned of as much tissue as possible, and frozen until further processing.

One otolith from each fish was set in epoxy resin (5:1 mixture of Buehler Epo-Kwick Resin and Buehler Epo-Kwick Hardener). A Buehler Isomet 1000 precision saw was used to remove a 0.6-mm transverse section containing the nucleus (Secor et al. 1992). Sections were mounted on glass slides and polished using wetted 600-grit sandpaper. Digital images of otolith thin sections were captured with a Nikon SMZ-1500 microscope equipped with top and bottom illumination; a SPOT Insight four-megapixel, color-mosaic, high-resolution digital camera; a computer; and SPOT image analysis software. Immersion oil was applied as necessary to improve clarity. Each reader was given a series of digital

Table 1. Mean coefficients of variation (coefficient of variation = 100 × standard deviation/mean; standard error in parenthesis), complete (i.e., all three readers agreed on an age) agreement, and partial (i.e., at least two of three readers agreed on an age) agreement for otolith and cleithrum age estimates by three readers of varying experience from northern pike *Esox lucius* collected from Devils Lake, North Dakota and Cable Lake, Wisconsin during June–August, 2011.

Lake	*N*	Structure	Mean coefficient of variation (%)	Complete agreement (%)	Partial agreement (%)
Devils Lake	66	Otoliths	17 (1.8)	17	92
		Cleithra	10 (1.4)	41	91
Cable Lake	45	Otoliths	10 (1.6)	40	100
		Cleithra	11 (1.9)	49	89

images (corresponding to each time the thin section was polished) to estimate age from thin-sectioned otoliths. Northern pike otoliths are very opaque relative to other fishes' otoliths, which made it difficult to clearly view all annuli at the same time (Figure 1). Annuli near the focus required a significant amount of polishing to improve visibility, which could result in polishing away outer annuli near the edge. Thus, age estimates were not an average among images but a composite age arrived at by examining all images in succession. Annuli were identified as alternating translucent and opaque zones (Quist et al. 2012).

Whole cleithra were immersed in near boiling water for 15–30 seconds to loosen excess tissue, which was removed by hand or with the aid of a coarse wire brush if needed. This process was repeated as necessary. Once clean, cleithra air-dried for 1–2 days before age estimation. Cleithra were placed in a black dish and immersed in water to improve visibility of annuli. Once immersed, cleithra were examined either with the naked eye under ambient light or using a fiber-optic light source (Figure 1). Annuli were identified as alternating translucent and opaque zones (Casselman 1979).

Three independent readers (i.e., individuals that estimated the age of a fish using a calcified structure) with different levels of experience estimated each fish's age. Reader 1 had experience estimating esocid age using cleithra, but had little experience estimating fish age using otoliths. Reader 2 had experience estimating fish age using otoliths, but had little experience estimating age of esocids using cleithra. Reader 3 had no previous experience estimating fish age using either structure, but received a brief introduction to estimating fish age before this study. Each reader examined structures independently of the other two readers, and had no knowledge of fish length when estimating ages.

Precision among all three readers' age estimates for each structure was quantified using coefficient of variation (CV):

$$CV = 100 \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

where X_{ij} is the ith age estimate of the jth fish, X_j is the mean age of the jth fish, and R is the number of times each fish has its age estimated (Campana et al. 1995). Mean CVs were compared between structures using a paired t-test ($\alpha = 0.05$). Complete agreement (i.e., all three readers assigned the same age) and partial agreement (i.e., at least two of three readers assigned the same age) were also used to compare the precision of age estimates derived from each structure (Isermann et al. 2010) and were compared between structures using a z-statistic ($\alpha = 0.05$). Age-bias plots were used to assess potential bias (i.e., systematic differences; Campana et al. 1995) when comparing cleithrum age estimates with otolith age estimates for each individual reader, and when comparing age estimates from an experienced reader with an inexperienced reader for each structure (Campana et al. 1995). Cleithrum ages were assumed to represent accurate estimates of northern pike age on the basis of previous validation studies (Babaluk and Craig 1990; Laine et al. 1991) because known-age fish were not available. Otolith age estimates were averaged for all fish of the same cleithrum age. Age-bias plots were constructed for each reader by plotting mean otolith ages (± 95% confidence intervals) against cleithrum age. Linear regressions were used to determine whether the slopes and intercepts of these relationships differed significantly from 1 and 0 respectively ($\alpha = 0.05$). Age-bias plots were constructed

