Age Estimation of Burbot Using Pectoral Fin Rays, Branchiostegal Rays and Otoliths

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Abstract

Throughout much of its native distribution, burbot (*Lota lota*) is a species of conservation concern. Understanding dynamic rate functions is critical for the effective management of sensitive burbot populations, which necessitates accurate and precise age estimates. Managing sensitive burbot populations requires an accurate and precise non-lethal alternative. In an effort to identify a non-lethal ageing structure, we compared the precision of age estimates obtained from otoliths, pectoral fin rays, dorsal fin rays and branchiostegal rays from 208 burbot collected from the Green River drainage, Wyoming. Additionally, we compared the accuracy of age estimates from pectoral fin rays, dorsal fin rays and branchiostegal rays to those of otoliths. Dorsal fin rays were immediately deemed a poor ageing structure and removed from further analysis. Age-bias plots of consensus ages derived from branchiostegal rays and pectoral fin rays were appreciably different from those obtained from otoliths. Exact agreement between readers and reader confidence was highest for otoliths and lowest for branchiostegal rays. Age-bias plots indicated that age estimates obtained from branchiostegal rays and pectoral fin rays were substantially different from age estimates obtained from otoliths. Our results indicate that otoliths provide the most precise age estimates for burbot.

Key words: Burbot, Age and growth, Precision, Age estimation, Otoliths, Wyoming

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Introduction

Burbot (*Lota lota*) is the only freshwater member of the family Gadidae (McPhail and Paragamian 2000). It has a circumpolar distribution rarely extending south of the 40th parallel N and occupies diverse lentic and lotic habitats throughout Europe, Asia and North America. Secure burbot populations exist in Alaska, much of Canada and several Eurasian countries (Latvia, Lithuania, Switzerland, Russia; Stapanian et al. 2010). However, across much of its native distribution, burbot populations are declining or completely extirpated as in many Eurasian countries (Tammi et al. 1999, Dillen et al. 2008, Stapanian et al. 2010), the United States and Canada (McPhail and Paragamian 2000, Stapanian et al. 2008, 2010). Therefore, the conservation of burbot is a major management focus of numerous natural resource agencies worldwide.

Effective management of fish populations requires knowledge about the most influential functions controlling productivity: recruitment, growth and mortality (Ricker 1975). Recruitment, often defined as the age a fish is recruited to a population or fishery, has obvious implications for fisheries management. Back calculations are ubiquitous in fisheries research and allow for estimates of growth given age and length data (Quist et al. 2012). Similarly, catch curves are essentially an age distribution by which inferences about mortality can be established (Chapman and Robson 1960). Regardless of the method used to evaluate population dynamics, accurate calculations of rate functions rely on precise and accurate age estimates.

Sagittal otoliths are the primary structure used to estimate the age of burbot. A number of studies have evaluated the precision and (or) accuracy of otoliths for estimating the age of burbot (McCrimmon and Devitt 1954, Guinn and Hallberg 1990, Stuby 2000, Edwards et al. 2011). Stuby (2000) validated age estimates of otoliths from burbot in the Fish Creek drainage, Alaska using oxytetracycline (OTC) and reported 100 percent accuracy in age estimates beyond the OTC mark. Unfortunately, the use of otoliths requires sacrificing fish. In areas where burbot conservation is a concern, managers are often unwilling to sacrifice burbot. Therefore, a non-lethal method for accurately and precisely estimating the age of burbot is highly desirable.

Few studies have evaluated the viability of non-lethal structures for estimating the age of burbot. Scales of burbot are generally disregarded for age estimation due to their small size, difficulty in reading and misrepresentation of annuli (McCrimmon and Devitt 1954, Guinn and Hallberg 1990). Pectoral fin rays have also been used to assess the age of burbot (McCrimmon and Devitt 1954, Giroux 2005). However, previous research suggests pectoral fin rays of burbot are difficult to read and consistently underestimate the age of burbot. To acknowledge declining burbot populations and conservation efforts around the world, non-lethal options for ageing burbot requires further research.

The goal of this study was to assess the precision of age estimates of burbot obtained from all structures while evaluating the accuracy of pectoral fin rays, dorsal fin rays and branchiostegal rays compared to otoliths.

