

PRECISION OF HARD STRUCTURES USED TO ESTIMATE AGE OF MOUNTAIN WHITEFISH (*PROSOPIMUM WILLIAMSONI*)

Carson J. Watkins^{1,4}, Tyler J. Ross², Ryan S. Hardy², and Michael C. Quist³

ABSTRACT.—The mountain whitefish (*Prosopium williamsoni*) is a widely distributed salmonid in western North America that has decreased in abundance over portions of its distribution due to anthropogenic disturbances. In this investigation, we examined precision of age estimates derived from scales, pectoral fin rays, and sagittal otoliths from 167 mountain whitefish. Otoliths and pectoral fin rays were mounted in epoxy and cross-sectioned before examination. Scales were pressed onto acetate slides and resulting impressions were examined. Between-reader precision (i.e., between 2 readers), between-reader variability, and reader confidence ratings were compared among hard structures. Coefficient of variation (CV) in age estimates was lowest and percentage of exact agreement (PA-0) was highest for scales (CV = 5.9; PA-0 = 70%) compared to pectoral fin rays (CV = 11.0; PA-0 = 58%) and otoliths (CV = 12.3; PA-0 = 55%). Median confidence ratings were significantly different ($P \leq 0.05$) among all structures, with scales having the highest median confidence. Reader confidence decreased with fish age for scales and pectoral fin rays, but reader confidence increased with fish age for otoliths. In general, age estimates were more precise and reader confidence was higher for scales compared to pectoral fin rays and otoliths. This research will help fisheries biologists in selecting the most appropriate hard structure to use for future age and growth studies on mountain whitefish. In turn, selection of the most precise hard structure will lead to better estimates of dynamic rate functions.

RESUMEN.—El salmón *Prosopium williamsoni* es una especie con amplia distribución en el oeste de América del Norte, cuya abundancia ha disminuido en algunas partes de su distribución debido a perturbaciones antropogénicas. En este estudio, analizamos la precisión de las estimaciones de edad a través de las escamas, radiografías de las aletas pectorales y otolitos sagitales de 167 ejemplares. Los otolitos y los radios de las aletas pectorales fueron montados en epoxy y seccionados antes de ser examinados. Las escamas se montaron sobre portaobjetos de acetato y analizamos las impresiones resultantes. Hicimos pruebas de confiabilidad entre lecturas (es decir, entre dos evaluadores), la variación entre lecturas y los niveles de confiabilidad entre lecturas con respecto a las estructuras duras. El coeficiente de variación (CV) en las estimaciones de edad resultó más bajo y el porcentaje de concordancia exacto (PA-0) resultó más alto en las escamas (CV = 5.9; PA-0 = 70%) en comparación con los valores relativos de las aletas pectorales (CV = 11.0; PA-0 = 58%) y a los otolitos (CV = 12.3; PA-0 = 55%). Los valores promedio de confiabilidad ($P \leq 0.05$) difirieron significativamente entre todas las estructuras, las escamas mostraron la confiabilidad promedio más elevada. La confiabilidad de lectura disminuyó con la edad del pez en las escamas y radiografías de la aleta pectoral, pero la confiabilidad de la lectura aumentó en los otolitos con la edad de los peces. En general, las estimaciones de edad fueron más precisas y la confiabilidad de lectura fue mayor en las escamas en comparación con las radiografías y los otolitos de la aleta pectoral. Esta investigación ayudará a los biólogos pesqueros en la selección de la estructura dura más apropiada a utilizar en futuros estudios de edad y crecimiento del salmón *Prosopium williamsoni*. A su vez, la selección de la estructura dura más precisa conducirá a una mejor estimación de las funciones de tipo dinámico.

The mountain whitefish (*Prosopium williamsoni*) is native to western North America and occupies a diversity of lentic and lotic systems from the McKenzie River in British Columbia, Canada, to the Colorado River in the United States (Behnke 2002). Compared to other salmonid species, mountain whitefish are not a popular game fish, thereby resulting in low interest in mountain whitefish populations by management agencies (Northcote and Ennis 1994). Historically, anglers have speculated

that interspecific competition existed between mountain whitefish and other, more desirable, salmonid species (Overton et al. 1978). Although this speculation has been unsubstantiated, the negative perception of mountain whitefish has led some management agencies to deplete mountain whitefish populations to reduce perceived competitive interactions with trout (Corsi 1956, Erickson 1971). Overall, little research has focused on mountain whitefish populations (Northcote and Ennis 1994, Meyer

¹Idaho Cooperative Fish and Wildlife Research Unit, Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Dr., MS 1141, Moscow, ID 83844.

