**Validity of age estimates from muskellunge (Esox masquinongy) fin rays and associated effects on estimates of growth**

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Canadian Journal of Fisheries and Aquatic Sciences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>cjfas-2018-0404.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>20-Mar-2019</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Crane, Derek P.; Coastal Carolina University</td>
</tr>
<tr>
<td></td>
<td>Cornett, Marinda; Coastal Carolina University</td>
</tr>
<tr>
<td></td>
<td>Bauerlien, Cory; Coastal Carolina University</td>
</tr>
<tr>
<td></td>
<td>Hawkins, Michael; Coastal Carolina University</td>
</tr>
<tr>
<td></td>
<td>Isermann, Daniel; U. S. Geological Survey, Wisconsin Cooperative Fishery Research Unit</td>
</tr>
<tr>
<td></td>
<td>Hansbarger, Jeff; West Virginia Division of Natural Resources</td>
</tr>
<tr>
<td></td>
<td>Kapuscinski, Kevin; Lake Superior State University</td>
</tr>
<tr>
<td></td>
<td>Meerbeek, Jonathan; Iowa Department of Natural Resources</td>
</tr>
<tr>
<td></td>
<td>Simonson, Timothy; Wisconsin Department of Natural Resources</td>
</tr>
<tr>
<td></td>
<td>Kampa, Jeffrey; Wisconsin Department of Natural Resources</td>
</tr>
<tr>
<td>Keyword:</td>
<td>AGE DETERMINATION &lt; General, validation, GROWTH &lt; General, muskellunge, fin rays</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue? :</td>
<td>Not applicable (regular submission)</td>
</tr>
</tbody>
</table>
Validity of age estimates from muskellunge *Esox masquinongy* fin rays and associated effects on estimates of growth

*Derek P. Crane*

Coastal Carolina University, Department of Biology, 107 Chanticleer Drive East, Conway, South Carolina 29526, USA. Email: dcrane@coastal.edu; phone: (843) 349-4065

Marinda R. Cornett

Coastal Carolina University, Department of Biology, 107 Chanticleer Drive East, Conway, South Carolina 29526, USA. Email: rincornett@gmail.com

Cory J. Bauerlien

Coastal Carolina University, Department of Biology, 107 Chanticleer Drive East, Conway, South Carolina 29526, USA. Email: cjbauerlien@gmail.com

Michael L. Hawkins

Coastal Carolina University, Department of Biology, 107 Chanticleer Drive East, Conway, South Carolina 29526, USA. Email: mlhawkins@coastal.edu

Daniel A. Isermann

U.S. Geological Survey, Wisconsin Cooperative Fishery Research Unit, College of Natural Resources, University of Wisconsin–Stevens Point, 800 Reserve Street, Stevens Point, Wisconsin 54481, USA. Email: dan.isermann@uwsp.edu
Jeff L. Hansbarger
West Virginia Division of Natural Resources, Forks of Coal State Natural Area, 50 Rocky Branch Road, Alum Creek, West Virginia 25003, USA. Email: jeff.l.hansbarger@wv.gov

Kevin L. Kapuscinski
Lake Superior State University, School of Biological Sciences, 650 W. Easterday Avenue, Sault Ste. Marie, Michigan, 49783, USA; kkapuscinski@lssu.edu

Jonathan R. Meerbeek
Iowa Department of Natural Resources, 122 252nd Avenue, Spirit Lake, Iowa, 51360, USA. Email: jonathan.meerbeek@dnr.iowa.gov

Timothy D. Simonson
Wisconsin Department of Natural Resources, 3911 Fish Hatchery Road, Fitchburg, Wisconsin 53711, USA. Email: Timothy.Simonson@wisconsin.gov

Jeffrey M. Kampa
Wisconsin Department of Natural Resources, 810 W Maple Street Spooner, Wisconsin 54801, USA. Email: jeffrey.kampa@wisconsin.gov

*Corresponding author
Abstract

Accurate age estimates are critical for understanding life histories of fishes and developing management strategies for fish populations. However, validation of age estimates requires known-age fish, which are often lacking. We used known-age (ages 1–25) muskellunge (*Esox masquinongy*) to determine the precision and accuracy of age estimates from fin rays. We also determined if fin location (anal or pelvic), fin ray number, and preparation methods affected accuracy and precision. Lastly, we determined if von Bertalanffy growth parameters estimated from fin ray ages were similar to parameters estimated from known ages. Precision and accuracy of age estimates from anal and pelvic rays were similar and estimates were relatively precise (coefficient of variation = 8.5%) and accurate (mean absolute difference from known age = 0.85 y) for ages 4–15, but ages were overestimated for younger fish and underestimated for older fish. Growth models based on estimated age were similar to models based on known age. Anal and pelvic rays offer a non-lethal alternative for age estimation of muskellunge ages 4–15, and for producing reliable estimates of growth.
Introduction

Accurate and precise estimates of age are essential for understanding life history characteristics (e.g., longevity, age-at-maturity) and population dynamics of animals (e.g., growth, mortality, recruitment [Quist and Isermann 2017]). Counting annuli in calcified structures such as scales, otoliths, cleithra, or fin rays and spines is the most common method for estimating age of fishes. Assessing precision of age estimates is relatively easy because it only requires repeated readings by independent readers and does not require known-age fish. Studies comparing precision of estimates from various calcified structures have been conducted for numerous fishes (Maceina et al. 2007). However, robust studies assessing accuracy of estimates (i.e., validation) are comparatively rare (Campana 2001; Maceina et al. 2007) because few or no known-age fish have been available for many species. Moreover, samples from known-age individuals representing the lifespan of moderately long-lived and long-lived fishes are exceedingly rare (Spurgeon et al. 2015). Marking fish with tags that have high retention rates (>90%; e.g., passive integrated transponders) has become increasingly common over the last 20 years, thus providing greater availability of known-age fish for some species.

Use of nonlethal structures for estimating age of some fishes has increased due to conservation concerns (e.g., sturgeons), management objectives (e.g., management of trophy fisheries), and societal concerns (e.g., concerns about sacrificing fish when most anglers practice catch and release for a particular species; Fischer and Koch 2017; Klein et al. 2017).

