Fisheries Management and Ecology, 2006, **13**, 31–37

Sectioned pelvic fin ray ageing of muskellunge *Esox masquinongy* from a Virginia river: comparisons among readers, with cleithrum estimates, and with tag–recapture growth data

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Abstract The potential utility of pelvic fin rays as ageing structures was evaluated for southern US muskellunge *Esox masquinongy* Mitchill populations by comparing age estimates among three readers and against cleithrum estimates, and by comparing observed length changes of tagged fish with changes predicted from growth equations based on pelvic fin ray age estimates. Mean coefficient of variation in age estimates among all readers and between the two readers with prior ageing experience was 17.8% and 5.6%, respectively. Exact and within 1-year agreement rates between pelvic fin rays and cleithra were 76% and 100%, respectively. Mean (± 2 SE) estimated absolute error between observed and predicted length changes for 13 tagged muskellunge was 30 ± 14 mm. This evaluation indicated that pelvic fin rays may prove to be a useful, non-lethal method for ageing muskellunge in southern US waters. Validation studies are still needed to ensure that growth rings form consistently throughout fish's life span.

KEYWORDS: ageing error, ages, cleithrum, muskellunge, pelvic fin rays, validation.

Introduction

The ability to ascribe accurate ages to fish is critical for many aspects of fish population assessment. Age data can be used in conjunction with length and catch information to estimate growth and mortality rates, which are necessary components for evaluating changes in population abundance, biomass and yield. Fish can be aged using a number of structures, including scales, rays, spines, cleithra and otoliths (DeVries & Frie 1996). Two important considerations when selecting a structure for ageing a sample of fish are whether the structure yields accurate estimates of fish age and whether the structure can be obtained without killing the specimens. The latter can be especially important for a species that occurs at low densities (e.g. threatened or endangered species), because killing large numbers of fish to estimate age could affect population viability.

The tendency for muskellunge *Esox masquinongy* Mitchill to occur at low densities often is cited as a factor complicating management of muskellunge stocks (Graff 1986; Strand 1986). The cleithrum, a bone partly comprising the pectoral girdle, has been widely used to age muskellunge. Although the cleithrum has been found to yield accurate estimates of muskellunge age (Casselman 1979 as cited in Casselman 1996), specimens must be killed to obtain this structure. Other structures that do not require killing specimens, including scales and fin rays, also have been used to age muskellunge (Johnson 1971; Fitzgerald, Margenau & Copes 1997; Larscheid, Christianson, Gengerke & Jorgensen 1999), but ageing accuracies associated with these structures can be low. Johnson (1971) found that although pelvic fin ray age estimates for muskellunge were more than 70% accurate for age 10 and younger fish, age estimates were < 30% accurate for age 13 and older fish. Scales were found to be reliable only for muskellunge up to age 4 (Fitzgerald *et al.* 1997).

As muskellunge longevity in northern areas such as Wisconsin and Ontario can be in excess of 30 years, the low accuracies observed for scales and pelvic fin

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rays, particularly for older fish, present strong arguments against the use of these structures for ageing muskellunge in northern latitudes. However, longevity of a species in northern hemispheres can decrease along north-to-south latitudinal gradients (Beverton 1987; Braaten & Guy 2002). Indeed, maximum ages reported for muskellunge in southern areas such as West Virginia, Kentucky and Virginia have ranged from 8 to 13 years (Miles 1978; Brewer 1980; Jenkins & Burkhead 1993). Although the accuracies of these age estimates may be questionable because in some cases they were determined from scales, these maximum age observations, in conjunction with Johnson's (1971) finding that pelvic fin rays can be more than 70% accurate for age 10 and younger fish, suggest that pelvic fin rays are a potentially useful, non-lethal method for ageing muskellunge in southern US (e.g. Kentucky, North Carolina, Tennessee, Virginia, West Virginia) water bodies.

The purpose of this paper was to explore the potential utility of pelvic fin rays as ageing structures for muskellunge from a southern US water body. Specific objectives were to: (1) assess reader precision in ageing muskellunge by pelvic fin rays; (2) compare pelvic fin ray and cleithrum age estimates; (3) compare parameter estimates of von Bertalanffy growth equations derived from pelvic fin ray and cleithrum age estimates; and (4) compare observed length changes for tagged and recaptured muskellunge to length changes predicted from pelvic fin ray growth models.

