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Comparison of precision and bias of scale, fin ray, and otolith age estimates for lake whitefish (*Coregonus clupeaformis*) in Lake Champlain

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ABSTRACT

We compared the precision, bias, and reader uncertainty of scales, dorsal fin rays, and otolith age estimates from 151 lake whitefish (*Coregonus clupeaformis*) from Lake Champlain, 2009. Mean and systematic differences in age estimates were compared among structures using consensus ages from two readers; precision of age structures was quantified through the use of age–bias plots, coefficient of variation, and percent agreement; reader confidence was indexed as a measure of overall reader uncertainty for each individual fish by structure. Mean age estimates based on otoliths were systematically higher (7.8 years) than based on scales (6.0 years) or fin rays (5.6 years). Ages determined using otoliths generated a wider range of ages and greater number of age classes (1–23 years, 20 age classes) when compared with scales (1–16 years, 15 age classes) and fin rays (1–14 years, 13 age classes). Otoliths were the most precise of the structures (CV = 4.7, compared with 7.4 for scales and 12.1 for fin rays). Percent agreement between readers indicated high precision and reproducibility of age estimates using otoliths. Percent reader uncertainty was lowest when using otoliths (7.6%) in comparison with fin rays (21.2%) or scales (26.8%). This study is the first evaluation of precision and bias of age structures for Lake Champlain's unexploited lake whitefish population and suggests that otoliths are the most appropriate structure for age estimation. However, the differences in age estimates from the three structures in this study emphasize the importance of validating aging structures to provide accurate age estimates for Lake Champlain.

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Introduction

Age data from fish populations are valuable for modeling population dynamics to understand trends in growth, age at maturity, and estimates of mortality (Campana, 2001). The use of inaccurate ages can cause severe errors in fish population management (Beamish and McFarlane, 1983; Yule et al., 2008). Given the importance of accurate estimates of age, in recent years fisheries scientists have focused on the importance of validating and comparing the various aging structures (Mills and Beamish, 1980; Beamish and McFarlane, 1983; Muir et al., 2008a; Bruch et al., 2009; Davis-Foust et al., 2009).

Age estimation has been examined in lake whitefish (*Coregonus clupeaformis*) since the late 1920s, initially using scales; scales are still used for routine lake whitefish (hereafter whitefish) age estimation by some state agencies. The scale method of age estimation and justification as published by Van Oosten (1923, 1929), using scales from whitefish held in the New York Aquarium (an artificial environment) for a known period of time; in this study he determined that annuli were formed annually. However, tagged whitefish in Little

Moose Lake, New York, failed to form a scale annulus between marking and recapture (Neth, 1955). Such discrepancies led fisheries professionals to begin to determine precision and accuracy of aging structures, using techniques later described by Campana et al. (1995). Aging using scales, fin rays, and otoliths have been compared for many whitefish populations (unexploited and exploited) to determine precision and evaluate ease of preparation. Scales commonly yield lower ages for whitefish in comparison to estimates from fin ray and/or otoliths (Barnes and Power, 1984; Mills et al., 2005; Hosack, 2007; Muir et al., 2008a). The few studies that compare fin ray to otolith age estimates reported little to no difference between the age estimates from these two structures (Mills and Chalanchuk, 2004; Muir et al., 2008b). Studies validating aging structures for whitefish are limited; however, Mills and Chalanchuk (2004) and Mills et al. (2004) validated otolith and fin rays for age estimation on unexploited whitefish using mark-recapture and successive removal of fin rays.

Concerns regarding the use of age estimates from structures that are not validated are well known (Beamish and McFarlane, 1983; Campana, 2001). However, validation requires capture and recapture of known-age individuals over multiple years to determine whether an annulus was formed each year following initial age estimation for multiple age classes. Because of the long time frame (years) and labor-intensive field work for validation, managers use other techniques to assess age estimation, such as age structure comparisons. Comparisons

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among aging structures are valuable because ages determined from different structures taken from an individual fish often do not agree, due to difficulty in identifying the first annulus, clustering of annuli on the edge of the structure, or poor preparation of the structure for aging (Campana, 2001; Mills and Chalanchuk, 2004). Clustering of annuli on the edge of a structure is common among older individuals with slow growth rates. Given that growth is variable among populations, the most precise and accurate aging structures may vary among bodies of water. Age-bias plots and the use of statistical tests such as correlation of variation and/or paired *t*-tests be used when trying to assess bias (systematic or random) and precision of fish aging structures (Campana et al., 1995).