Historically, scales were the most common nonlethal structure used to estimate age of fishes; however, use of scales for age estimation has decreased because estimates from scales have been observed to have lower accuracy (negative bias) and precision compared to other calcified structures for many fishes (McInerny 2017). Fin rays and spines have been used as nonlethal
structures to estimate age for a diversity of cartilaginous and bony fishes, and although age estimates from fin rays and spines have been found to be similar to age estimates from otoliths for several species, underestimation of age compared to otoliths has also been reported (Fischer and Koch 2017). Further, age estimates from fin rays and spines have not been validated for most fishes they have been used for (Campana 2001; Fischer and Koch 2017). For example, in a global review of freshwater fish validation studies, Spurgeon et al. (2015) only documented validation studies for five species using fin rays or spines from known-age fish. Of the validation studies for fin rays or spines identified by Spurgeon et al. (2015), only one study (Chilton and Bilton 1986) contained known-age fish representing the life span of the species (Chinook salmon [*Oncorhynchus tshawytscha*]), and that species is short-lived (average lifespan = 3-6 y; Behnke 2002).

The muskellunge (*Esox masquinongy*) is a moderately long-lived species (individuals age 10-15 y are commonly observed, maximum estimated age = 30 y [Casselman et al. 1999]). Counting annuli on cleithra has historically been the most common method for estimating age of esocids, largely because annual increment formation in cleithra has been validated for northern pike (*Esox lucius*; Casselman 1974, 1979; Babaluk and Craig 1990; Laine et al. 1991). However, age estimates from cleithra have not been validated for muskellunge. Further, removal of cleithra requires euthanizing fish, and sacrificing sufficient numbers of muskellunge to estimate population characteristics is inconsistent with muskellunge management objectives, which focus on developing and sustaining trophy fisheries where release rates by anglers are typically ≥ 99% (Casselman et al. 2017; Richards 2017; Tallion and Heinbuck 2017). Additionally, muskellunge naturally occur at low densities (e.g., median population density in high-density lakes in Wisconsin is only about 1.1 mature muskellunge/ha; T. Simonson, unpublished data); thus,
collecting a sufficient sample to estimate population characteristics using ages estimated from a lethal method may require several years of sampling to avoid negative effects on a fishery due to overharvest for scientific collection. Therefore, a nonlethal method for estimating age of muskellunge is desirable, but little validation of these methods has occurred (i.e., two studies; Johnson 1971 and Fitzgerald et al. 1997). Johnson (1971) and Fitzgerald et al. (1997) observed that muskellunge age estimates from scales were inaccurate. Johnson (1971) observed greater accuracy for age estimates made from sectioned fin rays compared to scales; however, he only evaluated accuracy of estimates for fish up to age 13 from northern Wisconsin and found that accuracy declined substantially for fish > age 9. Although known-age fish were not available, Brenden et al. (2006) demonstrated ages estimated from pelvic fin rays agreed with ages estimated from cleithra for 76% of muskellunge collected from the New River, Virginia, and observed differences were all within ± 1 y.

Choice of fin ray (anal, pectoral, or pelvic) and preparation techniques have varied among previous evaluations of muskellunge age estimation (Johnson 1971; Brenden et al. 2006; Ward et al. 2017). Additionally, the technologies (e.g., microscopes, lighting, computer software) used in the age estimation process have changed substantially since 1971, when the validation study of Johnson was completed, and some of these improvements likely provide for easier detection of annuli in fin rays, especially for older fish (i.e., > age 10). Tagging or marking of age-0 and age-1 muskellunge before stocking has become relatively common (Larscheid et al. 1999; Wolter et al. 2013; Rude et al. 2017), providing a larger pool of known-age fish that includes fish that are ≥ 10 y older than the oldest fish included by Johnson (1971). Consequently, evaluation of methodology (e.g., fin ray choice and preparation techniques) and additional assessment of the accuracy of fin ray age estimates for muskellunge are warranted and the
increased availability of known-age fish provides the opportunity to complete this type of
evaluation.

The goal of our study was to outline specific methods for estimating muskellunge age
from fin rays and to determine if fin rays provide age estimates that are sufficiently accurate to
describe age and growth for muskellunge populations. We used known-age muskellunge
obtained from 10 waters in five states to determine the precision and accuracy of anal and pelvic
fin ray estimates, which included assessment of accuracy for fish > age 15. We also determined
if reader experience, fin location (anal or pelvic), fin ray number, and different preparation
methods affected the accuracy and precision of age estimates. Lastly, we determined if von
Bertalanffy growth parameters estimated from fin ray ages were similar to parameters estimated
from known ages. Results from this work can be used to guide future population assessments
and research on muskellunge biology and ecology, and our approach can be used to investigate
the value of estimated ages from fin rays for other moderately long-lived fishes.

Methods

This study was reviewed and approved by the Institutional Animal Care and Use
Committee at Coastal Carolina University. Anal and pelvic fin rays were collected from
muskellunge in seven waters in the northern portion (Iowa and Wisconsin) of their distribution
and three waters in the southern portion of their distribution (southern Illinois, Kentucky, and
Virginia) during collection of brood stock and population assessments in spring 2015 and 2016
(Table 1, Fig. 1). Anal fin rays were collected from 317 fish; pelvic fin rays were only collected
from 50 fish. All samples were collected from fish of known age as determined by unique marks
or tags (combination freeze brand and passive integrated transponder [PIT] tag, PIT tag, vinyl
anchor tag, or fin clip) applied to fish before stocking that allowed for identification of individuals or specific year classes. We clipped fin rays from the anterior portions of the anal and pelvic fins as close to the body as possible and at a right angle to the rays using diagonal cutting pliers (Fischer and Koch 2017).

Assessment of methods for estimating age from fin rays

To provide guidance for sampling and preparation of fin rays, we investigated the effects of section width (0.3, 0.5, 0.7 mm), anal fin ray number (3 vs. 4; count does not include the first rudimentary ray), pelvic fin ray number (1 vs. 2), and fin location (anal vs pelvic fin). Because pelvic fin rays were only available for a subset of our total sample, we examined the effects of section width and section location for anal fin rays only. We used a stratified random sampling approach to select a subset ($n = 71$) of anal fin samples from Iowa fish because we had the largest number of samples and broadest age range for these populations. Both the third and fourth fin rays were available for all fish included in this sample. Strata were based on age groups (ages 3-5, 6-8, 9-11, 13-17, and 18-21), and the number of samples from each stratum (3-6) was based on availability of samples within each age range. Section widths were randomly assigned to individual fish ($n = 23$ for 0.3 and 0.5 mm; $n = 25$ for 0.7 mm), so that both the third and fourth rays from a given fish were sectioned at the same width.

