Notes

Comparison of Structures Used to Estimate Age and Growth of Yellowstone Cutthroat Trout

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Abstract

Understanding age and growth of fishes is critical for making meaningful management decisions. Obtaining useful information is dependent on using the best structure (e.g., scale, otolith). The objective of this study was to evaluate precision and reader confidence in age estimates from sagittal otoliths (i.e., whole, sectioned) and scales for Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* collected from Henrys Lake, Idaho. We also sought to compare growth estimates among structures sampled during annual gill net surveys in May 2019 and 2020. We removed sagittal otoliths and scales from 416 Yellowstone Cutthroat Trout. Two readers without prior knowledge of fish length independently aged scales, whole otoliths, and sectioned otoliths. Each reader also provided a confidence rating of 0 (not confident) to 3 (completely confident). Percent exact agreement between readers was highest for sectioned otoliths (85.3%), followed by scales (68.5%) and whole otoliths (66.1%). Average confidence rating was highest for sectioned (mean $\pm$ SD = 2.2 $\pm$ 0.6) and whole (1.4 $\pm$ 0.5) otoliths and lowest for scales (1.0 $\pm$ 0.2). Among structures, percent exact agreement (i.e., consensus age) was highest between whole and sectioned otoliths (66.7%), followed by scales and sectioned otoliths (58.9%). Exact agreement was lowest between scales and whole otoliths (51.2%). Differences in back-calculated length at age estimates between sectioned otoliths and scales were minimal, particularly for ages 1–4. Although sectioned otoliths required more time to prepare than scales or whole otoliths, sectioned otoliths produced the most precise age estimates for Yellowstone Cutthroat Trout, with the highest reader confidence.

Keywords: age; growth; structure; otolith; scale; whole; sectioned

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Introduction

Age and growth analyses provide valuable insight into the population dynamics of fishes and assist managers in making meaningful management, conservation, and restoration decisions (Kerns and Lombardi-Carlson 2017). Considering the precision and accuracy of ageing structures (e.g., scales, otoliths) is important when selecting the best structure for estimates of age and growth (Buckmeier 2002). Historically, scales have been the most common structure used for ageing fishes (Maceina et al. 2007; Quist et al. 2012; Kerns and

Lombardi-Carlson 2017). More recently, otoliths have replaced scales as the preferred structure for many species because they tend to be more accurate and precise than other structures (Phelps et al. 2017).

Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* is native to the Yellowstone River and the Snake River drainages in Wyoming, Idaho, Utah, and Nevada (Gresswell 2011). Although Yellowstone Cutthroat Trout is an important sport fish across its distribution, it is also considered a species of conservation concern due to population declines (Gresswell and Varley 1988; Meyer et al. 2006; Gresswell 2011). Managers have implemented a variety of conservation measures to mitigate negative effects on Yellowstone Cutthroat Trout, including habitat restoration and nonnative species removal (Gresswell 2011). Understanding the population dynamics of Yellowstone Cutthroat Trout populations is critical for evaluating the efficacy of conservation efforts and guiding future management decisions. One system where management of Yellowstone Cutthroat Trout is of high importance is Henrys Lake, Idaho.

Henrys Lake is a shallow, eutrophic lake located in eastern Idaho that is managed for trophy Yellowstone Cutthroat Trout, Rainbow Trout *Oncorhynchus mykiss* × Yellowstone Cutthroat Trout hybrids, and Brook Trout *Salvelinus fontinalis* (Irving 1955). In addition to providing a world-renowned trophy fishery, conservation of native Yellowstone Cutthroat Trout is also a management priority in the system. Yellowstone Cutthroat Trout have been monitored in Henrys Lake since the 1950s. Before 2002, evaluation of fish age and growth was done via scales. Since 2002, estimates of age of Yellowstone Cutthroat Trout in Henrys Lake have been done via whole sagittal otoliths. Scales regularly underestimate age of fishes, particularly salmonids (Schill et al. 2010; Quist et al. 2012; Phelps et al. 2017). Whole otoliths may also fail to provide accurate age estimates, and their use in estimating back-calculated lengths at age is questionable (Klumb et al. 2001; Long and Grabowski 2017). As such, the primary objective of this study was to evaluate precision in age estimates from scales and sagittal otoliths for Yellowstone Cutthroat Trout. Specifically, we sought to evaluate between-reader precision for each structure, as well as precision in consensus age estimates among structures. We also compared back-calculated lengths at age between sectioned sagittal otoliths and scales.