Study Area

The Green River is the largest tributary of the Colorado River and drains portions of Wyoming, Utah and Colorado (Wyoming Game and Fish Department 2010). The Green River originates in the Wind River Range of western Wyoming and flows for approximately 235 km before entering Fontenelle Reservoir (Wyoming Game and Fish Department 2010). From Fontenelle Reservoir, the Green River flows for about 150 km until it enters Flaming Gorge Reservoir at the Wyoming-Utah border. Flaming Gorge Dam was completed in 1962 impounding approximately 17,000 hectares of water with a maximum depth of 34 m (Teuscher and Luecke 1996). Flaming Gorge Reservoir is approximately 145 km long and encompasses portions of western Wyoming and northeastern Utah.

Methods and Materials

Burbot were sampled from the Green River using electrofishing in the summer and autumn of 2013. Electrofishing was conducted at night using a drift boat equipped with a 5,000 W generator and Smith-Root VVP-15B electrofisher (Smith-Root, Vancouver, WA). Electrofishing power output was standardized to 2,750 – 3,200 W (Miranda 2009). Burbot were sampled from Flaming Gorge Reservoir using trammel nets in the autumn of 2013. Trammel nets were 48.8 m long and 1.8 m wide, with 25.4-cm outer bar mesh and 2.5-cm inner bar mesh. Nine nets were set perpendicular to shore and fished for approximately 24 hours.

All burbot sampled were enumerated and measured to the nearest millimeter (total length). Up to ten burbot from each 10 mm length group were euthanized with an overdose of tricaine methanesulfonate (MS-222; Western Chemical, Ferndale, Washington). Sagittal otoliths, pectoral fin rays, dorsal fin rays and branchiostegal rays were removed from each fish in the field. Otoliths were accessed from the ventral surface and removed following Schneidervin and Hubert (1986). The left leading pectoral fin ray was removed by cutting at the insertion of the articulating process (Koch et al. 2008). The anterior-most dorsal fin ray was removed by cutting into the surrounding tissue and rotating the dorsal fin ray until it was pulled free. The ventral-most branchiostegal ray (largest) was removed by rotating the structure until it pulled free from the hyoid complex. Otoliths, pectoral fin rays, dorsal fin rays and branchiostegal rays were cleaned of tissue and stored in numbered scale envelopes and allowed to air dry.

Structures were mounted in epoxy and sectioned using a low speed saw (Buehler, Lake Bluff, Illinois; Koch and Quist 2007). Fin rays and branchiostegal rays were mounted in epoxy with the proximal end down in 2 ml centrifuge tubes following Koch and Quist (2007). Fin rays were cross-sectioned at the base of the structure. Upon initial examination, branchiostegal rays had a small protrusion near the proximal end of the structure which interfered with annuli identification. Therefore, branchiostegal rays were cross-sectioned immediately distal to the protrusion. Cross-sections of fin rays and branchiostegal rays measured approximately 0.7 mm in thickness. Otoliths were mounted in epoxy in 2 ml centrifuge tubes and transversely sectioned about the nucleus (Edwards et al. 2011). Otolith cross-sections measured approximately 0.5 mm in thickness. Cross-sections were examined using a dissecting microscope with transmitted light and an image analysis system (Image-Pro Plus; Media Cybernetics, Silver Springs, Maryland).

Annuli were enumerated independently by two readers without knowledge of fish length, sampling location, or prior age estimates. Both readers had experience enumerating annuli of various structures prior to the study. After each reader assigned an age, each age estimate was compared. If discrepancies existed between age estimates, the structure was re-aged by both readers and discussed in a mutual reading. If a consensus age could not be reached, the structure was removed from further analysis.

In addition to an age estimate, readers assigned a rating indicating their confidence in their age estimate (Fitzgerald et al. 1997, Koch et al. 2008, Spiegel et al. 2010). Following the rating criteria suggested by Spiegel et al. (2010), readers assigned a confidence rating that varied from 0 to 3. A confidence rating of 0 indicated the reader had no confidence in their age estimate; whereas, a rating of 3 corresponded to near absolute confidence in the reader's age estimate.