Materials and methods

Muskellunge were collected by boat electric fishing and angling from sites throughout much of the Virginia section of the New River from 2000 to 2003. The New River is a warmwater river located in southwestern Virginia, that currently supports one of the state's top trophy-producing muskellunge fisheries (J. Williams, Virginia Department of Game and Inland Fisheries, personal communication). Lengths of captured fish were measured to the nearest mm, and fish were sexed based on the shape of the urogential papilla (LeBeau & Pageau 1989). The longest leading pelvic fin ray from each fish was clipped with wire cutters as close to the body as possible and stored in a coin envelope for subsequent ageing. Twenty-one of the captured fish were killed and their cleithra removed and sent to Cleithrum Project (Casselman & Crossman 1986) researchers for ageing. Cleithrum aged muskellunge ranged in total length from 593 to 1230 mm.

Approximately 150 muskellunge were tagged and released with a T-bar anchor tag (Hallprint Pty Ltd, Victor Harbor, South Australia), passive integrated transponder (PIT) tag (Biomark Inc., Meridian, ID, USA), or radiotelemetry transmitter (Advanced Telemetry Systems, Isanti, MN, USA) so that precise growth estimates from recaptured fish could be obtained. T-bar anchor tag and radiotelemetry transmitter presence on captured fish were checked visually, while presence and identification of PIT tags were determined using a Mini-Portable Reader (Biomark Inc.). Frequencies of radiotelemetry transmitters were identified with a R2000 receiver (Advanced Telemetry Systems).

Pelvic fin rays were transversely sliced into thin sections using a hand-held rotary cutter, mounted on glass microscope slides with a quick-drying epoxy, and ground and polished with 600 and 1500 grit sandpaper. Fin-ray sections were viewed through a SZ60 stereo-zoom microscope (Olympus America Inc., Melville, New York, USA), which was connected to a SAC-410NA video colour camera (Samsung Electronics America, Ridgefield Park, NJ, USA). Digital images of the fin-ray sections were created using Image-Pro Plus software (Media Cybernetics, Silver Springs, MD, USA). Fin-ray sections and digital images were prepared only for muskellunge longer than 500 mm, as young esocids often can be aged by modal analysis of length–frequency histograms (Casselman 1996).

Three readers independently aged each fish from the digital images of the sectioned fin rays. Precision among readers was assessed using age-bias plots and by calculating mean coefficients of variation (CV) in age estimates (Campana, Annand & McMillan 1995). Ages assigned to individual fish for comparison with cleithrum estimates and for constructing growth equations were based on majority rule. If age estimates from at least two readers agreed for a particular fish, then that age was assigned to the fish. If age estimates from all three readers differed, then the readers collectively examined the sectioned fin ray image and tried to reach a consensus regarding age. Pelvic fin ray and cleithrum age estimates were compared by calculating the exact and within 1-year agreement rates between the structures.

Sex-specific von Bertalanffy growth equations from both pelvic fin ray and cleithrum age estimates were fit by non-linear regression using PROC NLIN in SAS (SAS Institute Inc., Cary, NC, USA). Sex-specific growth equations were estimated within the same statistical model by use of an indicator variable. Because of inconsistencies in the shape of the fin ray sections, lengths at current ages (as opposed to back-calculated lengths at age) were used when modelling growth from the pelvic fin ray age estimates. Back-calculated lengths at age were used for estimating growth equations from the cleithrum age estimates. Length and age data for sampled muskellunge that were assigned age 1 from modal analysis of length–frequency histograms were included in growth models. This was necessary to ensure estimates of the t_0 parameter were accurate. Differences in coefficient estimates of pelvic fin ray and cleithrum growth equations were tested using sum-of-square-reduction *F*-tests (Quinn & Deriso 1999).

Observed length changes in tagged and recaptured muskellunge were compared with predicted length changes to evaluate how well individual growth patterns corresponded to fitted pelvic fin ray growth equations. Predicted length changes were calculated using

$$\Delta L_i = (L_\infty - L_{1i}) \cdot (1 - e^{-\kappa \cdot \Delta t_i})$$

where ΔL_i is the predicted length change (mm), Δt_i is the elapsed time between marking and recapture (years), L_{1i} is length at initial marking (mm), and L_∞ (asymptotic length, mm) and κ (Brody growth coefficient) are von Bertalanffy growth model parameters (Quinn & Deriso 1999). Estimated absolute error and the ratio of estimated absolute error to fish length at recapture were used to evaluate similarity between observed and predicted length changes.

