Spring 5-15-2017

Use of First Pelvic, Second Pelvic, and Fourth Anal Rays for Estimating Age of Muskellunge (Esox masquinongy)

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2017

BY

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Marine Science Major

Submitted in Partial Fulfillment of the

Requirements for the Degree of Bachelor of Science

In the Honors Program at

Coastal Carolina University

May 2017

Abstract

While cleithra are the most popularly used calcified structure used to estimate age of Muskellunge (*Esox masquinongy)*, the use of fin rays as an age estimation structure has been investigated as a nonlethal alternative. My objectives were to: (1) to compare the accuracy and precision of estimating age using first pelvic, second pelvic, and fourth anal rays from 46 known age Muskellunge and (2) to analyze a larger sample (n =120) of fourth anal rays to estimate age. Ages were estimated blindly by three readers independently from all available structures. Different analyses of precision and accuracy had conflicting results, however, I would recommend the use of either the fourth anal ray or the second pelvic ray to estimate age.

Introduction

The freshwater sport fish *Esox masquinongy*, commonly known as Muskellunge or Muskie, is a freshwater esocid found only in North America (Ontario Ministry of Natural Resources 2011). Muskellunge are found in all of the Great Lakes, and have record lengths of up to 161.3 centimeters, making them a popular trophy fish in the recreational fisheries industry (Ontario Ministry of Natural Resources 2011). In 2011, approximately 1,642 anglers spent a total of 23,420 days fishing for Muskellunge and other popular Esocids (Northern Pike, Chain Pickerel, and Muskellunge hybrids), (United States Department of the Interior 2011). In 2010, a total of 19 states and the Canadian province of Ontario stocked Muskellunge into a total of 343 water bodies (Ontario Ministry of Natural Resources 2011). In 2006, $425 million was spent directly on Muskellunge fishing in Wisconsin (Simonson, WDNR 2012).

Methods of estimating age are critical for collecting age data, which is important to understanding species' life histories, whether for an ecological study or for fisheries management. Age estimation is important for determining year-clear, productivity, age of maturity, mortality rate, and growth rate, all of which are vital to understanding and managing a species. Most methods to collect structures needed to estimate age (eg. otolith or cleithra) are lethal. While methods using other structures may be non-lethal, they are not always valid or reliable for all species (ex. scales). For example, age of Muskellunge were estimated using scales by three experienced esocid scale readers with less than 50% accuracy for Muskellunge of 3-10 years old (Fitzgerald et al. 1997). Another thing to consider when deciding on a method for estimating age is whether a method has been validated. In other words, how accurate and repeatable is the method being used to estimate age. Beamish and McFarlane (1983) reviewed 500 papers published from 1907 to 1980 to highlight the importance of validating age estimation methods and how often validation is not considered. Beamish and McFarlane (1983) found that only 17 papers were actually successful in validating their aging methods and 170 papers did not attempt to validate their methods.

Brenden et al. (2006) investigated the use of the first pelvic ray for estimating age of Muskellunge in comparison to cleithra, and 76% of the estimates matched between cleithra and first pelvic ray, while 100% were estimated within one year (Brenden et al. 2006). However, Brenden et al. (2006) used unknown age fish, so accuracy could not be estimated. In this study, I investigated the potential for the use of sections from rays of both pelvic and anal fins as a non-lethal estimator of Muskellunge age. My objectives were (1) to compare the accuracy and precision of estimating age using first pelvic, second pelvic, and fourth anal rays from 46 known age Muskellunge and (2) to analyze a larger sample of age estimates using the fourth anal ray.

Methods

Sample Collection

Hatchery stocked Muskellunge of known age (freeze branded or PIT tagged) were collected by the Iowa Department of Natural Resources (IA DNR) from East Okoboji, West Okoboji, and Spirit lakes during the annual spring Muskellunge survey during 2015 and 2016. Anal (n = 120) and pelvic (n=46) fins were removed from the Muskellunge by IA DNR using side cutters at the base of each fin. Fins were then packaged individually in coin envelopes and sent to our lab at Coastal Carolina University (CCU).