Table 2. Summary statistics for slopes and intercepts of age-bias plots comparing sagittal otolith and cleithrum age estimates from inexperienced readers with age estimates from an experienced reader for each structure for northern pike *Esox lucius* collected from Devils Lake, North Dakota during June–August, 2011. t = t-statistic; df = degrees of freedom; P = P-value.

	Reader 1: otoliths		Reader 3: otoliths		Reader 2: cleithra		Reader 3: cleithra	
	Slope = 0.89	Intercept = 0.58	Slope = 0.86	Intercept = 1.44	Slope = 1.05	Intercept = −0.22	Slope = 1.00	Intercept = −0.17
t	1.97	1.34	2.55	3.51	1.01	−0.71	0.07	−0.58
df	9		9		8		8	
P	0.08	0.21	0.03	0.01	0.34	0.5	0.95	0.58

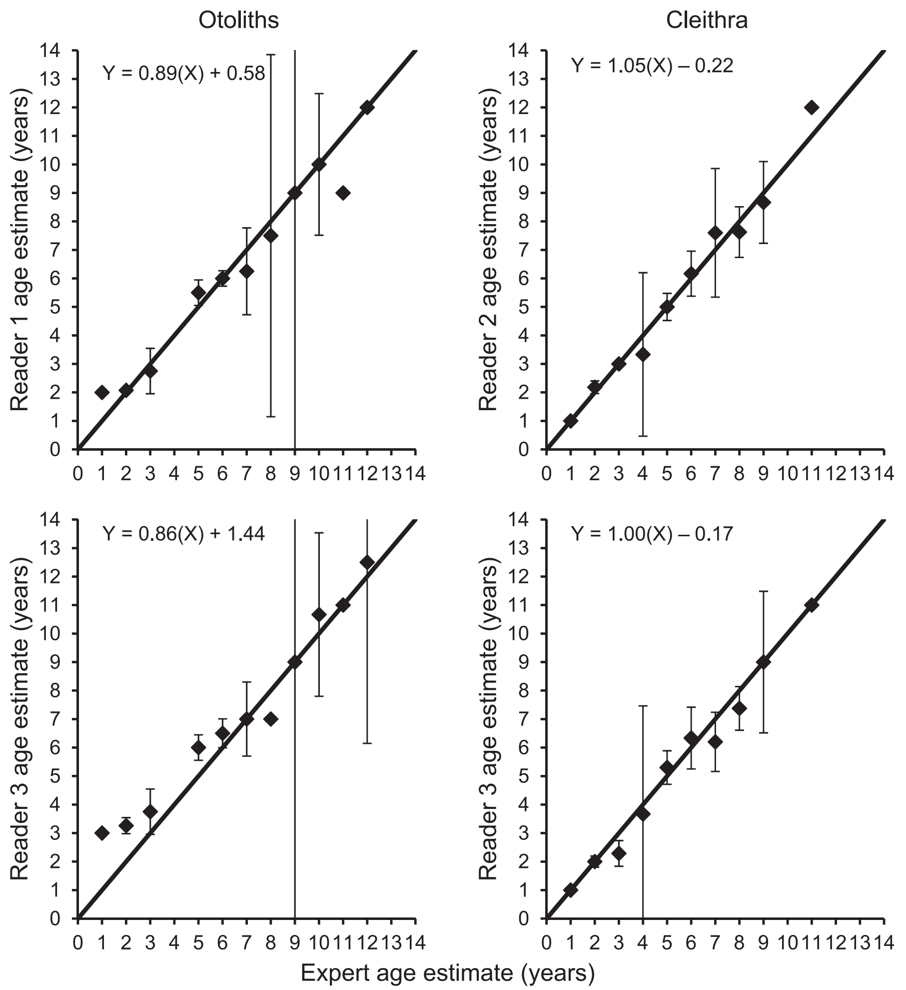

Figure 3. Age-bias plots comparing mean thin-sectioned otolith and cleithrum age estimates from the less-experienced readers with age estimates for the same structure from the experienced reader for northern pike *Esox lucius* collected from Devils Lake, North Dakota during June–August, 2011. Solid lines represent 1:1 lines and error bars represent 95% confidence intervals.

and evaluated in a similar manner to compare ages estimated by the experienced reader with the inexperienced readers for each structure (i.e., mean inexperienced age estimates plotted against experienced age estimates, and linear regression used to evaluate relationships). Reader 1 was considered the experienced reader for cleithra, and reader 2 was considered the experienced reader for otoliths.

Results

Devils Lake