²Idaho Department of Fish and Game, 2885 W. Kathleen Ave., Coeur d'Alene, ID 83815.

³U.S. Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Dr., MS 1141, Moscow, ID 83844.

⁴Present address: Idaho Department of Fish and Game, 2885 W. Kathleen Ave., Coeur d'Alene, ID 83815. E-mail: carson.watkins@idfg.idaho.gov

et al. 2009). More recently, however, natural resource managers have found value in using mountain whitefish as indicators of environmental change. For instance, McPhail and Troffe (1998) found that mountain whitefish are sensitive to habitat alteration, particularly from water development activities. Mountain whitefish typically inhabit large rivers where water development has occurred and where species of conservation concern or recreational value depend on the ecological role of mountain whitefish. Research has recently focused on understanding various characteristics (e.g., growth, age structure, age at maturity) of mountain whitefish populations (Meyer et al. 2009). Understanding more about the ecology of mountain whitefish has allowed researchers to better quantify local population-level influences from anthropogenic alterations and refine assessments of aquatic ecosystems (McPhail and Troffe 1998).

Knowledge about how fish populations are regulated is gained through information about age (i.e., longevity of a fish at a point in time) and growth (i.e., incremental change in body size as a function of time). Age data provide fisheries biologists with insight on dynamic rate functions (i.e., growth, recruitment, and mortality) that regulate fish populations (Ricker 1975). Quantifying dynamic rate functions generally requires the use of age data gleaned from hard structures. As such, obtaining accurate and precise estimates of rate functions is dependent on selecting hard structures that will yield the most precise age estimates within the constraints of the given project. Research projects are sometimes limited to using non-lethal hard structures because the study design (e.g., mark-recapture studies) does not allow for fish to be sacrificed. In addition to specific limitations, the accuracy and precision of age estimates from hard structures may vary among species and geographic regions. For example, Schramm and Doerzbacher (1985) reported that scales provided unreliable age estimates for black crappie (*Pomoxis nigromaculatus*) in the southeastern United States and suggested the use of otoliths. In contrast, Kruse et al. (1993) found that black crappie scales provided higher precision than otoliths among populations in the northern United States. Therefore, evaluating the precision of multiple structures across geographic regions is a necessary component of selecting a hard structure for age estimation.

Although a few studies have examined the population dynamics of mountain whitefish (Pettit and Wallace 1975, Meyer et al. 2009), no studies have evaluated the efficacy of hard structures used to estimate age. Previous studies have used scales (Pettit and Wallace 1975, Overton et al. 1978, Wydoski 2001) or sagittal otoliths (Meyer et al. 2009) to estimate ages of mountain whitefish, as both structures are commonly used to estimate age of many salmonids (e.g., rainbow trout [*Oncorhynchus mykiss*]; Schill et al. 2010). Although evaluating both precision and accuracy of hard structures is important (Beamish and McFarlane 1983), determining accuracy requires the use of known-age fish, which can be both costly and time consuming. Mountain whitefish of known age were not available in this study, so we were unable to assess accuracy. However, evaluating precision of age estimates provides biologists with guidance on selecting a hard structure that will reduce variation in age estimates and increase the repeatability of age and growth studies.

The objective of this study was to evaluate the precision and readability of scales, pectoral fin rays, and sagittal otoliths for estimating age of mountain whitefish. In light of recent and ongoing research related to mountain whitefish populations throughout the western United States, understanding which hard structures provide the most replicable age estimates with the highest level of confidence can improve efficiency for natural resource agencies undertaking age and growth projects. Increased precision in estimates of mountain whitefish age will lead to better estimates of population rate functions and allow managers to make more informed decisions.

METHODS

Fish Sampling and Hard Structure Processing

Mountain whitefish were collected on 2 February 2013 from the lower Kootenai River in Idaho using pulsed-DC, boat-mounted electrofishing. Electrofishing power output was standardized to 2750–3250 W based on ambient water temperature and conductivity (Miranda 2009). All individuals were measured to the nearest millimeter (total length), euthanized, and stored in a freezer for later processing. Five individuals per 1-cm length group were collected. Sagittal otoliths, scales, and pectoral

fin rays were later removed at the University of Idaho laboratory approximately 2–3 weeks after fish sampling. Otoliths were extracted following Schneidervin and Hubert (1986), cleaned, and placed in microcentrifuge tubes to dry. Approximately 10–20 scales were removed from the area directly ventral to the lateral line and posterior to the insertion of the pectoral fin on the left side of each fish. Pectoral fin rays were removed by cutting the left leading fin ray just proximal to the articulating process and rotating the fin ray out of the pectoral girdle following the procedure outlined in Koch et al. (2008). For smaller individuals (≤ 150 mm), the entire fin was removed so as to not damage the leading fin ray during removal.