Lake Champlain's unexploited whitefish population has been unstudied for almost 80 years and nothing is currently known regarding the population's age structure. The objectives of this study were to 1) compare age estimates from three different structures (scales, fin ray, and otoliths), 2) quantify precision of age estimates for each structure, and 3) quantify reader confidence in age determination for each structure from a relatively unstudied whitefish population from Lake Champlain. Mean and systematic differences in age estimates were compared among structures using consensus ages from two readers; precision of age structures were quantified through the use of age-bias plots and percent agreement; and reader confidence was indexed as a measure of overall reader uncertainty for each individual fish by structure.

Methods

Fish collection and processing

Whitefish were collected from 28 April through 13 November 2009 in Lake Champlain using a 7.6-m semi-balloon otter trawl (6.4-mm stretched-mesh cod end-liner with a chain footrope) and multiple graded monofilament bottom set gill nets to attempt to collect fish of various size classes. Gill nets were 1.8 m deep and 70.6–152.4 m long, and included panels of 7.6, 8.9, 10.2, 11.4, 12.7, 14, and 15.2 cm stretch mesh. Whitefish were sacrificed, measured (TL mm), weighed (nearest g), and three aging structures (scales, fin ray, and sagittal otoliths) were collected from each individual. Scales were removed from the region on the fish located between the posterior end of the dorsal fin and the lateral line, cleaned, dried, and mounted between microscope slides. The first three dorsal fin rays were removed at their bases and placed in scale envelopes to dry. Dried fin rays were embedded in epoxy covering the proximal joint and base of the ray. The rays were then cut into thin cross sections (~1.0 mm) at approximately a 90° degree angle nearest to the base of the ray using a Buehler low speed Isomet® saw with a diamond wafering blade. Sagittal otoliths were extracted and placed in individually labeled scale envelopes. Otoliths were placed in modeling clay for stability and transversely cut through the nucleus using a dremel tool with a 22.23 × 0.13 mm separating disk (Kingsley North, Inc.). Each otolith half was burned lightly on the cut surface to highlight the annuli, similar to the crack-and-burn technique (Schreiner and Schram, 2001).

Two readers estimated ages from all three structures without access to information on fish size or season collected, to avoid potential bias of interpretation. Scales were examined using a microfiche reader. A scale annulus was defined using the criteria of circuli crowding and “cutting over” described by Beamish and McFarlane (1983) and Muir et al. (2008a). Fin ray age was estimated by viewing the cross section with a dissecting scope at 18–110 magnification using transmitted light. A fin ray annulus was defined as the clear opaque zone or ring between the darker areas on the fin ray, which represent periods of growth (Mills and Beamish, 1980; Mills and Chalanchuk, 2004). Otolith age was estimated by viewing each of the burnt sections under reflected light with the same

dissecting scope used for estimation of fin ray age. An otolith annulus was defined as the complete distinct dark ring adjacent to a region of clear opaque growth (Muir et al., 2008a, 2008b). Age estimates using all three structures for each individual were blindly assigned by each reader; in situations of uncertainty a second age estimate was also recorded. Lastly, the two readers decided on a consensus age for each individual fish and structure. Consensus age was determined by comparing initial age estimates from each reader. If the estimates were equal, that age was used; if age estimates from the two readers differed by one year, the older age was used, and if ages differed by more than 1 year, the older intermediate age between the initial ages was used for analysis. For example, if reader estimates were 6 and 9 years for scales from the same fish, the consensus age recorded was 8 years. Older ages were assigned as the consensus age to compensate for the tendency for scales to commonly underestimate age (Power, 1978; Mills and Beamish, 1980; Barnes and Power, 1984; Mills and Chalanchuk, 2004; Muir et al., 2008a). The same method was used with fin rays and otoliths to remain consistent.

Data analysis

Mean consensus ages determined using three aging structures (scales, fin rays, and otoliths) were compared using paired *t*-tests. Precision and bias were quantified for the three structures using age-bias plots and by comparing percent agreement between readers and structures. Age-bias plots illustrate one age reading against another (reader to reader or structure to structure) and are interpreted through reference to the 1:1 equivalence (Campana et al., 1995). Precision was estimated by calculating the percent agreement between readers for each of the three structures, and calculating the coefficient of variation (CV; Campana et al., 1995). The CV formula for each individual was,

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

where *R* equals the number of times the age of each individual was estimated; *X_j* equals the mean age estimated for the *j*th fish, and *X_{ij}* is the *i*th age estimate for the *j*th fish (Chang, 1982). A symmetry test of age estimates by readers and structures was used to determine at what age readers and structures began to differ from equivalence using Bowker's (1948) test of symmetry, a technique modified by Hoenig et al. (1995). The measure of readability or confidence a reader had in each structure was estimated as percent uncertainty. Percent uncertainty was calculated by averaging the number of fish to which secondary ages were assigned for each structure. For example, if reader 1 was uncertain about 22 estimated ages and reader 2 was uncertain about 14 estimated ages using scales and the total number of individuals examined was 151, the percent uncertainty for scales would be the total number of uncertainties from reader 1 and 2 (i.e., 36) divided by 302.