Sample Preparation

To prepare samples for age estimation, we first excised out the fourth rays for each anal fin and both the first and second pelvic rays from each pelvic fin. We then cleaned the rays by gently scraping off dead skin with a scalpel. To set the samples, we cut plastic drinking straws (~1cm diameter) into shorter pieces (~4.5 cm) and stoppered one end with non-air drying modeling clay. We then placed the fin rays in the straws, pushed the tip of the ray into the clay (with the base facing upwards out of the clay), and minimized the contact between the fin ray and the inner wall of the straw. To guarantee identification of the samples, we wrote the FISH ID, provided on the envelope of the sample, on the side of the straw. We then poured epoxy mixed in a 5:1.08 ratio of resin to hardener into each straw until the entire ray was covered. We allowed samples at least twenty-four hours to dry. After samples were set, we removed them from the straws and placed them back into their matching Ziploc bag. After all rays were returned to their respective bags, we removed each individual ray and removed the jagged edge from the base of the ray using an IsoMet 1000 Precision Low-speed Saw, making sure to remove the least amount of ray possible. We then cut 1.2 mm sections from the base of each ray, placed the sections into centrifuge tubes labeled with corresponding FISH ID, and placed the centrifuge tubes into their matching Ziploc bag. We returned the remaining portions of epoxy cylinders with the non-sectioned portions of rays back into their respective envelopes in the event that we needed to re-section any samples.

Estimating Age

Three readers estimated age using the sections taken from the fourth anal ray, first pelvic ray, and second pelvic blindly, independently, and individually. We prepared each section sample for age estimation by removing it from its tube, wiping it with immersion oil (type A) on a Kimwipe, and placing it in a drop of immersion oil (type A) on a microscope slide. Sections were flipped in the immersion oil to ensure both sides were coated. We then put the slide under an Olympus SZX-10 zoom stereomicroscope with an Olympus DP-27 camera attachment in order to view the samples on a monitor using cellSens Standard software. To estimate age, we viewed the sections on the monitor and counted the annuli. Samples were viewed using dark field, oblique, or some combination of both lighting methods. We added one year to the number of annuli counted to account for the fact that samples were collected in spring, preventing the readers from seeing the most recent annulus. Age estimates were recorded on reader specific data sheets. We then wiped oil off the samples, dipped them in water with dish detergent, dried them with a clean kimwipe, placed then back into centrifuge tube, and returned them to their Ziploc bags. After all samples were aged by all three readers, I compiled the estimates into a Microsoft Excel spreadsheet to for later analysis.

Comparisons among structures

I compared age estimates for fourth anal, first pelvic, and second pelvic rays (n=46) to determine precision and accuracy across the varying fin rays. For all rays, I created age-agreement tables in R Studio (Version 1.0.136; R version 3.3.3; R Core Team 2017) for the estimations of age between readers using "ageprecision" function from the package 'FSA' (Ogle 2017). I ran summaries of the "ageprecision" for each of the treatments for "precision". From this information I quantified precision by recording the percent agreement and the average coefficient of variation (ACV), which is calculated by:

$$ACV = 100 * \frac{\sum_{j=1}^{n} \frac{s_j}{\bar{x}_j}}{n}$$

where $\bar{x}_j$ is the mean estimated age of jth fish, s_j is the standard deviation of the R age estimates for jth fish in a sample of n fish (Ogle 2016). I also created age-agreement tables in R studio (Version 1.0.136; R version 3.3.3; R Core Team 2017) for the estimations of age between readers and known age using "agebias" function in package 'FSA' (Ogle 2017) to test for accuracy of each ray. I ran two summaries of the "ageprecision" for each of the treatments: one summary for "absolute difference" and one summary for "precision". From the "precision" summary, I recorded the percent agreement between individual readers. From the "absolute difference" summary, I determined the average percent of age estimations across all readers that were 100% correct, within 1 year of known age, and within 2 years known age.

I created age bias plots for all possible combinations of readers for all rays in R Studio (Version 1.0.136; R version 3.3.3; R Core Team 2017) using the "agebias" function in package

‘FSA’ (Ogle 2017), to test for precision of age estimates from each ray. I also created age bias plots for readers and rays that compared each readers estimate to known age.