All structures were allowed to air-dry for at least 2 weeks. After drying, scales were pressed onto acetate slides and impressions were read with a microfiche projector. Pectoral fin rays were mounted in epoxy using 2-mL microcentrifuge tubes following Koch and Quist (2007). Cross sections (0.6 mm thick) were cut near the base of each fin ray just distal to the articulating process using an Isomet low-speed saw (Buehler Inc., Lake Bluff, IL). Whole otoliths were examined first, but readers quickly deemed them unreadable. Therefore, otoliths were mounted in epoxy using 2-mL microcentrifuge tubes. Transverse sections (0.6 mm thick) were cut using a low-speed saw with sections bracketing the nucleus. Otolith and pectoral fin ray sections were viewed using a dissecting microscope with transmitted light and an image analysis system (Image ProPlus, Media Cybernetics, Silver Spring, MD). Thin-sectioned structures were polished with 1000-grit sandpaper and covered in a single drop of immersion oil prior to reading.

Hard Structure Analysis

Annuli were enumerated on all structures independently by 2 readers. Readers had different levels of age-estimation experience using fin rays; however, both readers had substantial (>4 years) experience estimating age of fishes with scales and otoliths. Both readers received training on age estimation using mountain whitefish pectoral fin rays by a single experienced reader immediately prior to the study. Knowledge of fish length and age estimates from other readers were not shared between readers during the age estimation process to avoid bias. In addition to the presumptive

number of annuli, readers also assigned a confidence rating to each structure (Fitzgerald et al. 1997, Koch et al. 2008, Spiegel et al. 2010). Confidence ratings were used as a measure of the readability of individual structures. We followed the rating criteria from Spiegel et al. (2010) where confidence ratings were integers between 0 (no confidence) and 3 (complete confidence). When age estimates differed between readers, a consensus age was assigned based on a joint examination. If a consensus could not be reached, the age estimate was removed from further analyses.

Data Analysis

Age-bias plots were used to evaluate between-reader precision for each structure (Campana et al. 1995). Age-bias plots were created for each structure by plotting the age estimates from Reader 1 against the estimates from Reader 2. Precision in age estimates between readers was assessed by calculating the percentage of agreement (i.e., exact and within-1-year agreement) in age estimates for each structure. The coefficient of variation ($CV = [SD/mean] \times 100$) was calculated and used as another measure of precision in age estimates (Campana et al. 1995). The CV was estimated for each individual fish and structure and then averaged to provide an estimate of between-reader precision by structure. A Kruskal–Wallis test was used to evaluate whether median confidence ratings differed among structures. Dunn's post hoc multiple comparison test was then used to determine which pairs of structures displayed significant differences in confidence rating. A Type I error rate at $\alpha = 0.05$ was used for all statistical tests.

RESULTS

We estimated ages of 167 mountain whitefish varying between 110 mm and 465 mm in total length. Of the 167 fish, all were retained for age estimation using pectoral fin rays, 163 were retained for age estimation using scales, and 162 were retained for age estimation using otoliths. Several scales were removed from the sample because the scales were unreadable and several otoliths were lost or not recovered during fish processing. Age estimates varied from 1 year to 11 years for scales, 1 year to 12 years for pectoral fin rays, and 1 year to 11 years for otoliths.