To determine how ageing errors could affect estimation of growth equations, a simulation was conducted that involved generating random age errors, adding the random errors to the observed pelvic fin ray age estimates, and then re-estimating the pelvic fin ray growth equations based on the corrupted age data. The magnitude of the errors and the prevalence with which errors occurred were based on comparisons of the cleithrum and pelvic fin ray age estimates. The sequence of generating random age errors, adding the errors to the pelvic fin ray age estimates, and re-estimating the pelvic fin ray growth equations was repeated 1000 times. The observed ranges in coefficient estimates were used to evaluate how ageing errors affected coefficient precision.

Results

Pelvic fin rays were collected from 194 muskellunge. Age estimates for at least two of the three readers agreed for 87% of the fin ray sections examined. Exact agreement was 74% for Readers 1 and 2, 46% for Readers 1 and 3, and 49% for Readers 2 and 3. Within 1-year agreement was 94% for Readers 1 and 2, 76% for Readers 1 and 3, and 74% for Readers 2 and 3. Readers 1 and 2 gave consistent estimates for age 4 and younger fish, but Reader 2 typically underestimated age relative to Reader 1 for age 5 and older fish (Fig. 1). Relative to Readers 1 and 2, Reader 3 overestimated age for young fish but underestimated age for old fish. The mean CV in age estimates for all three readers was 17.8%. From a pairwise standpoint, the mean CV in age estimates was 5.6% for Readers 1 and 2, 17.5% for Readers 1 and 3, and 18.4% for Readers 2 and 3.

Age estimates agreed for 76% ($n = 16$) of the fish aged using both pelvic fin rays and cleithra. Pelvic fin rays overestimated age for two fish, and underestimated age for three fish relative to the cleithrum estimates. Pelvic fin ray and cleithrum age estimates disagreed only for age 3 and older fish. Within 1-year agreement of the pelvic fin ray and cleithrum age estimates was 100%.

Cleithrum and pelvic fin ray growth equations estimated for female muskellunge did not significantly differ ($F = 0.70$, $P = 0.551$). Although an overall difference in cleithrum and pelvic fin ray growth equations was detected for male muskellunge ($F = 16.13$; $P < 0.001$), comparisons of individual growth equation coefficients failed to detect any significant differences between the ageing structures (L_∞: $F = 0.00$, $P = 1.000$; κ: $F = 2.74$, $P = 0.100$, t_0: $F = 0.01$, $P = 0.925$).

Pseudo-r^2s for the pelvic fin ray and cleithrum growth models were 96.8% and 93.2%, respectively, indicating that the fitted growth models explained substantial amounts of variability in the observed length-at-age data. However, based on plots of predicted vs observed lengths at age (Fig. 2), it appeared that L_∞ for both sexes was poorly predicted with the fitted models. Asymptotic lengths equalled approximately 1060 and 1200 mm for male and female muskellunge, respectively, when estimated from both pelvic fin rays and cleithra. However, length data from the present sampling and from data obtained from local anglers and taxidermists indicated that both sexes routinely attained longer lengths. Consequently, the growth models were also estimated with κ and t_0 as the only model parameters to be estimated. Ford plots of length at age t vs length at age $t + 1$ based on both the cleithrum and pelvic fin ray age estimates indicated that asymptotic lengths of 1100 and 1300 mm were appropriate for male and female muskellunge; thus, these lengths were used in the growth equations as fixed values. Re-estimation of the pelvic fin ray and cleithrum growth models with the fixed asymptotic lengths visually improved model fit for the older age classes, and resulted in only marginal reductions in