Individual fin rays were excised and cleaned prior to being sectioned. To clean fin rays, we briefly soaked them in water to loosen excess skin and tissue and then removed the tissue with tweezers and a scalpel. Then, we mounted individual rays in clear epoxy (EpoKwick, Buehler, Lake Bluff, Illinois) following Koch and Quist (2007), except we used plastic drinking straws instead of centrifuge tubes to act as a mold for the epoxy. After allowing the epoxy to
cure for at least 24 h, we used an IsoMet 1000 Precision Low Speed saw (Buehler, Lake Bluff, Illinois) to cut sections at the assigned widths from the base of the rays. Three readers independently estimated ages for all sections. Reader experience for this portion of the study ranged from 6 mo (hundreds of structures viewed) to 20 y (>10,000 structures). All readers viewed a training set (samples not included in the study) of fin rays from known-age (ages 3-18) muskellunge before reading structures for this study. Prior to examination, sectioned fin rays were wiped with immersion oil (type A) to remove any residue from the sectioning process. Samples were then coated with immersion oil and viewed using a zoom stereo microscope (oblique, brightfield, or darkfield transmitted light) and associated camera and software. Readers were allowed to use the transmitted light setting(s) of their choice; however, switching back and forth between oblique and darkfield settings was helpful in identifying annuli near the edge for older fish. Ages were estimated by counting annuli (translucent zones), and the edge of the fin ray was counted as an annulus because fish were sampled early in the spring and the translucent zone corresponding to the previous annulus was not visible on the edge of the structure in most samples (Fig. 2). All samples of a specific section width were viewed before viewing samples of another section width, and the viewing order within each width treatment was haphazardly selected. Because readers for this portion of the study worked in three different labs, each reader used a different microscope, camera, and software to examine the structures. However, all viewing stations consisted of research grade equipment that is commonly used in age and growth labs, and we assumed that equipment did not affect the accuracy of estimates. All data were analyzed using R statistical software within RStudio (R Core Team, 2016; R Version 3.3.1; RStudio Team, version 1.0.153) and Microsoft Excel (Microsoft, Redmond,
Washington), and relationships were considered statistically significant at an alpha level of 0.05.

To determine if precision and accuracy of age estimates differed between the third and fourth anal fin rays or based on the width of fin ray sections, we first used the “FSA” package in R (Ogle 2018; function: “agePrecision”; version 0.8.20) and Excel to calculate a series of descriptive statistics for each specific fin ray number and section width treatment: (1) percent of samples that age estimates agreed among all readers (sample = individual fish-fin ray number-section width combination), (2) percent of samples with consensus agreement (at least 2 of 3 reader ages in agreement), (3) mean percent of estimated ages that were correct (calculated for each reader and then averaged across readers), and (4) mean percent of estimated ages that were within 1 y of known age (calculated for each reader and then averaged across readers). Next, we calculated coefficient of variation (CV; calculated for each sample), mean absolute difference in estimated age between reader pairs (MAD\text{reader}; absolute difference averaged across pairs for each sample) and mean estimated difference from known age (MAD\text{known}; averaged across readers for each sample). The CV provided a measure of precision that was scaled to the mean estimated age, and MAD\text{reader} and MAD\text{known} provided measures of average absolute disagreement between readers and accuracy of estimates in the original unit of years.

We used the “lme4” package (Bates et al. 2015; function = “lmer”; version 1.1-18-1) to fit linear mixed effects models to determine the effects of section width and anal fin ray number on CV, MAD\text{reader}, and MAD\text{known}. The CV, MAD\text{reader}, or MAD\text{known} were the response variables, and section width (categorical), ray number (i.e., third anal ray, fourth anal ray), and known age were the fixed effects. We did not include interactions in the models due to sample size limitations for each fin ray number and section width combination. Fish ID number was incorporated as a random intercept within each model. We included known age in the models to
test the assumptions that CV, MAD\textsubscript{reader}, and MAD\textsubscript{known} did not vary with age and to determine if mean values for CV, MAD\textsubscript{reader}, and MAD\textsubscript{known} (averaged across ages) could be reported or if age dependent values for the response variables were necessary (Bauerlien et al. 2018). An orthogonal quadratic term for known age was included using the “poly()” function in the analysis for MAD\textsubscript{known} because scatterplots of age and MAD\textsubscript{known} indicated a nonlinear relationship. We included fish ID number as a random effect because two samples were read for each fish (one from the third anal ray and one from the fourth anal ray), so estimates for each sample from an individual were not independent. We tested for relationships between the fixed effects and the response variables using the “anova” function (type III Wald $F$-test with Kenward-Roger estimated degrees of freedom [df]; see Singmann and Kellen In press) in the “lmerTest” package (Kuznetsova et al. 2017; version 3.0-1). When significant main effects were observed, we tested for differences among treatments at ages 5, 10, 15 and 20 using the “lsmeans” function in the “lsmeans” package (Lenth 2016; version 2.27-62). We specified “mode=kenward-roger” to use the Kenward-Roger method (Kenward and Roger 1997) to estimate denominator degrees of freedom and used the “tukey” option to adjust for multiple pairwise comparisons.

Because reader experience may affect interpretation of annuli from muskellunge fin rays (Brenden et al. 2006), we used a linear mixed effects model to compare that accuracy of age estimates (absolute difference from known age) among readers. Reader name was included as a fixed effect and fish ID number was again included as a random effect. This analysis was not included with the previously described analysis that examined the effects of ray number and section width because the response variables in those analyses were based on averaging across readers (CV and MAD\textsubscript{known}) or reader pairs (MAD\textsubscript{reader}), whereas this analysis focused on making comparisons among readers. All significance testing was completed as previously
described, except known age was not included in the model because all readers estimated ages for the same fish (i.e., we did not need to control for the effects of age on accuracy).