To compare fin rays, I estimated mean difference from known age, mean absolute difference from known age (MAD), and mean absolute difference between readers (MAD_reader). Because accuracy and precision were dependent on age, I created linear models for the relationships between (1) mean difference from known age, known age and fin ray, (2) MAD, known age, and fin ray, and (3) MAD_reader, known age, and fin ray, in order to determine if there was a significant difference among the slopes of the regression lines from the three different fin rays. In the event that slopes did significantly differ, I reported the F statistic, degrees of freedom and p-value from the linear models, and interpreted the slopes at various ages. In the event that slopes did not significantly differ, I tested the means with an ANOVA to see if there was a significant effect of fin ray on whichever relationship I analyzed; I reported the F statistic, degrees of freedom, and p-value from both the linear models and the ANOVA in these cases.

Analysis of Anal Ray Estimates

Because anal rays were determined to be more accurate and precise than first pelvic rays and we had a larger sample size (n=120) we conducted further analysis for fourth anal rays. The same statistical methods were used to assess fourth anal rays as were used to assess precision and accuracy of anal and pelvic rays from the 2016 samples.

Results

Comparison among Structures

For the 46 Muskellunge collected in 2016 that we were able to estimate age from the fourth anal, first pelvic and second pelvic, ACV was the highest for age estimates from the first pelvic ray (ACV = 10.4), followed by the fourth anal (ACV = 9.6), and the lowest was for second pelvic (ACV = 8.3) (Table 1). The 100% agreement with known age was the highest for second pelvic ray treatment (30.4%), followed by fourth anal ray (28.3%), and lowest for first pelvic ray (15.2%) (Table 1). The 100% agreement between readers, was again highest for the second pelvic ray treatment (30.4%), followed by fourth anal ray (28.3%) and lowest for first pelvic ray (15.2%) (Table 1.). The average percent of correct age estimates was equivalent for second pelvic ray and fourth anal rays (50.7%), and lower for the first pelvic ray (42%) (Table 1). The average percent of age estimates within 1 year of known age was highest for first pelvic rays (77.5%), and equivalent for the fourth anal rays and second pelvic rays (76.8%) (Table 1). Finally, the average percent of age estimates within 2 years was highest for the first pelvic ray treatment (84.8%), followed by the fourth anal ray (83.3%), and lowest for the second pelvic ray (81.9%) (Table 1).

Table 1. Precision and accuracy analysis summary for the comparison age estimations among multiple structures from the same Muskellunge (n=46).

Treatment	*n*	ACV	100% agreement b/t Readers	100% agreement	% correct	±1 yr.	±2 yr.
Anal (4th)	46	9.6	28.3	28.3	50.7	76.8	83.3
Pelvic (1st)	46	10.4	15.2	10.9	42	77.5	84.8
Pelvic (2nd)	46	8.3	30.4	30.4	50.7	76.8	81.9

I, Rin, had a negative bias compared to both Cory and Mike, and my bias was larger for all structures from fish of ten years or older (Figure 1). Cory had a positive bias compared to Mike when using both fourth anal rays and first pelvic rays for fish older than ten years old (Figure 1). Cory had a negative bias compared to Mike when using second pelvic rays for fish of ten years or older (Figure 1). Cory and Mike tended to agree more as their differences in estimates were typically small (Figure 1). Cory and Mike both underestimated structures from their true age for all structures that were age twelve or older (Figure 2), while I underestimated structures from age ten or older (Figure 2).