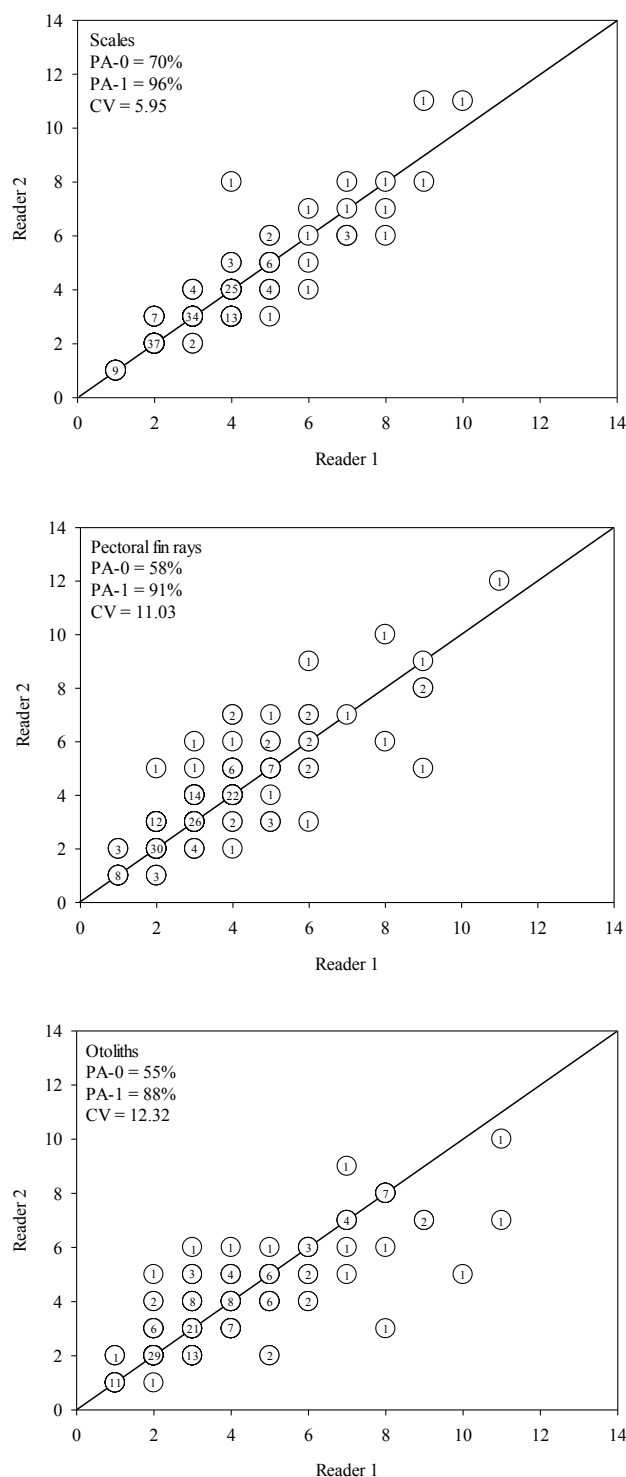


Fig. 1. Age-bias plots for ages assigned to scales ($n = 163$), pectoral fin rays ($n = 167$), and otoliths ($n = 162$) from mountain whitefish (*Prosopium williamsoni*) sampled from the Kootenai River, Idaho, in 2013. Precision between readers is indicated as percentage exact agreement (PA-0), percentage of agreement within 1 year (PA-1), and mean CV. Numbers in circles indicate the number of observations at each point.

Exact percentage of agreement (PA-0) between readers was highest for scales (70%), but lowest for pectoral fin rays (58%) and otoliths (55%). Percentage of agreement within 1 year (PA-1) increased to 96% for scales, 91% for pectoral fin rays, and 88% for otoliths. Scales also had the lowest mean CV (6.0), followed by pectoral fin rays (11.0) and otoliths (12.3). Examination of age-bias plots showed high concordance between readers for scales (Fig. 1). Less concordance between readers was observed for pectoral fin rays and otoliths (Fig. 1). Using pectoral fin rays and otoliths, Reader 1 tended to have lower age estimates compared to Reader 2. Median confidence ratings for scales were significantly higher than for pectoral fin rays and otoliths ($P = 0.002$). In addition, median confidence ratings for pectoral fin rays were significantly higher than for otoliths ($P = 0.03$).

DISCUSSION

The ability to precisely estimate age structure and dynamic rate functions of mountain whitefish populations is important for informing management. Although no previous studies have evaluated the precision of hard structures for estimating age of mountain whitefish, others have focused on salmonid species with similar ecology and life history (e.g., *Oncorhynchus* spp.; Hubert et al. 1987). Scales and sagittal otoliths have traditionally been used to estimate age of mountain whitefish (Pettit and Wallace 1975, Meyer et al. 2009, Benjamin et al. 2014), but the use of fin rays for estimating age of various fish species has increased in recent times (Quist et al. 2012).