To determine if accuracy and precision of age estimates differed between anal fin rays and pelvic fin rays and between pelvic fin rays 1 and 2, we examined rays removed from 50 Iowa muskellunge that both anal and pelvic rays were collected from. Based on the results of analyses described in the previous paragraph: (1) we only used the fourth anal fin ray to make comparisons with pelvic rays; (2) sections of pelvic rays were prepared using the same methods as described for anal fin rays, but all sections were cut from the base of the rays at a width of 0.7 mm, and (3) three readers with little experience were used to provide age estimates, so that age estimates were all made at the same laboratory. All readers were trained with the same set of known-age training samples as previously described, and age estimates from these three readers were used for the remainder of the study. We calculated the same statistics to describe precision and accuracy that were previously mentioned. We also compared age estimates across fin rays for each fish to calculate percent agreement in estimates between each fin ray. Lastly, we used linear mixed effects models to compare CV, MAD$_{\text{reader}}$, and MAD$_{\text{known}}$ among estimates from the fourth anal ray, and first and second pelvic rays. Fish ID number was included as a random effect because the fourth anal ray and first and second pelvic rays were used for each fish. Overall $F$-tests and post-hoc tests were also conducted as previously described, except the larger sample size for this portion of the study allowed for inclusion of a term for the interaction between known age and fin ray location.

*Overall precision, accuracy, and bias of ages estimated from anal fin rays*
We used anal fin rays from 317 muskellunge to investigate the overall precision, accuracy, and bias of age estimates; 105 samples from southern populations and 212 samples from northern populations. Known-age fish from northern populations were available from a broader age range (ages 3-25 y; median age = 7) compared to southern populations (ages 1-11 y; median age = 2 y; Fig. 3). Ages were estimated following the same protocols that were described previously, and we calculated the same statistics to describe precision and accuracy. To evaluate if there were systematic biases in age estimates for each reader, we created age difference plots that plotted the difference between estimated age and known age as a function of known age (Campana et al. 1995) using the FSA package (referred to as age-bias plots in the FSA package; Ogle 2018; function: “ageBias”; version 0.8.20). For each age difference plot, we used a built-in function within “ageBias” to test for differences between estimated age and known age for all known ages with at least three samples (one sample t-test adjusted for multiple comparisons using the Holm method; Ogle 2016). Next, we used linear regression (“lm” function in R) to test for and estimate effects of known age on CV, MAD_reader, and MAD_known. An orthogonal quadratic term was included for known age in all models because scatterplots of the response variables vs. known age indicated nonlinear relationships. Although we did not include a quadratic term in the analyses investigating the effects of fin ray preparation and selection on CV and MAD_reader, we included it here because there was a much larger sample size that included broader age range for this portion of the study, and nonlinear relationships between the response variables and known age were apparent. Unlike analyses for the methods portion of the study, we did not need to use a mixed model because only one estimate was provided for each fish by each reader and absolute differences were averaged across readers.
Effects of age estimation error on growth estimates

We fit separate von Bertalanffy growth models (Beverton and Holt 1957) for male \( n = 95 \) and female \( n = 83 \) muskellunge from three interconnected natural lakes in Iowa (Spirit, East Okoboji, and West Okoboji Lakes) based on known ages and ages estimated by each reader to determine if age estimation error affected growth estimates for this population. We fit separate models for male and female muskellunge because muskellunge display sexually dimorphic growth (Casselman et al. 1999) and models for each sex were defined by the function:

\[
L_t = L_\infty (1-e^{-K(t-t_0)}),
\]

where \( L_t = \) mean length-at-age \( t \) (TL in mm); \( L_\infty = \) maximum mean length; \( K = \) how quickly mean length approaches \( L_\infty \); and \( t_0 = \) a theoretical parameter that represents the age at a mean length of zero (Ogle et al. 2017). We fit separate models for each reader and based on known ages using the “nls” function in R, and selected starting values for \( L_\infty, K, \) and \( t_0 \) using the “vbStarts” function (Ogle 2018; “FSA” package; version 0.8.20). Next, we calculated bootstrapped 95% confidence intervals (CIs) for each parameter using “nlsBoot” (Ogle 2016). Finally, we plotted each sex-specific model to compare predicted lengths-at-age based on reader age estimates with lengths-at-age from the known-age models.

Results

Assessment of methods for estimating age from fin rays
Precision and accuracy of age estimates varied little between the third and fourth anal fin rays, and among section widths. The 0.7-mm sections cut from the fourth anal ray had the greatest percent of estimates that were agreed upon by all readers (28%), percent of consensus estimates (84%), percent of estimates that were correct (57%), and percent of estimates within 1 y (83%; Table 2). MAD\text{reader} increased with known age ($F = 41.90; df = 1, 67; p < 0.001$), but did not differ between fin ray numbers ($F = 1.38; df = 1, 70; p = 0.245$) or among section widths ($F = 0.03; df = 2, 66; p = 0.973$; Fig. 4A). The CV did not vary across ages ($F = 0.04; df = 1, 67; p = 0.840$) and did not differ between fin ray numbers ($F = 1.8; df = 1, 70; p = 0.18$) or among section widths ($F = 0.25; df = 2, 67; p = 0.780$; Fig. 4B). MAD\text{known} increased with age of fish ($F = 39.13; df = 2, 66; p < 0.001$), and differed between the third and fourth fin rays ($F = 6.19; df = 1, 70; p = 0.015$), but did not differ among section widths ($F = 3.10; df = 2, 66; p = 0.052$).

MAD\text{known} was less for the samples cut from the fourth compared to the third fin ray (Fig. 4C). However, coefficient estimates indicated that MAD\text{known} only differed by about 0.2 y between the third and fourth rays. Finally, MAD\text{known} differed among readers ($F = 10.46; df = 2, 379.27; p < 0.001$), and was greatest for the most experienced reader (MAD\text{known} for reader with 20 y experience = 1.42 y; MAD\text{known} for reader with 7 y experience = 1.05 y; MAD\text{known} for inexperienced reader = 0.94 y).