Reader bias was evaluated by plotting age estimates from reader one against reader two (Campana et al. 1995). Differences in confidence ratings by structure were evaluated using a Kruskal-Wallis test. A Tukey's honest significant difference post-hoc procedure was used to determine if confidence ratings between pairs of structures were significantly different. All statistical tests used a type I error rate at $\alpha = 0.05$. Between-reader precision for each structure was evaluated by calculating the coefficient of variation (CV; Campana et al. 1995). The CV was calculated as:

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

where X_{ij} is the ith age determination for the *j*th fish, X_j is the mean age of the *j*th fish and *R* is the number of times each fish was aged (Campana et al. 1995). The accuracy of age estimates for fin rays and branchiostegal rays was evaluated by comparing the consensus age estimates from each structure to the consensus age estimates from otoliths using age-bias plots. A CV was calculated for consensus age estimates of pectoral fin rays, dorsal fin rays and branchiostegal rays as an additional measure of accuracy. Concordance between consensus ages and reader bias was interpreted in reference to the equivalence line. In addition, variation in age estimates between readers and structure was assessed by calculating the percent agreement [exact (PA-0), within-1 year (PA-1)].

Results

Two readers estimated the age of 208 burbot from the Green River drainage, Wyoming (Table 1). Burbot averaged 418 mm in length and had a length distribution of 116 – 898 mm. Consensus age estimates varied from 0 – 11 for otoliths and branchiostegal rays and 0 – 10 for pectoral

Table 1. Sample size (n), and total length (mm) statistics of burbot sampled for age estimation from the Green River drainage, Wyoming (2013). Mean, standard deviation (SD), minimum (min), and maximum (max) lengths are provided (mm).

	Total Length				
Location	n	Mean	SD	Min	Max
Green River	128	422	138	116	686
Flaming Gorge Reservoir	80	411	127	285	898

fin rays (Fig. 1). A subset of 100 dorsal fin rays was independently read by both readers, but annuli were largely indiscernible. Therefore, dorsal fin rays were deemed a poor structure for estimating the age of burbot and removed from further analysis.

Readers were most confident in the age estimates for otoliths and least confident in the age estimates for branchiostegal rays (Table 2). Mean reader confidence was 2.9 (SD = 0.40) for otoliths, 1.6 (SD = 0.94) for pectoral fin rays and 1.3 (SD = 0.68) for branchiostegal rays. Confidence ratings of branchiostegal rays and pectoral fin rays were significantly different when compared to confidence ratings of otoliths ($P = 0.00$). Readers consistently reported lower confidence ratings for older fish (≥ 5 years old) using branchiostegal rays and pectoral fin rays than for younger fish. However, age estimates for otoliths were generally assigned high confidence rating by both readers regardless of the individual fish's presumptive age. For example, the mean confidence rating for branchiostegal rays was 1.27 (SD = 0.59) for fish with a consensus age ≥ five years; whereas, the mean confidence rating for otoliths was 2.81 (SD = 0.42) for fish with age estimates five years or older.

Exact agreement between age estimates of both readers (PA-0) was highest for otoliths and lowest for branchiostegal rays. Exact agreement between reader's age estimates was 90.4 percent for otoliths, 68.3 percent for pectoral fin rays and 58.4 percent for branchiostegal rays. Percent agreement between estimated ages within-1 year was 100.0 percent for otoliths, 93.3 percent for pectoral fin rays and 88.0 percent for branchiostegal rays. Between-reader CV was lowest for otoliths and highest for pectoral fin rays (Fig. 2). Age-bias plots indicated that concordance was highest between the age estimates of readers one and two for otoliths (Fig. 2). Age estimates using pectoral fin rays and branchiostegal rays showed high concordance between readers for fish less than 5 years old (i.e., consensus age). Relative to reader one, reader two tended to underestimate the age of older fish (> 5 years) using pectoral fin rays and branchiostegal rays.

Consensus age estimates from branchiostegal rays and pectoral fin rays tended to disagree with sectioned otoliths (Fig. 3). Branchiostegal rays and otoliths showed high concordance up to age 5. After age 5, branchiostegal rays tended to underestimate fish age when compared to age estimates obtained from otoliths. Age estimates from pectoral fin rays displayed little agreement with otoliths and consistently underestimated fish age. When compared to otoliths, exact agreement between consensus ages was 27.9 percent for branchiostegal rays and 11 percent for pectoral fin rays. Agreement within-1 year was 69.7 percent for branchiostegal rays and 39.0 percent for pectoral fin rays when compared to age estimates obtained from otoliths.

Discussion

Our findings support previous research suggesting sectioned otoliths provide precise age estimates for burbot. Stuby (2000) compared the readability of whole and sectioned burbot otoliths and observed higher readability in sectioned burbot otoliths.