Methods

We collected Yellowstone Cutthroat Trout from Henrys Lake during May 2019 and 2020 immediately following ice-out. We used a combination of Idaho Department of Fish and Game standard gill nets and customized American Fisheries Society experimental gill nets to sample fish. Idaho Department of Fish and Game gill nets were 45.7 m long and 1.8 m deep and had six panels of sequentially ordered mesh (i.e., 1.9-, 2.5-, 3.2-, 3.8-, 5.0-, and 6.4-cm bar-measure mesh). Customized American Fisheries Society gill nets were 24.4 m long and 1.8 m deep and had nine panels of randomly ordered mesh (i.e., 1.3-, 1.9-, 2.5-, 3.2-, 3.8-, 4.4-, 5.0-, 5.7-, and 6.1-cm bar-measure mesh). We set gill nets at dusk and pulled them at dawn to achieve approximately 12-h net sets. We measured total length (millimeters) and weight (grams) of all Yellowstone Cutthroat Trout. We removed scales and sagittal otoliths from 10 fish per centimeter length group (Quist et al. 2012; Miranda and Colvin 2017). We removed scales from the area just posterior to the pectoral fin, placed them in paper coin envelopes, and allowed them to air dry. We removed, cleaned, and stored otoliths in microcentrifuge tubes.

We mounted at least 10 scales per fish between two glass microscope slides and then viewed them by using a dissecting microscope with transmitted light. For each fish, we mounted one otolith in epoxy (Koch and Quist 2007), and a thin, transverse section (~0.65 mm) that included the nucleus was cut from each mounted otolith (Quist et al. 2012; Long and Grabowski 2017). We polished sections with progressively finer sandpaper as needed and viewed them by using transmitted light. The other otolith was left whole and viewed by using reflected light. We measured annual growth increments on scales and sectioned otoliths by using Image-Pro Plus software (Media Cybernetics, Rockville, MD).

Two readers independently estimated age of each structure. Reader 1 was relatively inexperienced, but received extensive training before this study. Reader 2 had experience using otoliths to estimate ages of fishes and received training on using scales for ageing. In addition to extensive instruction from an experienced reader, both readers reviewed a sample ($n = 82$) of known-age Yellowstone Cutthroat Trout from Henrys Lake for training purposes. Although known-age fish were age 2 and younger, they were helpful in training readers to identify the first annulus (a common source of error; Buckmeier et al. 2017; Long and Grabowski 2017). Because of the truncated age distribution and low sample size of known-age fish, we did not formally use the structures to evaluate accuracy. Readers independently provided age estimates without prior knowledge of fish length. Readers assigned each age estimate a confidence rating that varied from 0 (no confidence) to 3 (complete confidence; Fitzgerald et al. 1997; Koch et al. 2008). If age estimates differed between readers, the readers jointly examined the structure in an attempt to reach a consensus age estimate. Readers reached a consensus age for all fish.

We evaluated precision in age estimates by using various summarization techniques. We created age–bias plots (Campana et al. 1995; Buckmeier et al. 2017) for each structure by plotting the age estimates from reader 1 against those from reader 2. We summarized percent exact agreement (PA) and percent agreement within 1 y (PA-1) for each structure. We calculated the coefficient of variation (CV) as another estimate of precision in age estimates:

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

where X_{ij} is ith age estimate for the jth fish, X_j is the mean

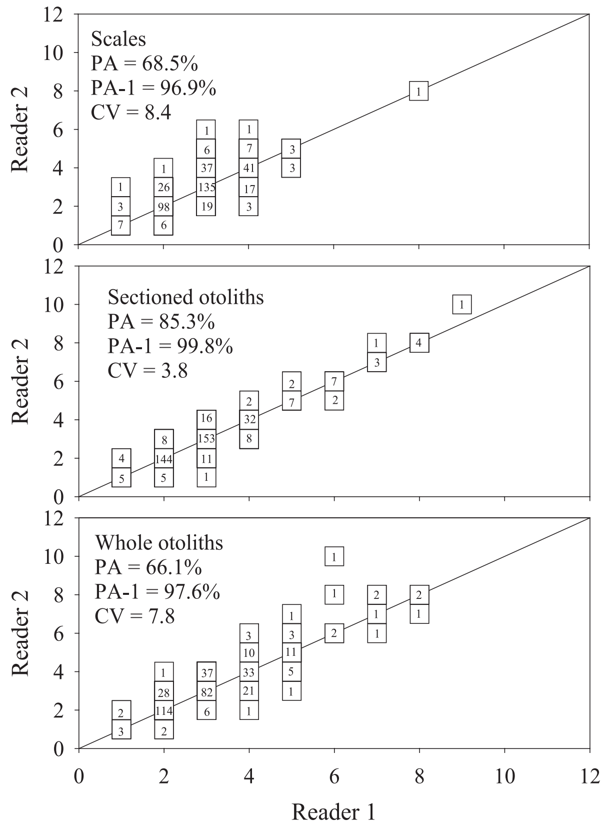

Figure 1. Comparison of reader age estimates for sectioned otoliths, whole otoliths, and scales from Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouveri* sampled from Henrys Lake, Idaho, 2019 and 2020. Percent exact agreement (PA), percent agreement within 1 y (PA-1), and the coefficient of variation (CV) are provided as measures of precision. Numbers inside boxes represent the observed number of fish.

age of the *j*th fish, and *R* is the number of times each fish is aged (Campana et al. 1995). Between-reader comparisons allowed us to assess the repeatability of age estimates across readers, as well as the general readability of each structure. The age of the vast majority of fish was unknown, but sectioned otoliths have been repeatedly shown to provide accurate age estimates (e.g., Haglund and Mitro 2017; Branigan et al. 2019). As such, comparing age estimates from scales and whole otoliths with estimates from sectioned otoliths likely provides insight into accuracy. Similar to the between-reader analysis, we created age–bias plots, calculated PA and PA-1, and estimated CV by using consensus age estimates to evaluate precision among structures.

We measured annuli along the anterior radius for scales and the dorsal radius for sectioned otoliths. We estimated back-calculated lengths for scales by using the Fraser–Lee method:

$$L_i = \frac{S_i}{S_c}(L_c - a) + a \qquad (2)$$

where L_i is the back-calculated length of the fish at the formation of the *i*th annulus, L_c is the length of the fish at capture, S_c is the radius of the scale at capture, S_i is the radius of the scale at the *i*th annulus, and *a* is the intercept of the regression of fish length at capture on hard-structure radius at capture (Shoup and Michaletz 2017). For otoliths, we estimated the back-calculated lengths by using the Dahl–Lea method:

$$L_i = \frac{S_i}{S_c} \times L_c \qquad (3)$$

where L_i is the back-calculated length of the fish at the formation of the *i*th annulus, L_c is the length of the fish at capture, S_c is the radius of the otolith at capture, and S_i is the radius of the otolith at the *i*th annulus. Accuracy of back-calculated length estimates is best evaluated with repeated captures of individual fish (e.g., Michaletz et al. 2009). Because we lacked such data, we compared back-calculated lengths at age with observed mean lengths at age at capture (i.e., based on otolith age) to provide insight into whether back-calculated length estimates were concordant with observed lengths. Similar comparisons of back-calculated lengths to lengths at age at capture are available for other species and systems (e.g., Schramm and Doerzbacher 1985; Maceina and Betsill 1987).

Results

In total, we sampled 416 Yellowstone Cutthroat Trout varying from 168 to 625 mm (mean $\pm$ SD = 375.2 $\pm$ 92.2 mm) in total length in 2019 and 2020 (Table S1, *Supplemental Material*). Between-reader agreement in age estimates varied across structures (Figure 1). Scales had the lowest PA (68.5%) and PA-1 (96.9%), followed by whole (PA = 66.1%; PA-1 = 97.6%) and sectioned (PA = 85.3%; PA-1 = 99.8%) otoliths. In addition to having the highest between-reader agreement, sectioned otoliths had the lowest CV (Figure 1).