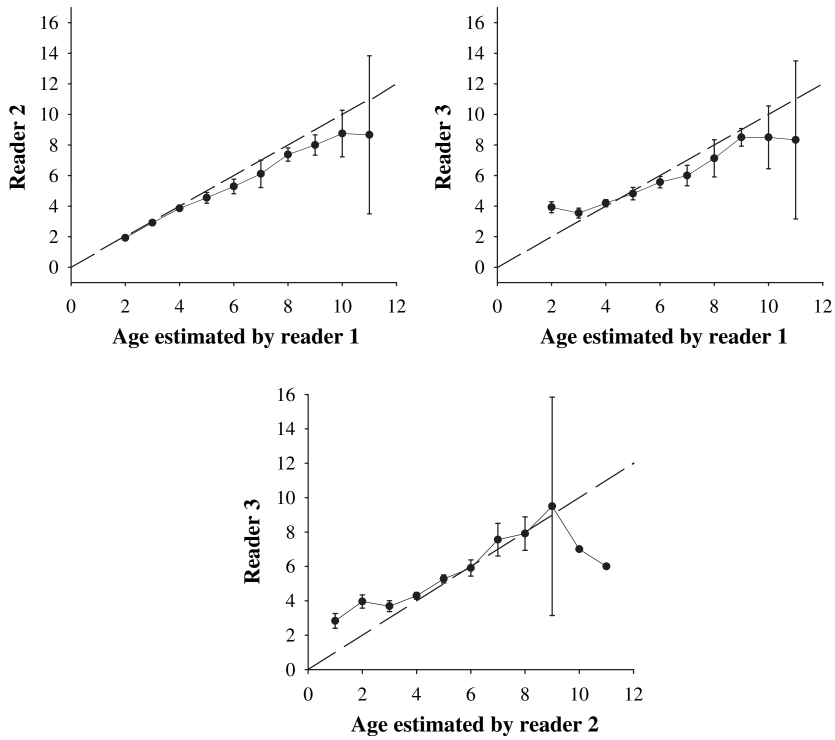

Figure 1. Pairwise age–bias plots calculated for the three readers who aged the muskellunge pelvic fin rays. Each plot indicates the mean age and associated 95% confidence interval for the ages assigned by one reader relative to the ages assigned by another reader. The dashed line represents perfect (1:1) agreement between the readers.

model pseudo-r^2s (Fig. 2). Pseudo-r^2 declined from 96.8% to 96.5% for the pelvic fin ray growth model, and from 93.2% to 92.9% for the cleithrum growth model.

Thirteen muskellunge were captured on multiple occasions when sampling. Elapsed time between tag and recapture dates ranged from 51 to 785 days. Observed length changes of recaptured fish ranged from 0 to 303 mm (Table 1). Length changes predicted from the two-parameter pelvic fin ray growth model (fixed L_∞) deviated from actual length changes by (−)43 to (+)99 mm, with an estimated absolute error of 30 ± 14 mm ($\bar{x}$ ± 2·SE). In terms of percentage of fish length at recapture, deviation of predicted from observed length changes was 3.7 ± 2.2% ($\bar{x}$ ± 2 · SE). Estimated absolute error for the original growth model was 26 ± 11 mm ($\bar{x}$ ± 2 · SE), indicating that the original growth model (unknown L_∞) better matched the observed growth of the tagged and recaptured fish than the re-estimated growth model (fixed L_∞).

Based on the observed agreement between the cleithrum and pelvic fin ray age estimates, errors of ± 1 year were randomly added to 24% of the pelvic fin ray age estimates for fish that were 3 years of age and older. When the growth equation for male muskellunge was estimated with the errors added to the pelvic fin ray age estimates, coefficient estimates ranged from 1036 to 1064 mm for L_∞, 0.452 to 0.582 for κ and −0.127 to 0.095 for t_0. For female muskellunge, coefficient estimates ranged from 1136 to 1209 mm for L_∞, 0.367 to 0.503 for κ and −0.195 to 0.065 for t_0.

Discussion

When fish age is estimated from structures such as scales, rays, or cleithra, two types of error (process and interpretive) can affect accuracy of the age estimates (Campana 2001). Process error results from growth rings not forming consistently on the structure throughout fish life spans, whereas interpretive error results from the inability of readers to distinguish annuli from other features on the structure (Campana 2001). To prevent process error, it is recommended that an age validation study be conducted for whichever structure is used to ensure that growth rings are formed at predictable and repeated frequencies (DeVries & Frie 1996; Campana 2001). Age validation studies can take a number of forms, such as release and recapture of known-age and marked fish and