Although there were some small discrepancies among metrics, precision and accuracy of age estimates from the fourth anal ray, first pelvic ray, and second pelvic ray were similar. We observed greater agreement among all readers and percent of estimates that were correct for estimates from the fourth anal ray and second pelvic ray compared to the first pelvic ray, but percent consensus agreement and percentages of estimates within 1 y of known age were similar among the three structures (Table 2). Although all structures had similar precision and accuracy,
age estimates frequently differed among structures for a given fish. Between structure age
estimates (anal vs. first pelvic, anal vs second pelvic, and first pelvic vs second pelvic) only
agreed for 40-44% of samples, but estimates were within 1 y for > 70% of samples. MAD$_{reader}$
increased with age for all structures (structure x known age interaction: $F = 7.69; df = 4, 96; p <$
0.001), and was lower for the first pelvic compared to the second pelvic and anal rays for age-15
and age-20 fish ($p < 0.05$; Fig. 5A), but did not differ among structures for other ages. Similarly,
there was a significant interaction between the known age of fish and structure type (anal, first
pelvic, second pelvic) in the model for CV ($F = 7.55; df = 2, 96; p < 0.001$); CV increased with
age for the second pelvic and anal rays, but declined slightly with age for the first pelvic ray (Fig.
5B). Ages estimated from the first pelvic ray had a greater CV than for ages estimated from the
second pelvic and anal rays for age-5 fish ($p < 0.05$), but lower CV for age-20 fish ($p < 0.05$; Fig.
5B). MAD$_{known}$ increased with age ($F = 147.61; df = 2, 47; p < 0.001$), but did not differ among
the first and second pelvic rays and the fourth anal ray ($F = 0.13; df = 2, 98; p = 0.876$; Fig. 5C).

Overall precision, accuracy, and bias of ages estimate from anal fin rays
Because precision and accuracy of estimates varied little between anal fin ray numbers or
among section widths, we included sections from the base of the third and fourth anal rays that
were used to investigate the effects of fin ray preparation to increase the sample size for this
portion of the study. Although only 48% of estimates were correct, 78% of estimates were within
1 y of known age (Table 2). Complete agreement among readers was low (20%), but consensus
was observed for 80% of fish (Table 2). When consensus was observed, 63% of estimates were
correct.
MAD\textsubscript{reader}, CV, and MAD\textsubscript{known} were all related to known age, ($p < 0.001$ for known age in all three models; Fig. 6). Estimates were positively biased for age-1-4 fish for two of three readers and negatively biased for all readers for fish > age 15 (Fig. 7). On average, estimated ages differed from known age by about 0.13-1.44 y for fish $\leq 15$ y, but MAD\textsubscript{known} increased substantially for fish $\geq 16$ y (Figs. 6 and 7). The CV was greatest for fish age 1-3 (means = 22-49%; Fig. 6), and MAD\textsubscript{reader} was about 1 y for fish in this age range (Fig.6). Mean absolute differences between reader pairs varied from about 0.1-1.8 y for fish ages 4-15, then increased to about 1.5-3.5 y for older fish. These differences in estimates between reader pairs corresponded to CVs that averaged 10% for fish ages 4-25 (Fig. 6).

The 95% CIs for von Bertalanffy growth function parameters for each readers’ models overlapped with 95% CIs for model parameters based on known age for both male and female muskellunge (Fig. 8). However, estimated age based on fin rays resulted in $L_\infty$ values that were consistently higher and $K$ and $t_0$ values that were consistently lower compared to estimates based on known age (Fig. 8). Averaged across readers, $L_\infty$ values based on ages estimated from fin rays were about 19 mm greater for male muskellunge and 40 mm greater for females compared to $L_\infty$ values estimated from known ages. For male muskellunge ages 3-16, predicted lengths-at-age from von Bertalanffy models based on anal fin ray age estimates were within 10 mm for 90% of estimates and within 5 mm for 69% of estimates from the known-age growth model. Eighty-three percent of predicted lengths-at-age for female muskellunge of the same age were within 15 mm of estimates from the known-age model (Fig. 8).

**Discussion**
We assessed precision, accuracy, and bias of age estimates for the practical lifespan of a moderately long-lived species using a nonlethal structure (we included muskellunge up to age 25, and muskellunge with an estimated age > 25 y have rarely been documented [Casselman et al. 1999; Casselman 2007]). Validation studies (excluding studies of increment formation) including known-age fish > age-10 are rare. For example, Spurgeon et al. (2015) only identified two validation studies for freshwater fishes that included fish > age-10 (largemouth bass [Micropterus salmoides] ages 1-16 [Buckmeier and Howell 2003]; golden perch (Macquaria ambiguа) ages 20 and 22 [Stuart 2006]), and Stuart’s (2006) study of golden perch only included two fish. Further, we are unaware of any other age validation studies using known-age freshwater fishes that included as broad of an age distribution as we included in our study (see Table 2 in Spurgeon et al. 2015). Given the increased use of nonlethal structures for age estimation, determining accuracy of estimates from these structures is necessary to ensure that estimates of population characteristics are based on reliable age data.