Confidence ratings varied from 0 to 2 for scales, from 0 to 3 for whole otoliths, and from 1 to 3 for sectioned otoliths. Average reader confidence was lowest for scales (1.0 $\pm$ 0.2) and whole otoliths (1.4 $\pm$ 0.5); sectioned otoliths had high confidence ratings (2.2 $\pm$ 0.6). The highest concordance between consensus ages was for whole and sectioned otoliths (PA = 66.7%; PA-1 = 96.8%; Figure 2). Consensus ages for scales were most similar to otolith ages for age-3 and younger fish (i.e., based on otolith age). Consensus ages for scales were less than those for otoliths when otolith ages were greater than age 4.

Mean back-calculated lengths at age were similar between scales and sectioned otoliths (Figure 3). Back-calculated lengths at age 1 and age 2 were higher for scales than for sectioned otoliths. After age 2, back-calculated lengths were similar between structures, albeit with high variation partly due to low sample size of age-4 and older fish (i.e., based on scale age). The back-calculated lengths from sectioned otoliths closely mirrored the observed lengths, particularly for age-5 and younger Yellowstone Cutthroat Trout.

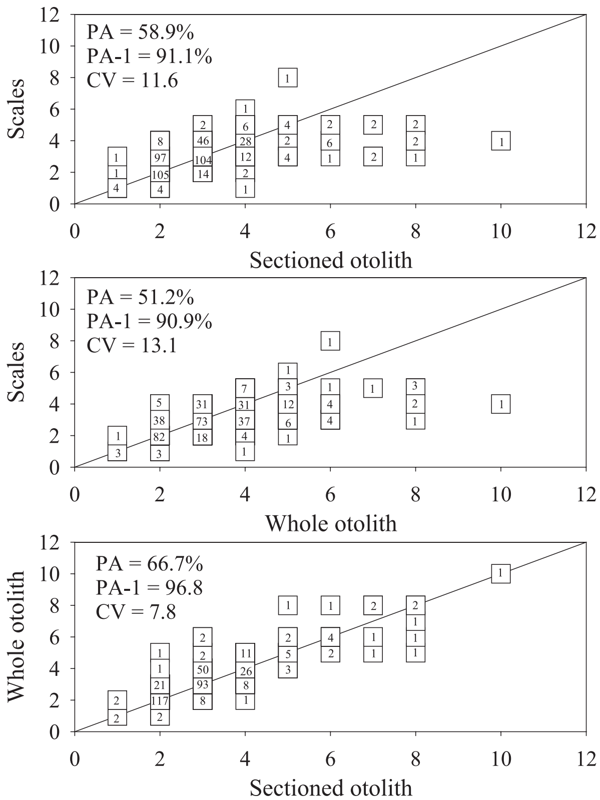

Figure 2. Comparison of sectioned otoliths, whole otoliths, and scale consensus age estimates from Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouveri* sampled from Henrys Lake, Idaho, 2019 and 2020. Percent exact agreement (PA), percent agreement within 1 y (PA-1), and the coefficient of variation (CV) are provided as measures of precision. Numbers inside boxes represent the observed number of fish.

Discussion

Sectioned otoliths produced the most precise age estimates with the greatest reader confidence. Validation of otoliths has not been conducted for Yellowstone Cutthroat Trout, but it has been shown to be an accurate ageing structure for a diversity of species, including salmonids (Schill et al. 2010; Haglund and Mitro 2017). Although we had a sample of known-age Yellowstone Cutthroat Trout, we did not formally assess accuracy of age estimates due to a truncated age distribution and low sample size. Nevertheless, all age assignments for known-age Yellowstone Cutthroat Trout from otoliths (whole and sectioned) were accurate.