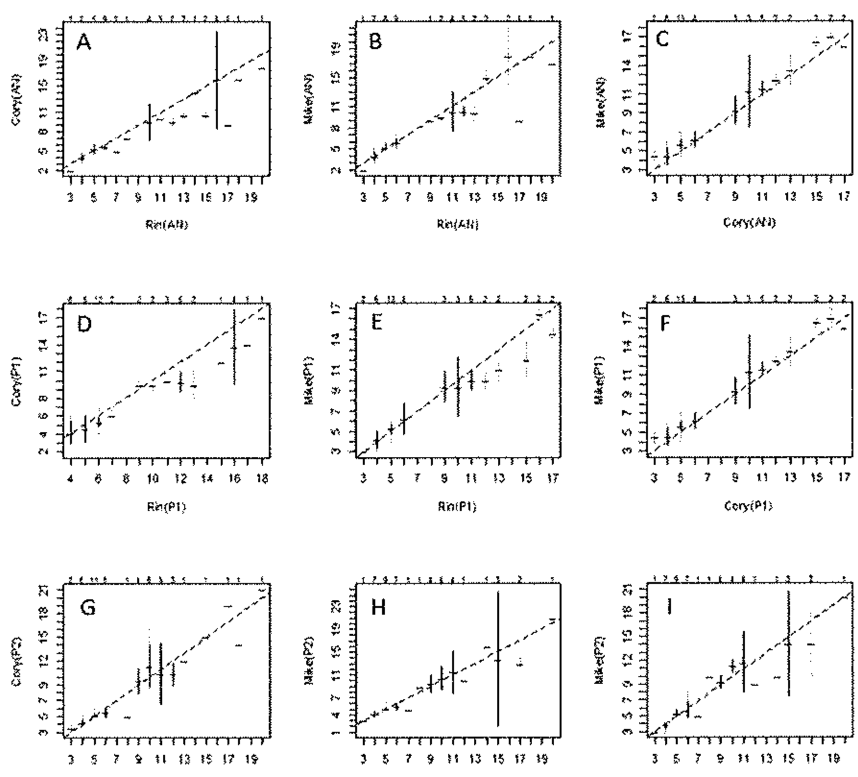

Figure 1. Age bias plots for the 46 Muskellunge from the three different fin ray estimates (fourth anal, first pelvic and second pelvic) among readers. Plots A- C are all possible combinations of readers, comparing our age estimates from each of the 46 fourth anal rays. Plots D-F are all possible combinations of readers, comparing our age estimates from each of the 46 first pelvic rays. Plots G- I are all possible combinations of readers, comparing our age estimates from each of the 46 second pelvic rays. The bars in black are the range, while the bars in gray are the 90% confidence intervals for each age.

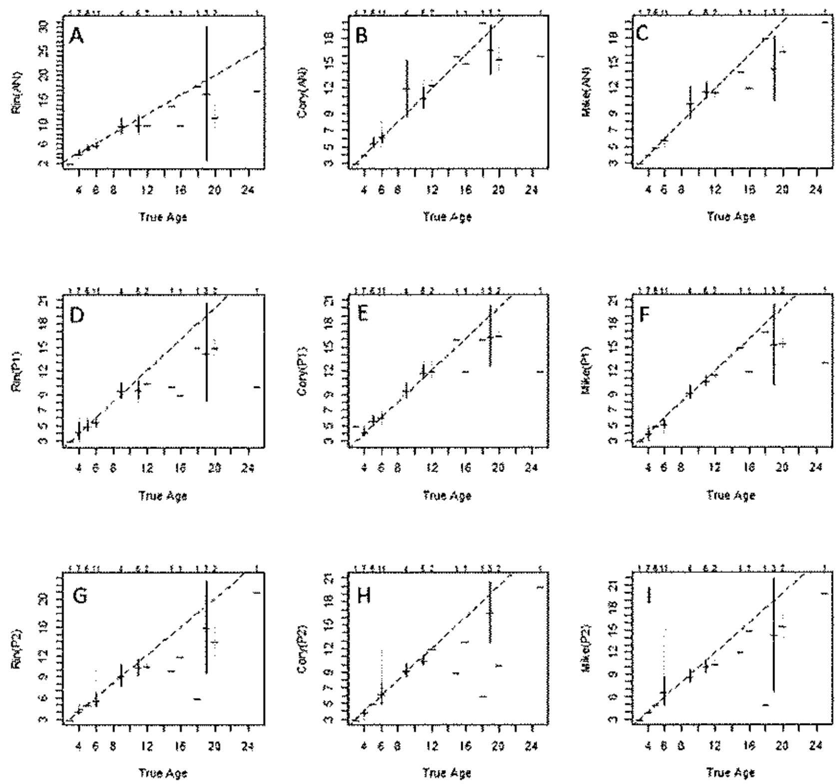

Figure 2. Age bias plots for the 46 Muskellunge from the three different fin ray estimates (fourth anal, first pelvic and second pelvic) in comparison to the known age. Plots A, B, and C are comparisons of all possible combinations of readers and known age for each of the 46 fourth anal rays. Plots D, E, and F are comparisons of all possible combinations of readers and known age for each of the 46 first pelvic rays. Plots G, H, and I are comparisons of all possible combinations of readers and known age for each of the 46 second pelvic rays. The bars in black are the range, while the bars in gray are the 90% confidence intervals for each age.