Ages were estimated for 66 northern pike from Devils Lake ranging from 32 to 101 cm total length (Figure 2; Table S1). Cleithrum age estimates ranged from age 1 to age 12, whereas otolith age estimates ranged from age 1 to age 13. Cleithra were more precise than otoliths for northern pike from Devils Lake, with cleithra having a mean CV of 10.2% (SE = 1.4; $t = -2.97$, $df = 65$, $P < 0.01$) compared with otoliths, which had a mean CV of 17.4% (SE = 1.8; Table 1). Cleithra also had a significantly higher complete agreement compared with otoliths ($z = 3.08$, $n = 66, 66$, $P < 0.01$; Table 1), whereas partial agreement was similar between cleithra and otoliths ($z = -0.31$, $n = 66, 66$, $P = 0.75$; Table 1).

For the readers with little or no experience estimating fish age using a given structure, cleithra were easier to use than otoliths for northern pike from Devils Lake (Table 2; Figure 3). Cleithrum age estimates from inexperienced readers (i.e., readers 2 and 3) were similar to those from the expert reader (Table 2; Figure 3). Otolith

Table 3. Summary statistics for slopes and intercepts of age-bias plots comparing sagittal otolith and cleithrum age estimates from three independent readers for northern pike *Esox lucius* collected from Devils Lake, North Dakota during June–August, 2011. t = t-statistic; df = degrees of freedom; P = P-value.

	Reader 1		Reader 2		Reader 3	
	Slope = 1.02	Intercept = 0.04	Slope = 1.04	Intercept = −0.13	Slope = 0.80	Intercept = 1.92
t	0.31	0.09	0.88	−0.37	1.86	2.59
df	8		9		9	
P	0.76	0.93	0.40	0.72	0.10	0.03