Age assignments from scales had the lowest reader confidence, lowest between-reader precision, and low concordance with otolith ages. Imprecise and inaccurate age estimates from scales are common and usually attributed to reabsorption, presence of false annuli due to stress (e.g., temperature, food availability), and crowding of annuli as fish growth slows with age (Quist et al. 2012; McInerny 2017). Only a few studies examined different structures for estimating age of Yellowstone Cutthroat Trout. Hubert et al. (1987) reported that agreement between age estimates from scales and otoliths was 56% for Yellowstone Cutthroat Trout from Yellowstone Lake and that scales tended to underestimate age beyond age 4. Kruse et al. (1997) evaluated scales and whole otoliths from Yellowstone Cutthroat Trout in the Greybull River, Wyoming. Scales were less precise than otoliths and tended to underestimate age. Several studies have shown similar results for salmonid (e.g., Bilton and Jenkinson 1969; Sharp and Bernard 1988; Stolarski and Hartman 2008; Zymonas and McMahon 2009; Schill et al. 2010; Stolarski and Sutton 2013; Watkins et al. 2015) and nonsalmonid (e.g., Isermann et al. 2003; Maceina and Sammons 2006; Vandergoot et al. 2008; Isermann et al. 2018) fishes. In addition to

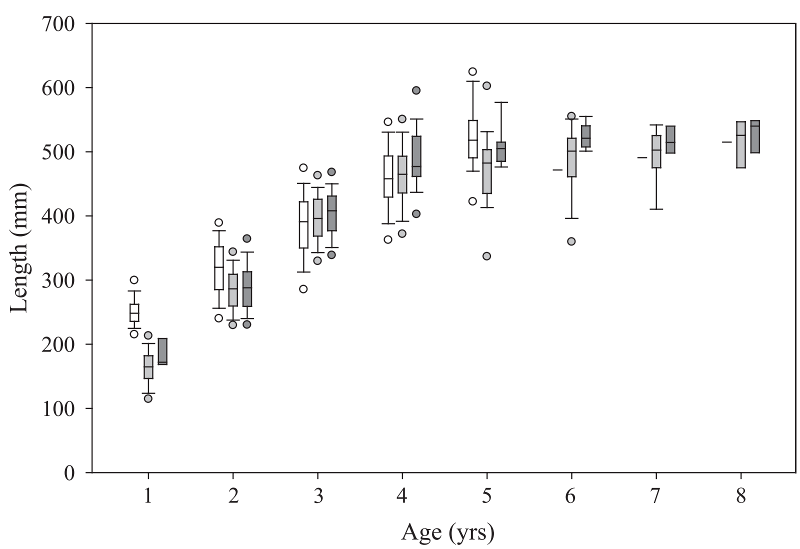

Figure 3. Back-calculated lengths for scales (open boxes) and sectioned otoliths (light gray boxes) and observed lengths at capture (dark gray boxes) for Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouveri* sampled from Henrys Lake, Idaho, 2019 and 2020.

differences between scales and otoliths, we noted differences between sectioned and whole otoliths.

Otoliths are widely accepted as the best structure for providing accurate age estimates (Long and Grabowski 2017). Although direct comparisons of accuracy by using different preparation techniques are lacking, several studies have shown discrepancies in age estimates based on the preparation technique of the otolith. Barber and McFarlane (1987) found that whole otoliths were more difficult to read and produced younger age estimates than sectioned otoliths from Arctic Char *Salvelinus alpinus* in Alaska and northern Canada. Newman et al. (2000) evaluated age estimates for three species of Red Snapper *Lutjanus* spp. from the Great Barrier Reef, Australia. Whole otoliths provided consistently lower and less precise age estimates than sectioned otoliths across species. Hyndes et al. (1992) found that whole otoliths consistently underestimated age compared with sectioned otoliths for Flathead *Platycephalus speculator* in an Australian estuary system. Although a few studies have shown similar age estimates between whole and sectioned otoliths (e.g., Long and Fisher 2001; Fernando et al. 2014; Isermann et al. 2018), most studies recommend using sectioned otoliths (e.g., Buckmeier and Howells 2003; Gallagher et al. 2016; Winkler et al. 2019). Our results are consistent with previous research in that sectioned otoliths for Yellowstone Cutthroat Trout in Henrys Lake were easier to read and had higher between-reader precision than whole otoliths. Whole otoliths are typically useful for fast-growing species or species with small otoliths that are difficult to section (Winkler et al. 2019). However, whole otoliths are problematic for slow-growing fishes and/or those for which the growth zones are obscured by the opaqueness of the otolith (e.g., large otoliths; Newman et al. 2000; Winkler et al. 2019). In addition, the edge periphery is often difficult to observe on whole otoliths from fishes with more spherical otoliths (Newman et al. 2010). After we collected the data for our study, we reexamined whole otoliths with knowledge of the sectioned otolith age estimate. Interpreting the periphery of whole otoliths was exceptionally difficult given the shape, and in most cases, we could not identify annuli on the whole otolith that were obvious on sectioned otoliths.