Age estimates from fin rays were relatively accurate and precise for muskellunge ages 4-15, but poorer precision and accuracy were observed for age estimates of younger and older fish. Conclusions about the use of fin rays for age estimation of esocids have varied (Johnson 1971; Brenden et al. 2006; Oele 2015; Bauerlien 2018). However, Johnson (1971) is the only study that assessed accuracy of age estimates from fin rays, and he observed that estimates were most accurate for fish up to age 9, but still within 1-2 y for fish ages 10-13 (the oldest fish examined in his study). Although our estimates were relatively accurate, Johnson (1971) observed greater accuracy of estimates for fish up to age 13, despite recent advances in viewing technology. The lower accuracy we observed may have been due to differences in formation of annuli or visibility of annuli across geographic locations (see below).
Ages tended to be overestimated for young fish in our study and underestimated for old fish. Overestimation of age for fish age 1-3 (and to a lesser extent age-4 fish) was likely the result of reader error and presence of false annuli (Fig. 2). Overestimation of age for these fish was most pronounced for one reader, suggesting reader error contributed to the relatively large MAD_{\text{known}} and positive bias for ages 1-4. After removing the reader with pronounced bias, MAD_{\text{known}} decreased from 0.55, 1.02, 1.17, and 0.50 y to 0.12, 0.55, 0.78, and 0.37 y for age 1-4 fish; however, a positive bias was still observed. Because alternating opaque and translucent zones in bony structures represent periods of fast and slow (or zero) growth (Buckmeier et al. 2017), false annuli can be formed due to additional factors other than annual growth cycles, such as cessation of feeding and growth during the summer due to thermal stress. Bregazzi and Kennedy (1980) hypothesized that false annuli observed in opercular bones and scales of northern pike (E. lucius) collected from a lake in southern England were due to warm water temperatures that resulted in cessation of growth at times during the summer, but continuation of growth during the winter. Similarly, Crane (unpublished data) observed putative false annuli in sectioned fin rays collected from muskellunge in eastern Kentucky streams at the southern range of the species. Most of the samples from fish age 1-3 in our study were from a single southern population; 98% (57 of 58) of samples from age-1 and age-2 fish and 81% (13 of 16 of samples) from age-3 fish were collected from Cave Run Lake, Kentucky. Errors in age estimation for fish from Cave Run Lake were predominantly positively biased, and may have been due to warm water temperatures during the summer causing thermal stress and cessation of growth. Summer surface water temperatures in Cave Run Lake are commonly ≥28 °C (T. Jackson, U.S. Army Corps of Engineers, unpublished data), which exceeds the realized thermal niche for muskellunge (22.3 °C ± 1.8 °C; Cole and Bettoli 2014). Because most fish we considered to be
from southern populations were from a single reservoir, it is unknown if these results are applicable to other southern populations, or at larger geographic scales. Although we had limited overlap in ages between northern and southern samples, post-hoc analyses of age-4 fish from northern (n = 22 from Wisconsin and Iowa) and southern (n = 19 from Kentucky and Virginia) populations suggested that age estimates from southern populations may be less precise and accurate than northern populations. Consensus among readers (100% vs. 79%), 100% agreement (64% vs 26%), and correct assignment of age (71% vs. 47%) were observed for a higher proportion of samples from northern populations compared to southern populations. Further, MAD\text{reader} (0.30 y vs. 0.91 y), CV (6% vs. 17%), and MAD\text{known} (0.36 y vs. 0.65 y) were lower for estimates from northern populations. Additionally, Johnson (1971), who studied muskellunge from Wisconsin populations, observed high accuracy of estimates (>95% correct) for age 1–4 fish based on pelvic fin rays.

Underestimation of age was most apparent for fish >age 15 in our study; however, we had small sample sizes for ages 12-15 (1-4 fish per age class), and we had no fish from southern populations that were >age 11. Underestimation of age based on hard structures such as fin rays is not uncommon (Buckmeier et al. 2017), especially for species that experience slow growth at older ages, because slow growth can result in compressed annuli that are difficult to discern (Buckmeier et al. 2017). Agencies currently managing muskellunge in the southern part of their distribution (Kentucky Department of Fish and Wildlife, North Carolina Wildlife Resources Commission, Virginia Department of Game and Inland Fisheries, West Virginia Division of Natural Resources) have recently or are currently stocking tagged or marked fish. Collection of marked fish from southern populations and targeted sampling of younger fish from northern populations in the next decade will provide the data necessary to make more thorough
comparisons related to formation of annuli and age estimation between northern and southern populations of muskellunge.

Despite large deviations in estimated age from known age for older muskellunge from the Iowa lakes used to compare von Bertalanffy growth models, ages estimated from fin rays were adequate for parameterizing the models, and estimated lengths-at-age differed little from predicted lengths-at-age based on the models developed using known-age fish. Positive bias in $L_\infty$ values was observed because ages were underestimated for older fish. However, $L_\infty$ values for estimated and known-age models were still similar. Our models suggested that muskellunge growth slowed substantially after ages 11-14 for males and 14-16 for females. Therefore, a 3-4 y error in estimated age represented little change in mean estimated length-at-age for the age range of fish that had a negative bias in estimated age. For example, mean estimated length of female muskellunge from the known-age model only increased 16 mm from age-18 to age-22.

Similarly, Casselman (2007) observed that samples only needed to include muskellunge up to age 8 (95% CI = 6-10 y) to estimate $L_\infty$ values that fell within the 95% CIs for $L_\infty$ from a given population. Although age estimates from fin rays may be adequate for growth models, we did not have the data to assess how error associated with these structures may affect estimates of mortality or analyses related to year class strength. Casselman et al. (2017) cautioned against using ages from fin rays to estimate mortality from catch curves because fin rays often result in underestimation of age for older fish. Underestimation of age truncates the age distribution and depending on the age structure of the population, can result in under- or overestimation of mortality. Similarly, we caution against using age estimates from fin rays to assess year class strength and relate year class strength to environmental or biological variables because (1) these analyses may be sensitive to small errors in age assignment due to variability in environmental
and biological conditions from year to year and (2) age estimates are not accurate enough to
confidently assign individuals to year classes.

Standardizing and evaluating methods used to estimate ages is essential for accurately
estimating population characteristics of fishes, and for making valid comparisons among studies.
Comparisons between rays from different fins or different ray numbers within a fin are rarely
made, but we found that precision and accuracy of age estimates were similar between pelvic and
anal fin rays and ray numbers we examined. Although we observed statistical differences in
accuracy between the third and fourth anal rays, estimated differences in accuracy between the
two rays and plots of fitted relationships between MAD_{known} and known age indicated that these
differences were minimal and not likely to be biologically meaningful. Although estimates from
the anal and pelvic rays had similar levels of accuracy and precision, readers in this study
preferred viewing anal rays because the paired halves from anal rays were symmetrical in shape,
whereas paired halves from pelvic rays were asymmetrical.

Our overall estimates of precision and accuracy were likely conservative, and two
methodological adjustments and addition of samples from younger fish to the training set may
improve these measures. First, precision and accuracy of estimates may be improved by looking
at multiple sections from rays or sections from multiple rays for a given fish. In our overall
analysis of accuracy and precision we only examined one section for each fish due to the time
required to process the number of samples we examined. If multiple sections are examined,
readers can select the section with the best contrast between opaque and translucent zones for
age estimation. Second, we have observed that fin rays sectioned without any cleaning (i.e.,
encased in dried tissue) have minimal refraction of light around the edges of the ray because the
tissue blocks transmission of light. Refraction of light can obscure annuli near the edge of rays,
so limiting transmission of light around the edge can improve readability of structures. Although cleaning structures is not required when sectioning fin rays that are dried and not embedded in epoxy, it can be problematic when sectioning structures embedded in epoxy on a low speed saw, which is best for sectioning rays from young fish due to their small diameter and fragility. Because the blade of a low-speed saw is lubricated with water during sectioning, rehydrating tissue surrounding the ray during this process can cause the ray to break free from the epoxy it is embedded in or it may result in tissue folding over the edge of the ray. An alternative for decreasing light refraction near the edge of rays may to be embed cleaned rays in epoxy that is dyed an opaque color. Finally, our training set did not include any age 1 or 2 fish, and ages for these fishes were commonly overestimated. Correctly identifying the first annulus is sometimes challenging and training of readers with a reference collection that also includes these age classes may improve the accuracy and precision of estimates for young fish.