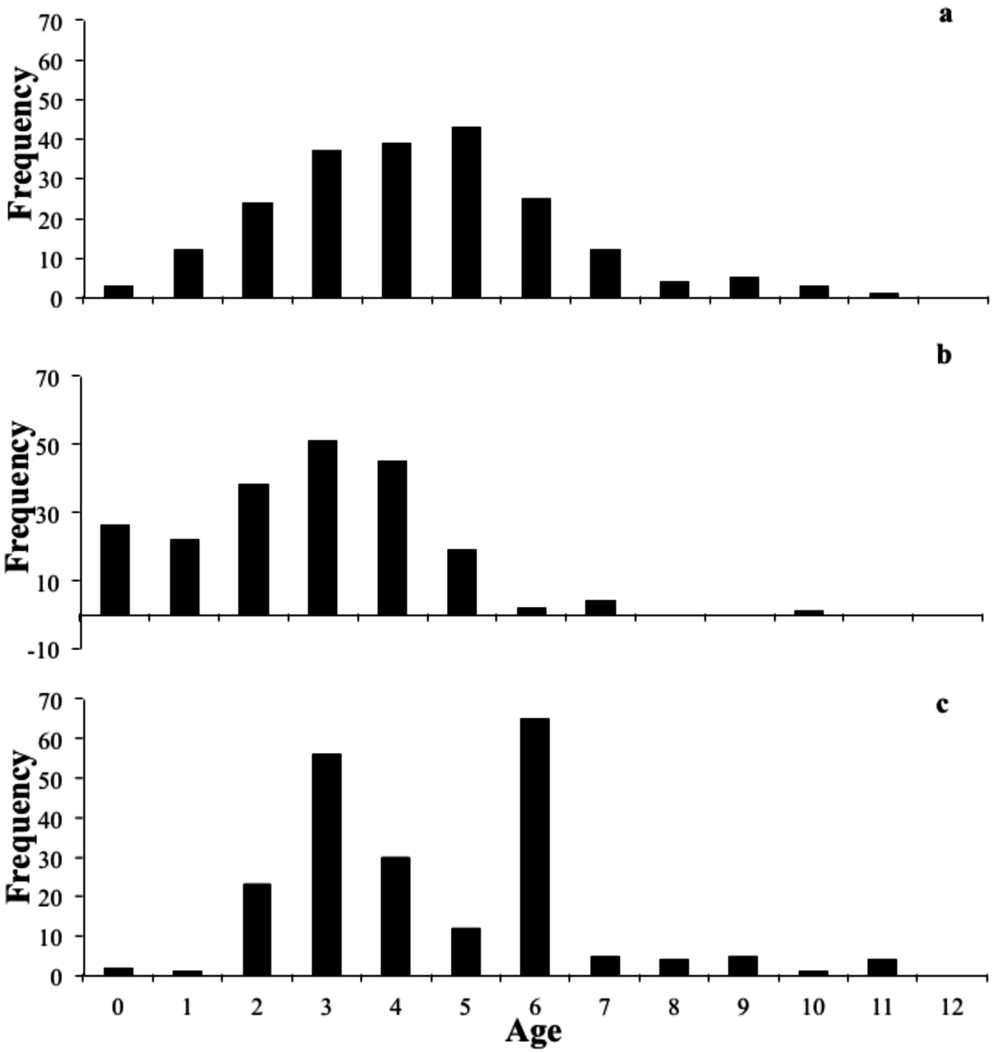

Figure 1. Age-frequency distributions for branchiostegal rays (a), pectoral fin rays (b), and otoliths (c) from burbot collected from the Green River drainage, Wyoming (2013).

Table 2. Percent confidence rating for reader one and two by structure for burbot collected from the Green River drainage, Wyoming (2013). Each structure represents the same individual fish with the sample size included in parenthesis.