Research validating growth from ageing structures is exceptionally rare. This rarity is largely a function of the difficulty in acquiring a growth history for individual fish than can be compared with estimates from an ageing structure. Klumb et al. (2001) raised Bluegill *Lepomis macrochirus* × Green Sunfish *Lepomis cyanellus* hybrids in the laboratory and applied different back-calculation models to measurements obtained from scales and whole otoliths. Back-calculated lengths by using information from whole otoliths were generally less accurate than those based on data from scales. The curvature associated with whole otoliths, coupled with a linear growth model, likely contributed to the poor performance of otoliths in their study (also see Isermann et al. 2018). In contrast to the general paucity of growth validation studies, several studies compared back-calculated lengths among structures (e.g., Wahl et al. 2009; Homer et al. 2015). In Henrys Lake, scales produced back-calculated lengths that were approximately 85 mm greater than those from sectioned otoliths at age 1 and about 30 mm at age 2. After age 2, back-calculated lengths were similar between structures. Compared with scales, back-calculated lengths from sectioned otoliths were most concordant with observed lengths. Specifically, compared with mean lengths at capture (i.e., at ice-out when annuli were likely being formed), the difference between observed lengths and back-calculated lengths for otoliths varied from 5.2 to 25.2 mm for age-1 to age-4 Yellowstone Cutthroat Trout. For the same ages, differences between the observed length and back-calculated length from scales varied from 16.6 to 65.6 mm among fish. Although length at age at capture introduces error from variation in growth among years, estimates from sectioned otoliths seem reasonable given growth of Yellowstone Cutthroat Trout in the system.

The Idaho Department of Fish and Game transitioned from using scales for age and growth assessments to whole otoliths in 2002. Yellowstone Cutthroat Trout grow fast in Henrys Lake and experience relatively high natural mortality (McCarrick 2021). As such, nearly all of the Yellowstone Cutthroat Trout ($>$ 90%) are age 4 or younger. Scales provided age and growth data that were similar to that from otoliths, at least up to age 4. Consequently, we reached similar conclusions regarding population rate functions (i.e., fast growth, high mortality) by using scales and otoliths. Even though conclusions are similar between structures, using the best structure and preparation technique is important, particularly if population rate functions change abruptly. Otoliths require sacrificing fish and require more effort to prepare than other structures. However, sectioned otoliths were easier to read and provided the most precise estimates of age compared with scales and whole otoliths. Although back-calculated lengths for scales and sectioned otoliths were similar, sectioned otoliths provided estimates that were most similar to observed lengths. To our knowledge, this study represents the most comprehensive evaluation of ageing structures for Cutthroat Trout. Additional research on other Yellowstone Cutthroat Trout populations and Cutthroat Trout subspecies, particularly efforts focused on validating age and growth estimates, is greatly needed to aid in management and conservation efforts.

Supplemental Material

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

Table S1. Datafile titled "Yellowstone Cutthroat Trout Data Raw" including total length (millimeters), collection year, age estimates for each reader, confidence ratings for each reader, and consensus age estimate by structure for Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouveri* sampled from Henrys Lake, Idaho. Structures include scales, whole otoliths, and sectioned otoliths.

Available: https://doi.org/10.3996/JFWM-21-095.S1 (34 KB XLSX)

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