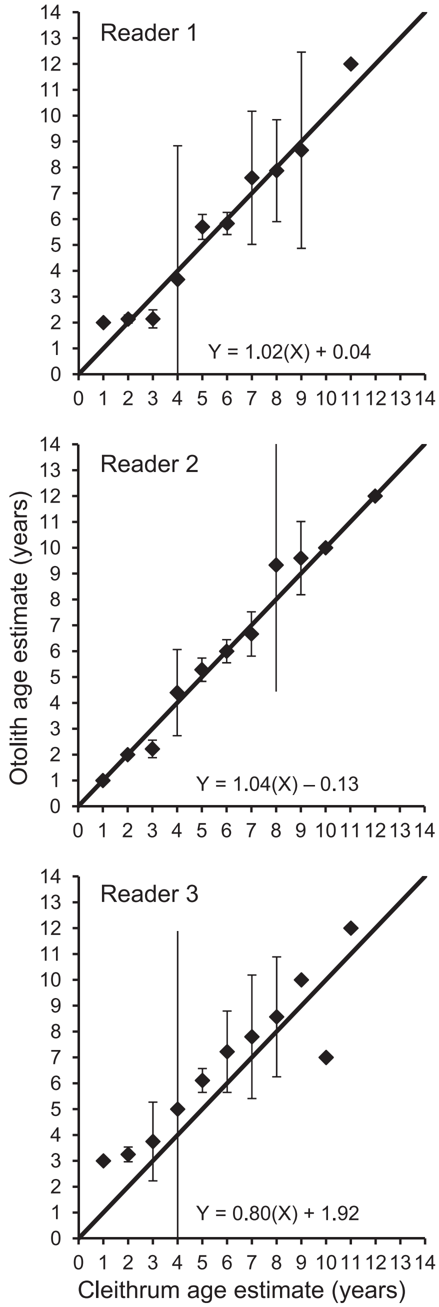

Figure 4. Age-bias plots comparing mean thin-sectioned otolith age estimates with cleithrum age estimates for three independent readers for northern pike *Esox lucius* collected from Devils Lake, North Dakota during June–August, 2011. Solid lines represent 1:1 lines and error bars represent 95% confidence intervals.

age estimates from reader 1 were similar to those from the expert reader (Table 2; Figure 3). However, the inexperienced reader (i.e., reader 3) overestimated ages relative to the expert reader when using otoliths (Table 2; Figure 3).

Mean otolith age estimates were generally similar to most cleithrum age estimates for northern pike from Devils Lake (Table 3; Figure 4). Otolith age estimates were similar to cleithrum age estimates for readers with previous age estimation experience (i.e., readers 1 and 2; Table 3; Figure 4). However, reader 3 (no previous age estimation experience) overestimated otolith age relative to cleithrum age (Table 3; Figure 4).

Cable Lake

Ages were estimated for 45 northern pike from Cable Lake ranging from 27 to 52 cm total length (Figure 2; Table S1). Cleithrum age estimates ranged from age 1 to age 9, whereas otolith age estimates ranged from age 1 to age 7. Cleithra were less precise than otoliths for northern pike from Cable Lake, with cleithra having a mean CV of 11.1% (SE = 1.9) compared with otoliths, which had a mean CV of 10.3% (SE = 1.6; Table 1). However, this difference was not statistically significant (t = 0.34, df = 44, P = 0.73). Cleithra had a higher complete agreement compared with otoliths, although this difference was not significant (z = 0.85, n = 45, 45, P = 0.40). In contrast, otoliths had a significantly higher partial agreement rate than cleithra (z = −2.30, n = 45, 45, P = 0.02; Table 1).

For readers with little or no experience estimating fish age using a given structure, cleithra and otoliths provided similar age estimates relative to the expert reader for northern pike from Cable Lake (Table 4; Figure 5). Cleithrum age estimates from inexperienced readers (i.e., readers 2 and 3) were similar to those from the expert reader (Table 4; Figure 5). Otolith age estimates from inexperienced readers (i.e., readers 1 and 3) were similar to those from the expert reader (Table 4; Figure 5).

Mean otolith age estimates differed significantly from cleithrum age estimates for northern pike from Cable Lake for two readers (Table 5; Figure 6). Otolith age estimates were similar to cleithrum age estimates from an experienced reader (i.e., reader 1; Table 5; Figure 6). However, otolith age estimates from the expert otolith reader (i.e, reader 2) underestimated northern pike age relative to cleithrum age estimates (Table 5; Figure 6). Further, otolith age estimates from the inexperienced reader (i.e., reader 3) were dissimilar to cleithrum age estimates (Table 5; Figure 6).

Discussion

Cleithra provided more precise age estimates than otoliths for northern pike from Devils Lake, North Dakota, but were similar for northern pike from Cable Lake, Wisconsin. Age estimates from both structures were less precise than precision levels reported for a variety of other fishes (Campana 2001). Campana (2001) reported a median CV of 7.6% on the basis of 117 published precision values, and suggested that a CV of 5% can serve as a reference point for fishes of moderate longevity and reading complexity. Because otoliths have never been formally evaluated for esocid age estimation, it is impossible to compare the precision of these results with other studies. However, the mean CVs for age estimates from thin-sectioned northern pike otoliths were higher than those reported for other fishes. Walleyes from South Dakota that had ages estimated

Table 4. Summary statistics for slopes and intercepts of age-bias plots comparing sagittal otolith and cleithrum age estimates from inexperienced readers with age estimates from an experienced reader for each structure for northern pike *Esox lucius* collected from Cable Lake, Wisconsin during June–August, 2011. t = t-statistic; df = degrees of freedom; P = P-value.