	Confidence Rating			
	0	1	2	3
Branchiostegal rays				
Reader 1	5% (11)	62% (129)	29% (61)	4% (7)
Reader 2	10% (21)	54% (113)	29% (61)	7% (13)
Pectoral fin rays				
Reader 1	11% (23)	45% (94)	32% (67)	12% (24)
Reader 2	14% (28)	32% (67)	27% (56)	27% (57)
Otoliths				
Reader 1	0% (0)	2% (4)	13% (28)	85% (176)
Reader 2	0% (0)	1% (2)	11% (24)	88% (182)

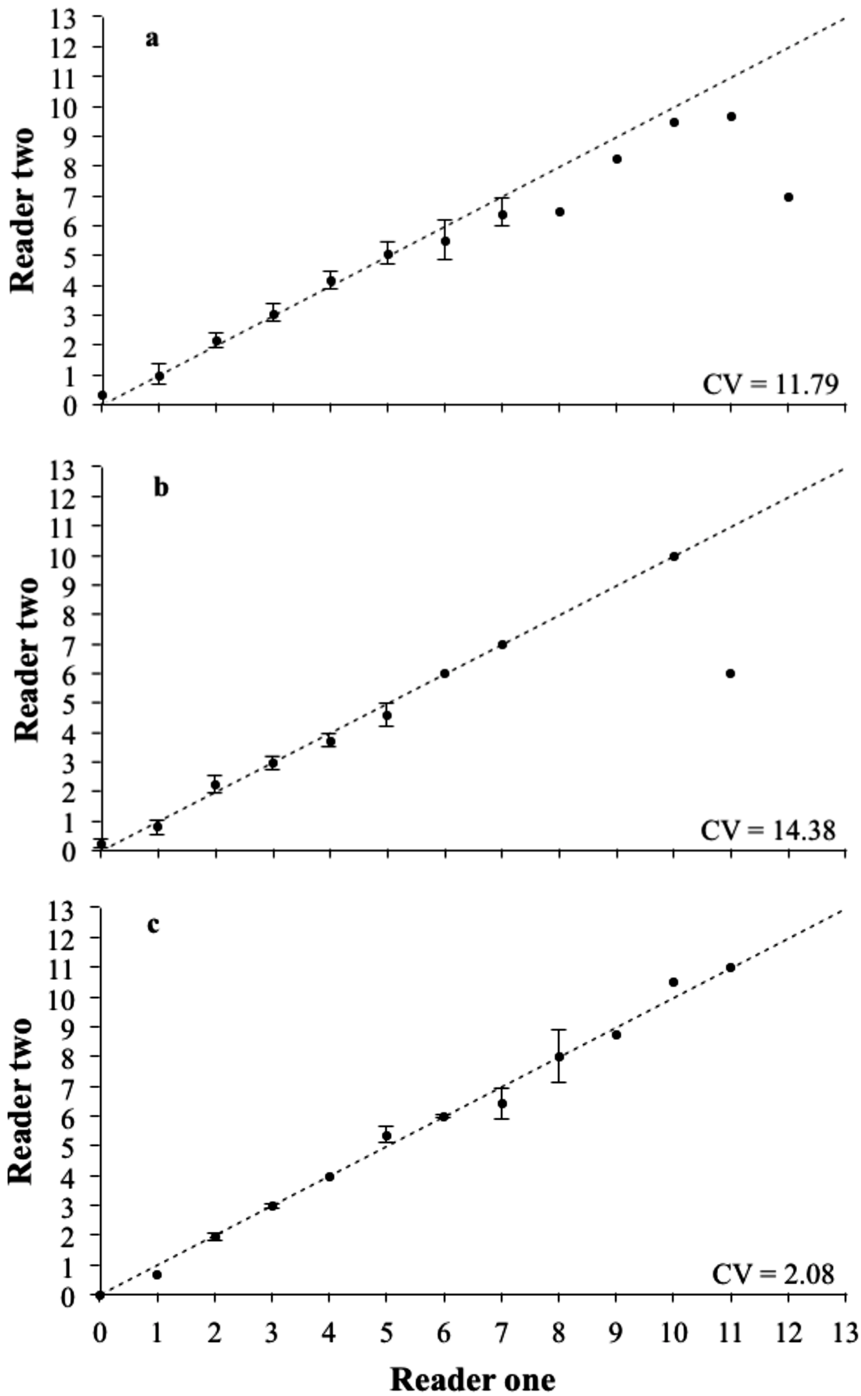

Figure 2. Assigned ages of reader one and two for branchiostegal rays (a), pectoral fin rays (b), and otoliths (c) from burbot collected from the Green River drainage, Wyoming (2013). Dashed lines represent exact agreement and error bars represent 95% confidence intervals. The mean coefficient of variation (CV) for each structure is provided.