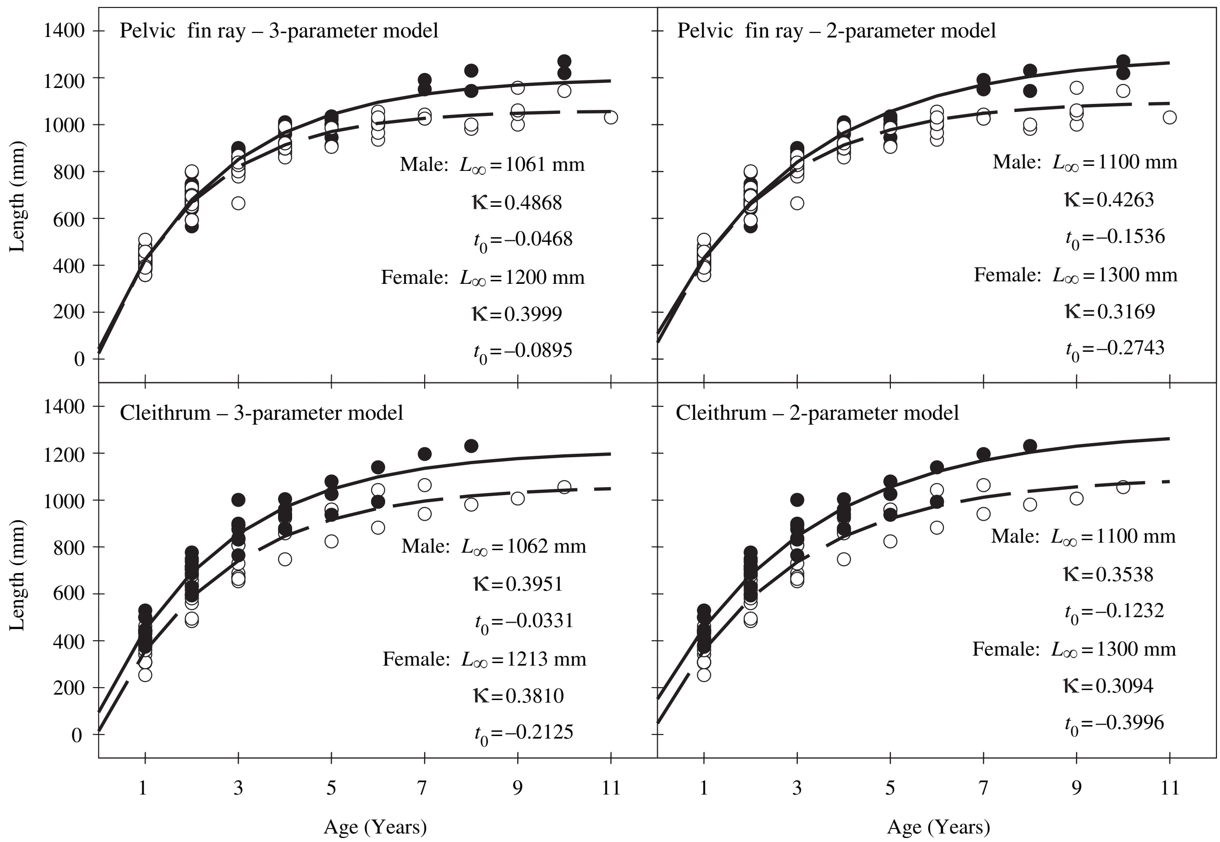

Figure 2. Predicted and observed lengths at age for the von Bertalanffy growth equations fit to the pelvic fin ray and cleithrum age estimates (solid dots and line = female growth equation; open dots and dashed line = male growth equation).

radio-chemical dating of calcified structures (Campana 2001).

None of the methods that were used in this research validated pelvic fin rays as ageing structures for muskellunge because actual ages of fish were unknown. However, assuming that the cleithrum age estimates were accurate, then the 76% exact and 100% within 1-year agreement rates between the cleithrum and pelvic fin ray age estimates, as well as the finding of no significant differences between the coefficients of the cleithrum and pelvic fin ray growth equations, support the contention that pelvic fin rays are potentially useful structures for ageing muskellunge in southern US water bodies. The similarity between observed length changes for tagged and recaptured fish, and length changes predicted from pelvic fin ray growth equations also is indicative of the potential utility of pelvic fin rays as ageing structures.

The precision among readers that was observed in the present study (mean CV = 17.8%) suggests that ageing of pelvic fin rays may be prone to interpretive errors. A mean CV of 17.8% is near the lower-end of precision levels reported by Campana (2001). Several steps are suggested to improve precision among readers for those wishing to age muskellunge with pelvic fin rays. First, it would be beneficial for all readers to have some prior ageing experience, whether it is with fin rays, scales, or some other ageing structure. Although none of the readers in the present study had prior ageing experience with fin rays, Readers 1 and 2 had previously aged fish using scales or otoliths. Conversely, Reader 3 had no prior ageing experience. Although all readers were aware of differences between annuli and false-annuli, Reader 3 appeared more likely to count what the other readers identified as false annuli when estimating ages for younger fish. This caused the precision levels of Reader 3 with the other readers to be lower then the observed precision level between Readers 1 and 2 (mean CV of 17.5% and 18.4% vs a mean CV of 5.6%). The precision level between Readers 1 and 2 (CV = 5.6%) was closer to the reference precision level (CV = 5%) identified by Campana (2001). It is also recommended that strict criteria be developed as to how readers will