Finally, there was not a significant difference found among the fin rays for the relationship between mean difference from known age and known age. ($F_{5, 132}$ = 33.38, p-value = < 0.001; Figure 3), or for the relationship between MAD and known age ($F_{5, 132}$ = 36.17, p-value = < 0.001; Figure 3). There was also not a significant effect of fin ray on relationship between mean difference and known age ($F_{2,134}$ = 1.293, p-value = 0.278; Figure 3) or of fin ray between MAD and known age ($F_{2,134}$ =0.444, p-value = 0.643). However, there was a significant difference found among treatments for the relationship of known age vs. MAD_reader ($F_{5, 132}$ = 25.16, p-value = < 0.001; Figure 3). Fourth anal rays were more precise than second pelvic rays, which were more precise than first pelvic rays, for structures up to known age of 7 (Figure 3). Second pelvic rays were more precise than first pelvic rays, which were more precise than fourth anal rays, for structures of known age 7 to 9 (Figure 3). First pelvic rays were more precise than second pelvic, which were more precise than fourth anal rays for structures of known age 9 and up (Figure 3).

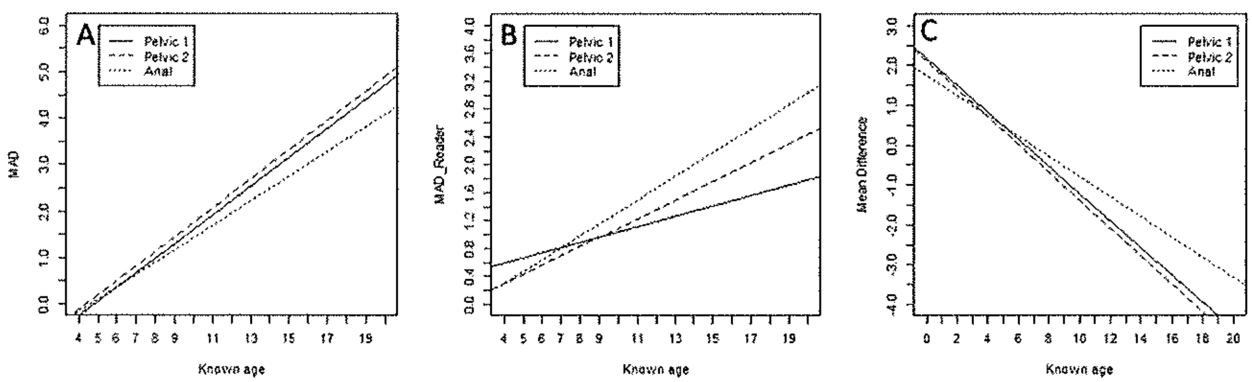

Figure 3. Plot A is the regression lines for each of the three treatments comparing Mean Absolute Difference (MAD) to Known Age. Plot B is the regression lines for each of the three treatments comparing Mean Absolute Difference between readers (MAD_reader). Plot C is the regression lines for each of the three treatments comparing Mean Difference to Known Age.

Anal Ray Estimates

For the 120 Muskellunge collected from 2015-2016 that we were able to estimate age from the fourth anal, ACV was 9.4, the 100% agreement was 27.5%, and the 100% agreement between readers was 29.2% (Table 2). The average percent of correct age estimates was 50% (Table 2). The average percent of age estimates that were within 1 year was 76.7% (Table 2). The average percent of age estimates that were within 2 years was 85% (Table 2).

Table 2. Precision and accuracy analysis summary for the age estimations from the fourth anal rays collected in 2015-2016 (n = 120).