Results and discussion

Age was estimated from scales, fin rays, and otoliths using two readers from 151 whitefish collected in 2009. Mean total length of whitefish was 436 mm (SE = 9.7 mm, range = 135–658 mm). Mean age estimates based on otoliths were systematically higher (7.8 years) than based on scales (6.0 years) or fin rays (5.6 years). Mean otolith age was significantly greater than mean scale (*P* < 0.001, *df* = 150) and fin ray age (*P* < 0.001, *df* = 150). Mean scale age was also significantly greater than mean fin ray age (*P* < 0.001, *df* = 150). Ages determined using otoliths generated a wider range of ages and greater number of age classes (1–23 years, 20 age classes) when compared with scales (1–16 years, 15 age classes) and fin rays (1–14 years, 13 age classes).

Between-reader bias did not occur when using otoliths, scales, or fin rays for age determination. Age estimated by reader 2 plotted against age estimated by reader 1 did not stray significantly from the equivalence line, indicating unbiased estimates between readers for the same structure (Fig. 1). Otoliths were the most precise of the structures ($CV = 4.7$), with the tightest groupings of age estimates along the equivalence line, even for older fish (Fig. 1). Percent agreement between readers indicated high precision and reproducibility of age estimates using otoliths; the two readers completely agreed for 62.3% of the fish, and their age estimates were within 1 year for 94% of the fish. Differences in age estimates between readers never differed by more than 2 years when using otoliths (Table 1). Fin rays and scales were less precise ($CV = 7.4$ and 12.1), especially for individuals estimated to be ≥ 9 years old (Fig. 1). Fin ray and scale ages also had a lower percent agreement (56.3 and 45.7%, respectively) between readers and the differences in age estimates between readers were also greater; in two instances, scale ages assigned by readers differed by 7 years (Table 1).

Prior studies have emphasized the importance of making comparisons with experienced readers (e.g., 10+ years experience, Mills and Beamish, 1980); however, agencies frequently do not have experienced readers and age estimation is done by seasonal employees.

Table 1

Percent agreement between two readers for three aging structures (otoliths, fin rays, and scales) from 151 whitefish collected in Lake Champlain, 2009. Percent reader uncertainty is the average number of fish to which secondary age estimates were assigned for each structure and is a measure of reader confidence, with the lowest percent indicating the highest confidence.

Between-reader difference (years)	Otoliths	Fin rays	Scales
0	62.3	56.3	45.7
± 1	94.0	88.7	75.5
± 2	100	97.4	90.7
± 3		98.7	96.0
± 4		100	97.4
$> \pm 4$			100
% Reader uncertainty	7.6	21.2	26.8

Therefore, we argue that the best structure, in the absence of validation, is one that does not require extensive prior experience to estimate age with the highest confidence and precision. We used percent reader uncertainty to determine reader confidence with each structure. Percent reader uncertainty was lowest when using otoliths (7.6%) in comparison with fin rays (21.2%) or scales (26.8%; Table 1). The majority (62.2%) of uncertainties recorded by readers were from individuals greater than 450 mm (TL). Mean total length of fish with