	Reader 1: otoliths		**Reader 3: otoliths**		**Reader 2: cleithra**		**Reader 3: cleithra**	
	Slope = 0.76	**Intercept = 1.45**	**Slope = 0.74**	**Intercept = 1.44**	**Slope = 1.39**	**Intercept = −0.81**	**Slope = 1.40**	**Intercept = −1.12**
t	1.84	2.47	1.77	2.22	1.85	−0.91	1.65	−1.09
df	5		5		3		3	
P	0.13	0.06	0.14	0.08	0.16	0.43	0.20	0.35

using otoliths had a mean CV of 2.5% (Isermann et al. 2003). Mean CVs of otolith age estimates for several catostomid (i.e., suckers) and cyprinid (i.e., minnows) species in the upper Colorado River basin ranged from 1.2 to 9.5% (Quist et al. 2007). Otolith age estimates for yellow perch from Lake Erie had a mean CV of 1.0% (Vandergoot et al. 2008). The mean CVs reported for other esocid age estimation studies that used cleithra were near 5% (Laine et al. 1991; Robinson 2005; Faust 2011). Cleithrum age estimates of northern pike from Squeers Lake, Ontario had a mean CV of 1.2% (Laine et al. 1991). Cleithrum age estimates for muskellunge from across North America archived by The Cleithrum Project had a mean CV of 5.4% (Robinson 2005), whereas muskellunge from northern Wisconsin that had ages estimated in a similar manner had a mean CV of 8.0% (Faust 2011). The lower precision levels reported here for northern pike age estimates may reflect the relative inexperience of two of the three readers for either structure, as any measure of precision will be artificially inflated by any bias present among readers (Campana 2001).