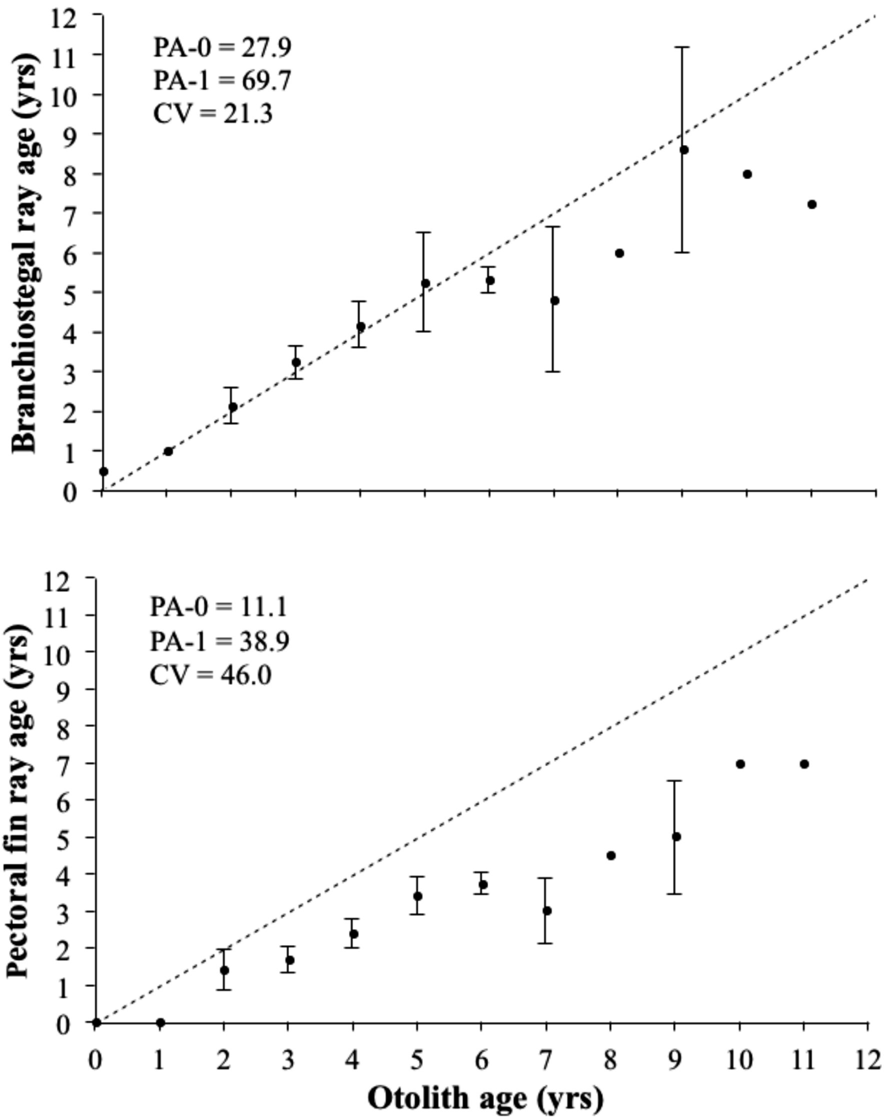

Figure 3. Age-bias plots for consensus ages assigned to branchiostegal rays and pectoral fin rays compared to otoliths for burbot collected from the Green River drainage, Wyoming (2013). Dashed lines represent exact agreement and error bars represent 95% confidence intervals. Precision between structures is indicated as exact (PA-0) and within-1 year (PA-1) agreement and mean coefficient of variation (CV).

More recently, Edwards et al. (2011) compared precision in age estimates using whole, cracked and sectioned otoliths and reported sectioned otoliths provided the most precise age estimates. Although we did not specifically address the precision of sectioned otoliths compared to other preparation techniques, otoliths appear to be a precise structure for ageing burbot regardless of the processing methodology. The relative ease of use and precision of otoliths will likely cement their use as the primary ageing structure for estimating the age of burbot.