Treatment	*n*	ACV	100% agreement b/t Readers	100% agreement	% correct	±1 yr.	±2 yr.
Anal (4th)	120	9.4	29.17	27.5	50	76.7	85

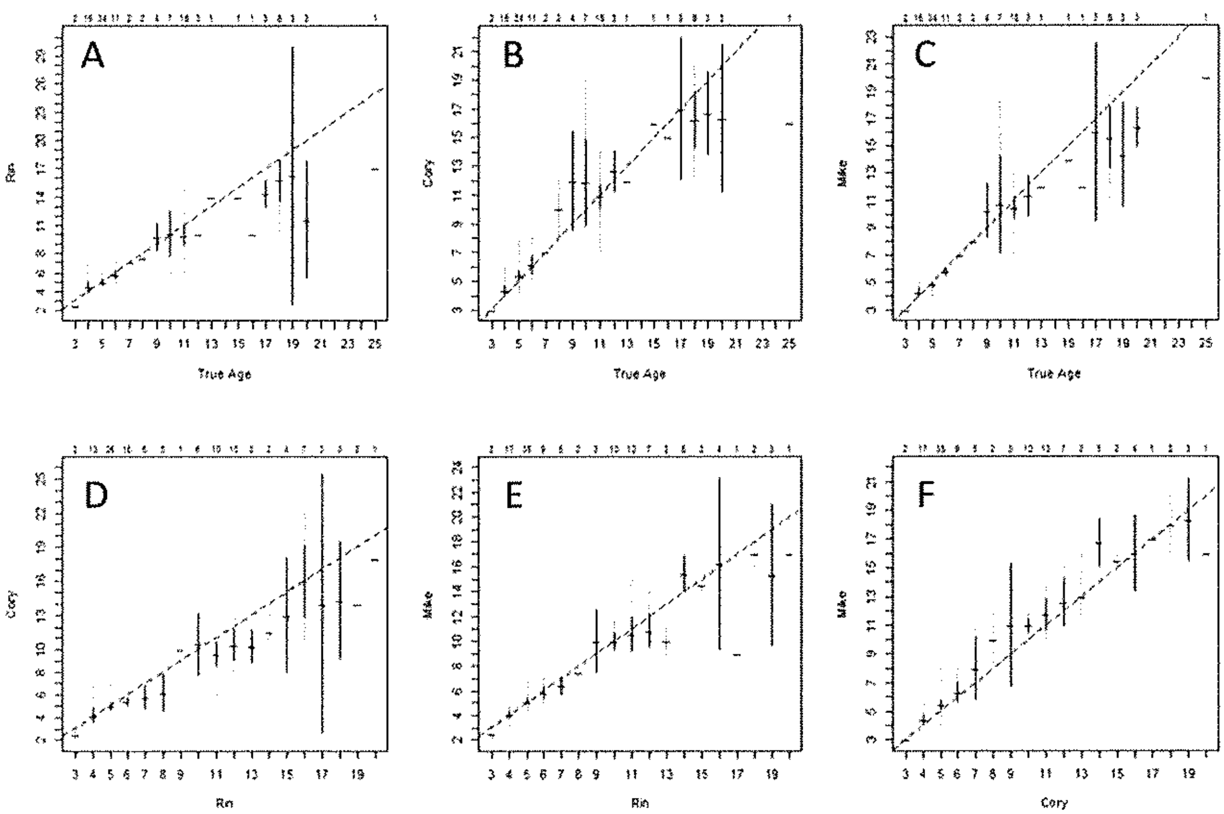

Figure 6. Plots A-C are age bias plots for all possible combinations of readers and known age for the 120 anal rays. Plots D-F are age bias plots for all possible combinations of readers for the 120 anal rays. The bars in black are the range, while the bars in gray are the 90% confidence intervals for each age.

Discussion

The coefficient of variation for an age estimation methodology is recommended to be 5% or lower for fish that have a moderate longevity and whose structures are complex to read (Campana 2001). The coefficient of variation (CV) used by Campana (2001) differs slightly from the *average* coefficient of variation (ACV) used here. All rays examined in our study had an average coefficient of variation > 5%, with the second pelvic ray having the lowest at 8.3% (Table 1). In the sample of 120 Muskellunge aged using fourth anal rays only, the ACV was 9.4% (Table 2), indicating that sample size was likely not the issue for the fourth anal ray treatment.