Table 1. Observed length changes of marked and recaptured muskellunge and length changes predicted from two-parameter (fixed L_∞) pelvic fin ray growth equations. Estimated error is the absolute value of the difference between observed length change and predicted length change, and relative estimated error is the estimated error/recapture length

Sex	Length at tagging (mm)	Elapsed time (years)	Observed length change (mm)	Predicted length change (mm)	95% CL of predicted length change (mm)	Estimated error (mm)	Relative estimated error (%)
Female	1219	0.90	37	20	19–21	17	1.35
Female	1010	0.28	44	24	23–26	20	1.90
Female	958	0.98	48	91	87–95	43	4.27
Female	995	0.97	55	81	77–84	26	2.48
Female	1054	1.03	36	69	65–72	33	3.03
Female	352	0.76	303	204	194–214	99	15.11
Female	364	1.17	299	289	276–302	10	1.51
Male	376	0.94	293	238	225–251	55	8.22
Male	650	1.86	220	246	235–257	26	2.99
Male	825	0.14	5	16	15–17	1	1.33
Male	669	1.11	158	162	154–171	4	0.48
Male	985	1.00	0	40	38–42	40	4.06
Male	682	2.15	258	251	240–261	7	0.74
						$\bar{x} = 30$ mm	$\bar{x} = 3.65\%$

distinguish between the nucleus of the pelvic fin ray and the first annulus. Many of the discrepancies between Readers 1 and 2 stemmed from disagreements as to whether a particular feature was a nucleus or first annulus; thus, establishing strict criteria in advance should lead to improved precision and accuracy. With populations that are enhanced or maintained through stocking, marking fish early in life with a chemical mark, such as oxytetracyclene, also could assist readers in distinguishing between the nucleus and the first annulus.

The maximum age of muskellunge observed in the present study was 11 years, which is within the range reported for southern US muskellunge stocks (Miles 1978; Brewer 1980; Jenkins & Burkhead 1993). Given these maximum age estimates, there may be some concern that ageing errors of even 1 year may be too large and that pelvic fin rays simply should not be used to age muskellunge even in southern US regions. The significance of the errors will be situationally dependent. The simulation of how ageing errors affected growth equation estimation found that estimates of L_∞ could range from 1036 to 1064 and 1136 to 1209 mm for males and females, respectively, and estimates of κ could range from 0.452 to 0.582 and 0.367 to 0.503 for males and females, respectively. In some cases, such estimate ranges may be sufficiently precise given the management question at hand. In other cases, such observed ranges may be too large. Fishery managers ultimately will need to decide for themselves whether the potential for ageing errors outweighs the benefit of not needing to sacrifice specimens to obtain age estimates.

The purpose of this research was to conduct an initial evaluation concerning the usefulness of pelvic fin rays as ageing structures for southern muskellunge. Pelvic fin rays and cleithra had exact and within 1-year agreement rates of 76% and 100%, respectively, and there was little observed difference in growth equations estimated from the different structures. Additionally, length changes predicted from growth equations based on pelvic fin ray age estimates deviated from observed length changes an average of 30 mm for muskellunge that were tagged and recaptured. These findings support the conclusion that pelvic fin rays are useful structures for ageing muskellunge from southern US waters. Precision associated with pelvic fin ray age estimates can be low particularly if readers have little or no prior ageing experience; thus, readers should receive extensive training in fish ageing before attempting to age muskellunge by fin rays. Age validation studies still need to be conducted to ensure that growth rings form consistently on pelvic fin rays throughout muskellunge life spans.

Acknowledgments

Funding for this research was provided by the Acorn-Alcinda Foundation and the Virginia Department of Game Inland Fisheries through Federal Aid in Sportfish Restoration Project F-122-R. The authors thank S. Hale and S. Staples for assisting with the fin ray ageing, J. Casselman for conducting the cleithrum ageing, A. Scott for assisting with angling collections, and J. Williams for assisting with electrofishing collections.

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