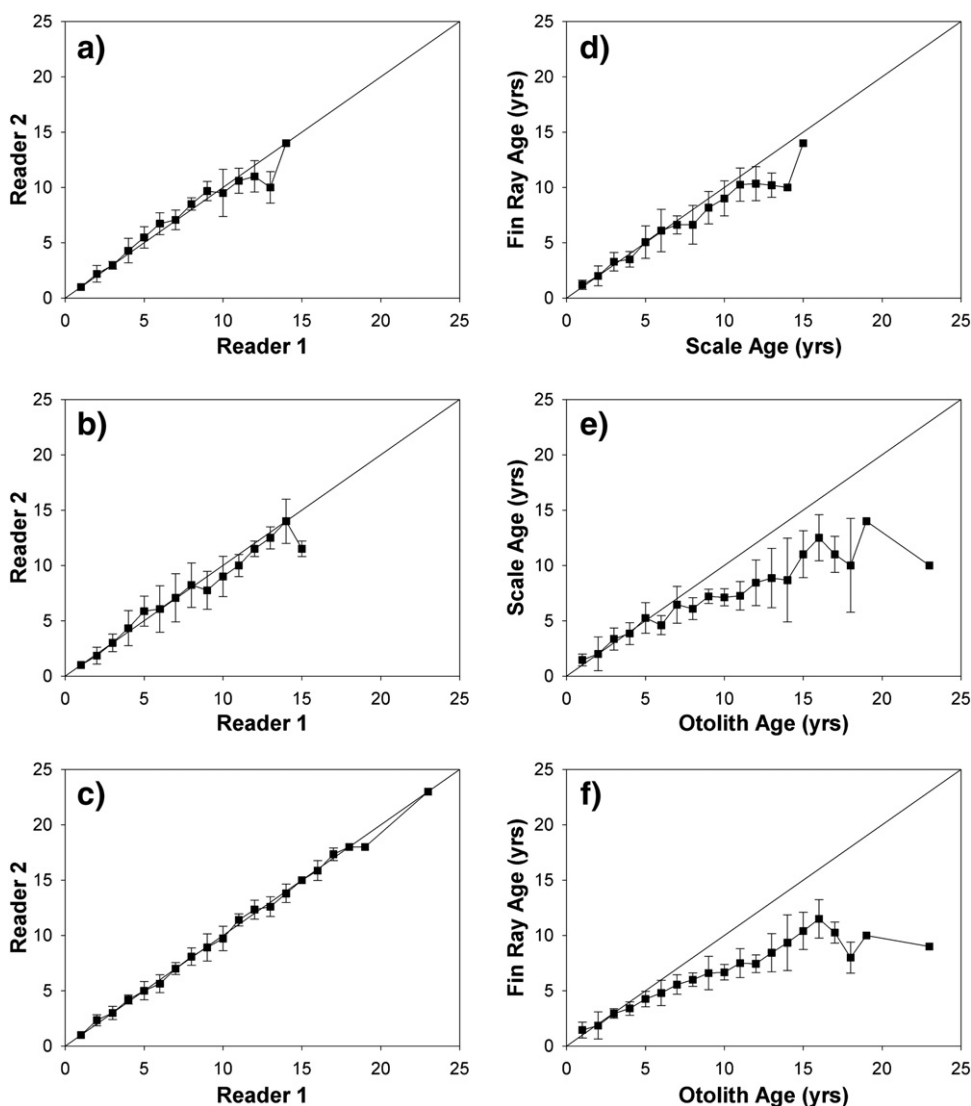


Fig. 1. Age-bias plots comparing age estimates from two readers using (a) fin rays, (b) scales and (c) otoliths, and comparing estimated ages between (d) fin rays vs. scales, (e) scales vs. otoliths and (f) fin rays vs. otoliths for 151 whitefish collected in Lake Champlain, 2009. Error bars indicate \pm SD about the mean; the solid line represents 1:1 equivalence between readers or structures.

secondary ages assigned using otoliths was lower (460 mm) than fin rays (466 mm) and scales (483 mm), indicating that readers had higher confidence estimating age for larger individuals with otoliths. Otoliths give the most reproducible age with the highest level of confidence; therefore, they are particularly valuable when age estimation is done by inexperienced readers. For example, Robillard and Marsden (1996) reported that precision increased based on experience, but precision using otoliths was higher than scales even for the most inexperienced readers.

Otolith age estimates were consistently greater beginning with age 4 and older fish when compared with fin rays ($X^2=10.07$, $df=5$, $P=0.07$) and age 8 and older fish when compared with scales ($X^2=21.18$, $df=16$, $P=0.17$; Fig. 1). Scale age estimates were symmetric with fin ray ages ($X^2=35.43$, $df=30$, $P=0.23$) with scales yielding slightly greater estimates beginning at age 7 (Fig. 1). However, this trend was not as distinct as the differences in ages when comparing otolith ages to fin rays or scales.

Our results indicate that age estimates based on scales, fin rays, and otoliths yield significant differences in mean age for whitefish in Lake Champlain. These results are consistent with the general understanding that scale age estimates are frequently lower than otoliths-based estimates for whitefish (Power, 1978; Barnes and Power, 1984; Mills et al., 2005; Hosack, 2007; Muir et al., 2008a). Similarly, otolith age estimates for yellow perch (*Perca flavescens*) and freshwater drum (*Aplodinotus grunniens*) were regularly greater than estimates using scales (Robillard and Marsden, 1996; Davis-Foust et al., 2009). However, we were surprised to find that our age estimates from fin rays and otoliths differed significantly, which was in contrast to unexploited whitefish populations in Canada (Mills and Chalanchuk, 2004; Mills et al., 2004). The differences in age estimates from the three structures in this study further emphasize the importance of validating aging structures for different populations throughout the native whitefish range to provide accurate age estimates. Without validation, managers should use caution when deciding which structure should be used when assigning fish age for the use in population modeling.

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