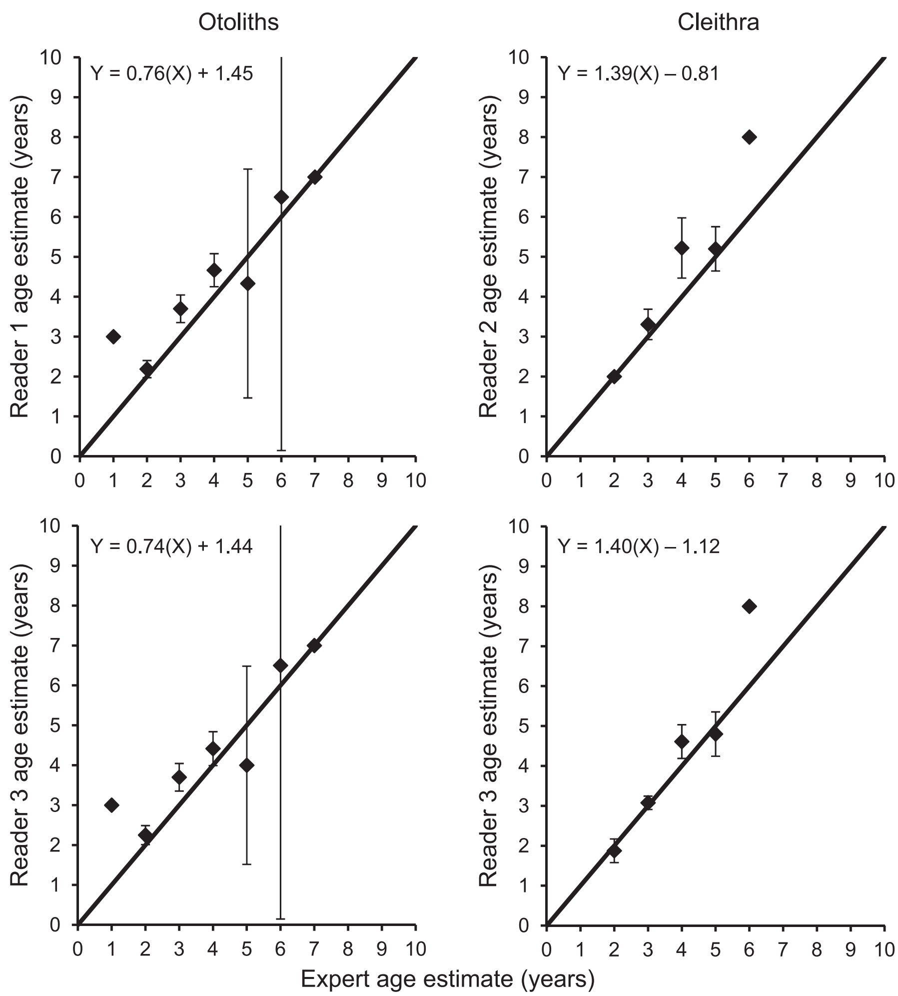

Figure 5. Age-bias plots comparing mean thin-sectioned otolith and cleithrum age estimates from the less-experienced readers with age estimates for the same structure from the experienced reader for northern pike *Esox lucius* collected from Cable Lake, Wisconsin during June–August, 2011. Solid lines represent 1:1 lines and error bars represent 95% confidence intervals.

Table 5. Summary statistics for slopes and intercepts of age-bias plots comparing sagittal otolith and cleithrum age estimates from three independent readers for northern pike *Esox lucius* collected from Cable Lake, Wisconsin during June–August, 2011. t = t-statistic; df = degrees of freedom; P = P-value.

	Reader 1		Reader 2		Reader 3	
	Slope = 0.95	Intercept = 0.18	Slope = 0.68	Intercept = 0.38	Slope = 0.69	Intercept = 1.00
t	0.36	0.30	0.74	0.74	4.02	2.74
df	3		6		5	
P	0.74	0.79	0.01	0.49	0.01	0.04

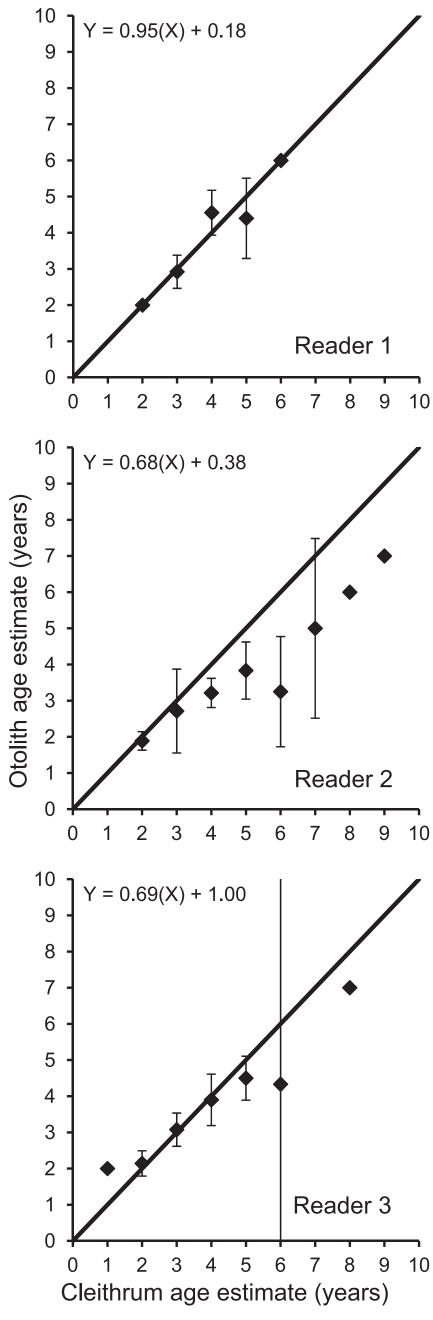

Figure 6. Age-bias plots comparing mean thin-sectioned otolith age estimates with cleithrum age estimates for three independent readers for northern pike *Esox lucius* collected from Cable Lake, Wisconsin during June–August, 2011. Solid lines represent 1:1 lines and error bars represent 95% confidence intervals.