Anal and pelvic fin rays are an acceptable non-lethal alternative for estimating ages of muskellunge ages 4-15, and for producing reliable growth estimates. However, the broadest age range of fish included in this study was from the northern portion of the muskellunge’s distribution in the U.S. Additional research including a broader age range of fish from multiple southern populations is necessary to better understand the applicability of our findings over a larger geographic distribution. Collecting adequate samples from known-age fish is warranted to also evaluate how age estimation error affects mortality estimates and indices of year class strength, because increased accuracy of estimates for population characteristics will ultimately result in improved understanding of muskellunge biology and more effective management actions. Finally, we encourage researchers to strive to investigate ways for improving methodology of selecting, preparing, and viewing non-lethal structures for age estimation.
Acknowledgments

This work was funded by a grants from the Hugh C. Becker Foundation and Coastal Carolina University. We thank Tim Parks, Michael P. Rennicke, Jason Hallacher, Tom Timmerman, Shawn Hirst, and other employees of Iowa Department of Natural Resources, Illinois Department of Natural Resources, Kentucky Department of Fish and Wildlife, Virginia Department of Game and Inland Fisheries, and Wisconsin Department of Natural Resources for collecting samples for this study. We also thank an anonymous reviewer for their thoughtful comments. We thank Derek Ogle and Travis Brenden for reviews of this manuscript and meaningful conversations we have had with them about statistical analyses used to investigate precision and accuracy of age estimates. We thank John Casselman for his insight on age estimation of muskellunge. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References


**Table 1.** Summary of samples from known age fish that were provided for use in investigating the accuracy and precision of age estimates of muskellunge based on anal fin rays.

<table>
<thead>
<tr>
<th>State</th>
<th>Northern or southern</th>
<th>Water</th>
<th>Male</th>
<th>Female</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>N</td>
<td>Clear Lake</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>IA</td>
<td>N</td>
<td>East Okoboji Lake</td>
<td>42</td>
<td>37</td>
<td>-</td>
<td>79</td>
</tr>
<tr>
<td>IA</td>
<td>N</td>
<td>Spirit Lake</td>
<td>38</td>
<td>40</td>
<td>-</td>
<td>78</td>
</tr>
<tr>
<td>IA</td>
<td>N</td>
<td>West Okoboji Lake</td>
<td>15</td>
<td>6</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>IL</td>
<td>S</td>
<td>Lake Kinkaid</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>KY</td>
<td>S</td>
<td>Cave Run Lake</td>
<td>17</td>
<td>12</td>
<td>60</td>
<td>89</td>
</tr>
<tr>
<td>VA</td>
<td>S</td>
<td>South Fork Shennandoah/Shennandoah River</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>WI</td>
<td>N</td>
<td>Fox River</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>WI</td>
<td>N</td>
<td>Lake of the Pines</td>
<td>9</td>
<td>3</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>WI</td>
<td>N</td>
<td>Sand Lake</td>
<td>6</td>
<td>-</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>142</strong></td>
<td><strong>104</strong></td>
<td><strong>71</strong></td>
<td><strong>317</strong></td>
</tr>
</tbody>
</table>
Table 2. Descriptive statistics of precision and accuracy of ages estimated from fin rays of muskellunge. Two tests of preparation methods and fin ray selection were conducted based on a subsample of fin rays from Iowa fish. An assessment of overall accuracy and precision of anal fin rays was conducted based on a larger sample size of fish from 10 waters in five states (Illinois, Iowa, Kentucky, Virginia, and Wisconsin). 100% agreement (%) = estimated age was the same for all readers; Consensus (%) = the percentage of samples that at least two of three readers had the same estimated age; Correct (%) = the mean percentage of samples that readers correctly estimated the age; ±1 y (%) = the mean percentage of samples that the estimated age was within 1 y of the known age.

<table>
<thead>
<tr>
<th>Fin ray</th>
<th>n</th>
<th>100% agreement (%)</th>
<th>Consensus (%)</th>
<th>Correct (%)</th>
<th>±1 y (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effects of anal fin ray number and section width</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ray3_0.3mm</td>
<td>23</td>
<td>13</td>
<td>52</td>
<td>41</td>
<td>72</td>
</tr>
<tr>
<td>Ray3_0.5mm</td>
<td>23</td>
<td>9</td>
<td>61</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Ray3_0.7mm</td>
<td>25</td>
<td>16</td>
<td>60</td>
<td>47</td>
<td>69</td>
</tr>
<tr>
<td>Ray4_0.3mm</td>
<td>23</td>
<td>13</td>
<td>74</td>
<td>41</td>
<td>75</td>
</tr>
<tr>
<td>Ray4_0.5mm</td>
<td>23</td>
<td>13</td>
<td>65</td>
<td>39</td>
<td>71</td>
</tr>
<tr>
<td>Ray4_0.7mm</td>
<td>25</td>
<td>28</td>
<td>84</td>
<td>57</td>
<td>83</td>
</tr>
<tr>
<td><strong>Anal vs pelvic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anal</td>
<td>50</td>
<td>26</td>
<td>74</td>
<td>51</td>
<td>79</td>
</tr>
<tr>
<td>First pelvic</td>
<td>50</td>
<td>12</td>
<td>74</td>
<td>42</td>
<td>79</td>
</tr>
<tr>
<td>Second pelvic</td>
<td>50</td>
<td>30</td>
<td>72</td>
<td>54</td>
<td>81</td>
</tr>
<tr>
<td><strong>Overall precision and accuracy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (anal rays)</td>
<td>317</td>
<td>20</td>
<td>80</td>
<td>48</td>
<td>78</td>
</tr>
</tbody>
</table>
Fig. 1. Locations where muskellunge were collected for age estimation. 1 = West Okoboji Lake, 2 = Spirit Lake, 3 = East Okoboji Lake, 4 = Clear Lake, 5 = Sand Lake, 6 = Lake of the Pines, 7 = Fox River, 8 = Kinkaid Lake, 9 = Cave Run Lake, 10 = South Fork Shenandoah River. Basemap provided by Esri Inc. (Redlands, CA)