Our results suggest that age estimates from pectoral fin rays of mountain whitefish were less precise than age estimates from scales and only slightly more precise than those from otoliths. These results contradict those of Zymonas and McMahon (2009) who found that age estimates derived from pelvic fin rays of bull trout (*Salvelinus confluentus*) were nearly as precise as those derived from otoliths and more precise than those derived from scales. Mills and Chalanchuk (2004) reported that fin rays had high accuracy in estimating age of lake whitefish (*Coregonus clupeaformis*); however, exact age agreement between fin rays and otoliths was relatively poor (49%). Muir et al. (2008) reported that pectoral fin rays (CV = 4.9)

provided more precise age estimates for lake whitefish than scales (5.4) and otoliths (5.4), which is also inconsistent with our results. We found that pectoral fin rays and otoliths had poor exact percent agreement (<60%) and high CVs (>11.0). Both readers in our study reported difficulty identifying annuli of pectoral fin rays among all age classes.

Otoliths are generally considered the most reliable hard structure for estimating age of fishes (Quist et al. 2012), particularly salmonids (Schill et al. 2010). Consequently, we expected that mountain whitefish otoliths would provide the most precise age estimates. However, between-reader agreement was lower and the CV was higher for otoliths compared to pectoral fin rays and scales. We found that otoliths were consistently assigned the lowest confidence ratings by both readers, particularly for younger fish. Both readers had difficulty identifying annuli in most otoliths. In fact, it was common for age estimates of the 2 readers to differ by 4 or more years. Scales have traditionally been the most commonly used hard structure for estimating age of fishes because they are nonlethal and easy to process. However, scales do not always form distinguishable annuli and are known to provide imprecise age estimates for many fishes (Schramm and Doerszbacher 1985, Marwitz and Hubert 1995, Sylvestre and Berry 2006, Quist et al. 2007, 2012). Issues related to poor readability and inconsistent annulus formation can cause erroneous age estimates, which can influence subsequent age and growth analyses (Quist et al. 2012).

In this study, scales from mountain whitefish had higher between-reader concordance than pectoral fin rays and otoliths (Fig. 1). Hubert et al. (1987) reported that scales of cutthroat trout (*Oncorhynchus clarkii*) from Yellowstone Lake lacked precision and were more prone to creating age bias than otoliths, pectoral fin rays, or dorsal fin rays. Schill et al. (2010) also reported that scales were not a viable option for estimating age of rainbow trout populations in high desert streams of Idaho and suggested that otoliths be used to obtain age estimates. Both of these studies provide results that are inconsistent with our findings. Several studies focused on salmonid species support the concerns associated with using scales to estimate age (Stolarski and Hartman 2008, Zymonas and McMahon 2009,

Schill et al. 2010). Contradictory to previous studies, however, we found that scales of mountain whitefish displayed the highest exact agreement (70%), highest within-1-year agreement (96%), and lowest CV (6.0).

Scales consistently received the highest confidence ratings by both readers, particularly for young fish (i.e., age 4 and younger). Readers using scales were also more confident in identifying early annuli among all age classes. In general, our results showed that reader confidence decreased in relation to age for scales and pectoral fin rays. However, reader confidence increased in relation to age for otoliths. Similar trends have been reported in previous studies showing that scales provide reliable age estimates for young age classes only (Jackson et al. 2007). Our study provides evidence that readers are less confident in assigning ages to scales from older mountain whitefish, but that precision was not influenced by age. Age-bias plots indicated that precision was not related to estimated age using scales. Although otoliths showed a pattern of increasing reader confidence in relation to assigned age for both readers, our results showed that precision was poor for all age classes using otoliths. Age estimates from otoliths tended to have poor between-reader agreement and high variation for both younger and older age classes.

The study herein sought to evaluate the relative precision and readability of scales, pectoral fin rays, and sagittal otoliths from mountain whitefish. Overall, this study showed that scales provided the most concordance between 2 readers and readers had the most confidence in assigning ages to scales. Our results contradict previous studies that have compared the precision of hard structures for other salmonid species. These studies found that otoliths provide the most precise age estimates. Many previous studies of mountain whitefish have been conducted using otoliths but have failed to account for between-reader variation and readability. Results of this study should be useful to fisheries scientists across western North America conducting age and growth studies on mountain whitefish populations. Future studies focused on mountain whitefish population dynamics can expect repeatable results and more precise estimates of rate functions by using scales, which will ultimately allow fisheries scientists to better

quantify factors influencing mountain whitefish population structure and dynamics.

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