Otoliths and cleithra provided similar age estimates for northern pike from both lakes, which is in contrast to previous studies that compared age estimates from otoliths and cleithra for a variety of fish species (McCarthy and Minckley 1987; Sharp and Bernard 1988; Baker and Timmons 1991). McCarthy and Minckley (1987) concluded that thin-sectioned otoliths provided the only clear indications of annual growth for razorback sucker *Xyrauchen texanus* from Lake Mohave, Arizona and Nevada after comparing age estimates from otoliths, cleithra, and five other calcified structures. Cleithra could not be used to estimate age of lake trout *Salvelinus namaycush* from Summit Lake, Alaska, with otoliths and opercular bones providing the most precise age estimates (Sharp and Bernard 1988). Similarly, cleithra produced highly variable age estimates when compared with otolith age estimates for Arctic char *S. alpinus* from the Wood River system, Alaska (Baker and Timmons 1991).

We recommend that cleithra be used for age estimation of northern pike. Esocid cleithra do not require specialized equipment such as a low-speed saw or digital imaging software for preparation before age estimation. Preparation time for cleithra is also less than for otoliths. Qualitatively, otoliths may need several weeks to allow sufficient time for drying after removal, epoxy setting and embedding, and sectioning and photographing. Cleithra can be cleaned immediately after removal, dried for a short time ($<$ 1 week), and age estimated after the drying period. Additionally, precision of cleithrum age estimates remained relatively unchanged for two different northern pike populations, with Devils Lake northern pike being fast-growing and large-bodied, whereas Cable Lake northern pike were slow-growing and small-bodied. However, cleithra may not be the best structure for all scenarios. For example, other structures such as fin rays may be necessary for age estimation of northern pike if fish cannot be sacrificed (Johnson 1971; Brenden et al. 2006). Cleithra are also relatively more difficult to estimate growth with than other structures (e.g., fin rays). Cleithrum annuli must be marked and measured manually with a digital caliper (Faust 2011), whereas structures such as fin rays can be more readily used in conjunction with digital imaging software to estimate growth. Differences may also exist in cleithrum age estimates between sexes (Laine et al. 1991). All readers should go through a training period to become familiar with structures used for northern pike age estimation (Campana 2001; Maceina et al. 2007).

This study represents an initial attempt to compare age estimates from otoliths and cleithra for northern pike, but several questions remain unanswered. Northern pike from Devils Lake, North Dakota and Cable Lake, Wisconsin were relatively young. Inclusion of additional populations containing older individuals may reveal greater differences in precision of age estimates between otoliths and cleithra, and possibly great differences at older ages. Cleithra have been validated for northern pike (Babaluk and Craig 1990; Laine et al. 1991), but otoliths have never been validated for any species of Esocidae, and as a result the accuracy of otolith age estimates is unknown. Future studies should strive to address these issues.

Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content or functionality of any supplemental material. Queries should be directed to the corresponding author for the article.

Table S1. All data are organized in a single tab, ''Age estimation data.'' The Age estimation data tab contains a unique identification code for each northern pike *Esox lucius* sampled, each northern pike's total length (cm), sample location (i.e., Devils Lake, North Dakota; Cable Lake, Wisconsin), the calcified structure used to estimate age (i.e., cleithrum; otolith), age estimate (years) for three independent readers, and precision of age estimates (i.e., coefficient of variation; complete reader agreement; partial reader agreement) for northern pike collected from Devils Lake, North Dakota and Cable Lake, Wisconsin during June–August, 2011. All headings and terms used in this data file are defined in the first tab, ''Data dictionary.''

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