Branchiostegal rays are routinely used to estimate the age of certain species and families of fish, e.g. gar (*Lepisosteidae* spp.; Love 2004, Glass et al. 2011, Buckmeier et al. 2012.) However, outside of select families of fish, limited knowledge exists regarding the use and practicality of estimating the age of fishes using branchiostegal rays. Bulkley (1960) evaluated the accuracy of age estimates obtained from whole branchiostegal rays of lake trout (*Salvelinus namaycush*) from Lake Michigan and reported 81 percent exact agreement between the known age and presumptive age estimates of branchiostegal rays. In the current study, age estimates obtained from branchiostegal rays of burbot were relatively inaccurate with only 27.9 percent exact agreement and 69.7 percent agreement within-1 year between branchiostegal and otolith age estimates. The discrepancy between burbot and lake trout in precision using branchiostegal rays is likely due to differences in species-specific morphology and processing methodology. Previous research generally used the largest pair of branchiostegal rays and estimated age using whole branchiostegal rays (Bulkley 1960, Netch and Witt 1962, Love 2004, Murie et al. 2009, Glass et al. 2011, Buckmeier et al. 2012). The majority of branchiostegal rays used in previous research exhibited thin, translucent distal ends which allowed for easy identification of annuli on whole branchiostegal rays (Netch and Witt 1962). The branchiostegal rays of burbot were relatively uniform in shape and annuli were not discernible under transmitted or reflected light using whole branchiostegal rays. As such, cross-sectioned branchiostegal rays were used in our study because they exhibited discernible annuli. Due to the paucity of information surrounding the use of branchiostegal rays for age estimation, it is difficult to know if another processing method (e.g., staining, clearing) might result in increased precision. Additionally, it is unclear if branchiostegal rays are truly a non-lethal ageing structure. To our knowledge, no research has evaluated the lethality of branchiostegal removal. Glass et al. (2011) posited that the removal of branchiostegal rays was lethal to spotted gar (*Lepisosteus oculatus*). Bulkley (1960) did not specifically assess survival of lake trout from which branchiostegal rays had been removed, but mentioned the potential for decreased survival for non-lethal removal of a single branchiostegal ray. Branchiostegal rays were easily removed from burbot, suggesting that a single branchiostegal ray could be carefully removed from anesthetized burbot without lethal repercussions. Further research to assess the potential use of branchiostegal rays as a non-lethal ageing structure for burbot may be warranted.

Fin rays are a common non-lethal structure used for estimating the age of fishes. Zymonas and McMahon (2009) reported that pelvic fin rays provided precise age estimates and were a viable non-lethal alternative to otoliths when estimating the age of bull trout (*Salvelinus confluentus*). Similarly, Quist et al. (2007) reported nearly identical age estimates between fin rays and otoliths for five catostomid species from the Little Snake River drainage, Wyoming. However, results regarding the accuracy and precision of age estimates obtained from fin rays are variable and tend to be species-specific. For instance, fin rays collected from pallid sturgeon (*Scaphirhynchus albus*) and white sturgeon (*Acipenser transmontanus*) provided inaccurate and imprecise age estimates (Rien and Beamesderfer 1994, Hurley et al. 2004). Results from our study support previous research suggesting age estimates obtained from burbot fin rays are relatively inaccurate when compared to age estimates obtained from otoliths. Giroux (2005) reported consistent underestimation of age using pectoral fin rays when compared to otoliths for burbot collected from British Columbia lakes. Additionally, pectoral fin ray age estimates obtained from burbot in our study had a mean CV of 14.38 indicating relatively imprecise age estimates. Campana (2001) suggested a CV ≤ 8 as an acceptable level of precision for most age estimation studies. Thus, the imprecision and inaccuracy of age estimates obtained from pectoral fin rays likely preclude their use as a valuable ageing structure for burbot.

The successful management of burbot relies on ageing structures that provide precise and accurate age estimates. To date, no non-lethal structures have been identified for estimating the age of burbot. In areas where managers are unwilling to sacrifice burbot, other age estimation methods will need to be used. Unfortunately, alternative age estimation methods rely on repeated sampling events and large sample sizes (e.g., mark-recapture of known aged fish, length-frequency analysis; Quist et al. 2012). Most management agencies will likely be unwilling or unable to bear the financial cost associated with repeatedly targeting a single species as in a mark-recapture study. Furthermore, low relative abundance in systems focused on burbot conservation will largely preclude the use of length-frequency analysis due to low sample sizes. Until future research identifies a viable non-lethal option, accurate and precise age estimates of burbot will likely rely on otoliths. Therefore, managers will need to weigh the loss of fish from a system to the relative importance of information gained from accurate and precise age data.

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