Disregarding that the CV and the ACV are technically different calculations, the ACV for each treatment would indicate that none of the treatments are precise enough to be credible or valid, according to the 5% premise set by Campana (2001). I disagree with this for several reasons. First, the Muskellunge is considered a long lived fish (Faust et al. 2013), and the 5% reference point of CV is for fish of moderate longevity, which is not specifically defined by Campana (2001). For example, most sharks are long lived species and Campana (2001) found that most labs using vertebrae to estimate the age of sharks used a CV reference point of 10%, as opposed to 5%. Second, another criteria for the use of the 5% reference point of CV is that the complexity of reading the structure be moderate, which is a vague criteria. It is not clear what moderate complexity of reading a structure would be. While I did not find reading most of the structures to be easy, I did not find a majority of them to be difficult, with the most difficult being older fish; in discussing the process of reading with the other readers, they agree. While this could be what is meant by moderate complexity, I am unsure.

Third, and most importantly, neither the ACV nor CV considers the known age of the fish whose age was being estimated in their calculation. It is important to consider all age groups within a species when validating age estimation methodologies (Beamish and McFarlane 1983), and this is applies to precision as well as accuracy. The significant difference among treatments for the relationship between MAD_reader and known age indicates that precision is dependent on treatment and known age of the fish. The plot of regression lines for all fin ray treatments between MAD_reader and known age (Figure 3B) provides a visual representation of this change in precision as age increases. This relationship is also apparent in the age bias plots for all three treatments comparing the estimated age of each structure (Figure 1); as the known age of a fish increases, age estimates tended to vary more among readers. Therefore, I do not believe that the use of the ACV, nor the CV, is the best calculation of precision for any of the fin ray treatments used in this study for estimating the age of Muskellunge due to their lack of consideration of known age of a fish. I suggest that a new calculation of precision that incorporates the known age of each fish be created in place of ACV and CV.

The lack of significant difference among treatments for the relationship between MAD and known age indicates that accuracy is independent of fin ray used as an estimator. However, based on my personal experience and the experience of the other readers in this study, it was much more difficult to estimate the age of a fish correctly when the known age of the fish was over 10 years old. While the average of age estimates that were within two years of known age was highest for estimates using the first pelvic ray (Table 1), this was the hardest structure to age due to the lack of symmetry in the shape of the first pelvic ray and their tendency to require to be cut twice in order to be readable. Also, the average of age estimates that were within two years of age for estimates using the second pelvic and fourth anal rays were not lower than that of the first pelvic ray treatment, and all three treatments had a high percent of correct age estimates within two years of known age (Table 1). However, the easiest fish to age in this study were those of six years of age or younger. This would potentially make this age estimation method, regardless of which of the three fin ray treatment one chooses, useful for models that focus more on younger fish or fish that are just entering the spawning stock.

The most commonly used age estimation method for Muskellunge is by using cleithra (Casselman 1983). Scale estimations have repeatedly been found to match cleithra estimations up to 10 years of age, after which the scales under estimated (Casselman 1979; Fitzgerald et al. 1997). Scale estimations have also been found to match cleithra estimates about half the time (Fitzgerald et al. 1997). Scales are often hard to read and often have areas of reabsorption (Casselman 1979), which is likely the cause of their inaccuracy. Otoliths of esocids are not practical for age estimation due to their small size which makes them hard to collect, and the fact their drying time can be up to several weeks (Faust et al. 2013). Fin rays may be a practical alternative structure for use in estimating age of Muskellunge because they are not as challenging to collect as cleithra, are easier to read than cleithra, and do not require the sacrifice of the Muskellunge. Because Muskellunge are long-lived (Casselman 1979; Faust et al. 2013) and occur in naturally low densities, a nonlethal method for estimating age is desirable. I would recommend the use of either the fourth anal ray or the second pelvic ray to estimate age, because these structures were the most clearly readable, the most symmetric, did not require recutting sections as often as the first pelvic and took less time to age in comparison to first pelvic.

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