Fig. 2. Images of sectioned anal and pelvic rays from muskellunge viewed using transmitted light. Panel A was illuminated by placing the contrast turret on the microscope base between the darkfield and oblique illumination positions. Darkfield illumination was used for panels B, C, and D. Yellow circles denote presumed annuli (including the edge). (A) Anal ray from age-17 female from East Okoboji Lake, Iowa. Readers estimated the age to be 15, 17, and 18 y. (B) Anal ray from age-4 male from Cave Run Lake, Kentucky, illustrating false annuli that were observed in some sectioned fin rays from this population. Yellow and red circles denote two different potential counts of translucent zones, with red circles denoting translucent zones that likely represent true annuli. Readers estimated the age to be 7, 5, and 6. (C) First pelvic ray from an age-6 male collected from West Okoboji Lake, Iowa. Readers estimated the age to be 5, 6, and 5. (D) Second pelvic ray from an age-11 male collected from West Okoboji Lake, Iowa. Readers estimated the age to be 9, 10, and 11.

Fig. 3. Age (known) distribution of muskellunge (n = 317) used to investigate the precision and accuracy of age estimates based on sectioned anal fin rays. Muskellunge were collected from 10 waters across five states (Kentucky, Illinois, Iowa, Virginia, and Wisconsin). Fish collected from waters in Iowa and Wisconsin were classified as northern populations and fish from Kentucky, Illinois, and Virginia were considered southern populations.
Fig. 4. Relationships between the (A) mean absolute difference in estimated age between pairs of readers ($MAD_{\text{reader}}$), (B) CV, and (C) mean absolute difference from known age ($MAD_{\text{known}}$), with known age of muskellunge collected from Spirit, East Okoboji, and West Okoboji lakes in Iowa ($n = 71$). Ages were estimated from sectioned anal fin rays and compared across six treatments to examine effects of ray number (3 vs. 4) and section width (0.3, 0.5, 0.7 mm) on precision and accuracy of estimates. $MAD_{\text{reader}}$ and CV were used to determine precision of estimates, and $MAD_{\text{known}}$ was used as a measure of accuracy.

Fig. 5. Relationships between the (A) mean absolute difference in estimated age between pairs of readers ($MAD_{\text{reader}}$), (B) CV, and (C) mean absolute difference from known age ($MAD_{\text{known}}$), with known age of muskellunge collected from Spirit, East Okoboji, and West Okoboji Lakes in Iowa ($n = 50$). Ages were estimated from thin sections cut from the base of the fourth anal and first and second pelvic rays to test for differences in precision and accuracy of age estimates from these fin rays. $MAD_{\text{reader}}$ and CV were used to determine precision of estimates, and $MAD_{\text{known}}$ was used as a measure of accuracy. Note that points are jittered due to overlapping data.

Fig. 6. Relationships between the (A) mean absolute difference ($MAD_{\text{reader}}$) in estimated age between pairs of readers, (B) CV, and (C) mean absolute difference from known age ($MAD_{\text{known}}$), with known age for 317 muskellunge collected from 10 waters across five states (Kentucky, Illinois, Iowa, Virginia, and Wisconsin). Ages were estimated from thin sections cut from the base of the third or fourth anal rays. $MAD_{\text{reader}}$ and CV were used to determine
precision of estimates, and \( \text{MAD}_{\text{known}} \) was used as a measure of accuracy. Note that points are jittered due to overlapping data.

**Fig. 7.** Age difference plots for ages estimated from anal fin rays of muskellunge \( (n = 317) \). Points and bars represent the mean difference and range of differences between a reader’s estimate and known age of fish. Open circles indicate values that are significantly different from zero \( (\alpha = 0.05) \). The x-axis histogram displays the distribution of known-age fish and the y-axis histogram displays the frequency of varying differences in estimates from known age. Note: Known ages with an \( n < 3 \) were not tested for a difference from zero.

**Fig. 8.** Comparisons of von Bertalanffy growth curves for muskellunge from three interconnected lakes in Iowa (Spirit, East Okoboji, West Okoboji Lakes), based on known age and three readers’ estimates of age from anal fin rays. Parameter estimates for the models and 95% CIs are displayed in the tables above. \( L_t = \text{mean length-at-age } t \) (TL in mm); \( L_\infty = \text{maximum mean length} \); \( K = \text{how quickly mean length approaches } L_\infty \).
A

Overall mean absolute difference between reader pairs

B

Overall CV

C

Overall mean absolute difference from known age

MAD_{reader} (y)

MAD_{known} (y)

Known age (y)
677x431mm (96 x 96 DPI)
### Male-smolt stage

<table>
<thead>
<tr>
<th>Known age</th>
<th>Reader 1</th>
<th>Reader 2</th>
<th>Reader 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{\text{w}}$</td>
<td>1042 (1025–1064)</td>
<td>1974 (1006–1137)</td>
<td>1061 (1020–1116)</td>
</tr>
<tr>
<td>$K$</td>
<td>0.231 (0.180–0.287)</td>
<td>0.177 (0.120–0.241)</td>
<td>0.105 (0.112–0.257)</td>
</tr>
<tr>
<td>$z$</td>
<td>-1.8 (3.33–0.73)</td>
<td>-3.33 (3.92–1.69)</td>
<td>-2.62 (3.21–1.07)</td>
</tr>
</tbody>
</table>

### Female-smolt stage

<table>
<thead>
<tr>
<th>Known age</th>
<th>Reader 1</th>
<th>Reader 2</th>
<th>Reader 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{\text{w}}$</td>
<td>1261 (1225–1304)</td>
<td>1291 (1259–1378)</td>
<td>1316 (1254–1408)</td>
</tr>
<tr>
<td>$K$</td>
<td>0.191 (0.138–0.244)</td>
<td>0.177 (0.118–0.238)</td>
<td>0.138 (0.086–0.199)</td>
</tr>
<tr>
<td>$z$</td>
<td>-1.8 (3.84–0.81)</td>
<td>-1.81 (4.18–0.18)</td>
<td>-3.28 (4.48–1.24)</td>
</tr>
</tbody>
</table>