



MANAGEMENT BRIEF

Age Validation of Brown Trout in Driftless Area Streams in Wisconsin using Otoliths

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Abstract

Accurate age estimation is important for understanding salmonid population dynamics and managing fisheries. In this study, we validated the age of Brown Trout *Salmo trutta* in four Driftless Area streams of southwestern Wisconsin using otoliths from known-age fish. We tagged and released known age-1 Brown Trout with coded wire tags in spring 2010–2015. Age-1 Brown Trout were identified by length and verified by identifying a single annulus in a subsample of otoliths. Brown Trout were recaptured 1 to 5 years after tagging to extract otoliths for aging and coded wire tags to identify known age. We used three readers to independently estimate age using otoliths for 249 Brown Trout, and we quantified bias and precision. Complete agreement (all readers assigned the same age) was 74%, partial agreement (at least two readers assigned the same age) was 98%, and consensus agreement with known age (all readers agreed on a consensus on age when assigned ages differed) was 93% with a coefficient of variation of 9.4%. Consensus agreement by age varied from 81% for age 3 ($n = 31$) to 98% for age 1 ($n = 132$) and 100% for age 5 ($n = 6$). We conclude that using otoliths is a valid approach for estimating the age of Brown Trout ages 1–5 in productive streams as found in Wisconsin's Driftless Area and that the use of multiple readers and consensus agreement can improve aging accuracy.

Age estimation is crucial for understanding fish population dynamics, stock structure, and life history (Beverton and Holt 1957; Ricker 1975; Maceina et al. 2007). Fish age is typically determined by counting periodic growth increments in calcified structures such as otoliths. Seasonal changes in the accretion of calcium carbonate, the protein matrix, and trace amounts of other chemicals on the margins of otoliths may form a record of fish age when deposition results in annuli formation. However, age determination is subject to error in the process of growth increment formation, which may obscure indications of fish age, and to error in the

interpretation of fish age attributable to the subjectivity of the reader (Campana 2001). Accuracy in the interpretation of annuli in otoliths and the precision of said interpretation among multiple independent readers is important. Bias in reading otoliths that results in the underestimation of fish age, for example, may lead to overly optimistic estimates of rate parameters such as growth (Campana 2001) and can compromise population dynamics studies and impede the attainment of management objectives (DeCicco and Brown 2006; Yule et al. 2008).

Studies comparing salmonid age estimation using different calcified structures, such as otoliths, fin rays, and scales, have generally shown otoliths to provide better accuracy and precision (Graynoth 1996; Stolarski and Hartman 2008; Herbst and Marsden 2011). While some studies have shown scales to provide accurate or precise age estimates for some salmonids less than 3 years old (Hining et al. 2000; Stolarski and Hartman 2008), other studies have shown scales to be unacceptable because of age underestimation (Beamish and McFarlane 1987; Graynoth 1996) and high error rates (Schill et al. 2010; Aymes et al. 2016) as compared with age estimation using otoliths. Otoliths are generally acknowledged to provide better accuracy and precision compared with other calcified structures for salmonid age estimation, particularly in slow-growing salmonid populations (Maceina et al. 2007; Horká et al. 2010; Jonsson and Jonsson 2011).

The validation of fish age is critical but often neglected. Reviews of aging studies have shown progress in validating the use of different calcareous structures for estimating fish age (Beamish and McFarlane 1983; Campana 2001). However, many studies fall short of validating fish age by only validating the periodicity of increment formation (Hall 1991; DeCicco and Brown 2006; Zymonas and McMahon

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2009). One of the most rigorous methods for validating fish age involves the release of known-age and tagged fish into the wild (Campana 2001). This method validates both fish age and the periodicity of increment formation. Limitations of this method include the obvious need of a source of known-age and tagged fish, and the recapture of older tagged fish at large for many years may be low or zero. Age estimation should be validated by species for the aging structure used and by region or population because of potential differences in growth rates and their effect on increment formation in calcareous structures.

Aging studies for Brown Trout *Salmo trutta* have been limited. The counting of annuli in calcified structures to estimate the age of Brown Trout dates back to the early 20th century (Jonsson and Jonsson 2011). Burnet (1969) validated the use of fin rays for aging Brown Trout in a spring-fed stream in New Zealand, but Shirvell (1981) found that the first annulus in fin rays may not be apparent in older Brown Trout. Aymes et al. (2016) validated the formation of the first annulus and subsequent annuli in Brown Trout otoliths in a 25-month study in a Kerguelen Islands stream and observed high error rates in age estimates derived from scales compared with otoliths. Studies that validate Brown Trout age using otoliths are needed to ensure the accuracy of age estimation for Brown Trout in fisheries of different regions where the species occurs.

The Wisconsin Department of Natural Resources manages stream fisheries for Brown Trout, which were first introduced to the state in 1887. Brown Trout are managed as a desirable sport fish in many Wisconsin streams but may threaten

populations of native Brook Trout *Salvelinus fontinalis* in others (Mitro 2016). Accurate age data for Brown Trout are critical for understanding life history, growth, and population dynamics and are therefore necessary to successfully manage Brown Trout fisheries. In this study, we used the release of known-age and tagged fish to validate the age of Brown Trout in Driftless Area streams in Wisconsin using otoliths.

METHODS

The four study streams were located in the Driftless Area in southwestern Wisconsin and included Ash Creek, Big Spring Branch, Elk Creek, and Timber Coulee Creek (Figure 1). The Driftless Area lacks the glacial drift deposits found in surrounding areas following the Wisconsin glaciation. This area is characterized by karst topography with an abundance of groundwater-fed streams that support productive coldwater trout fisheries. The Driftless Area extends into adjacent parts of Minnesota, Iowa, and Illinois.

We captured Brown Trout using a single-pulsed DC backpack electrofishing unit, shocking at approximately 200 V and 2 A. Captured fish were held in containers with well-oxygenated, circulating water that was pumped from the stream. All Brown Trout collected in April were measured for total length (TL; mm) (Figure 2), age-1 Brown Trout identified by size were tagged, and all fish were released back into the stream. Over the course of the study we tagged 4,172 Brown Trout during spring at age 1 in the four study streams, rather than during summer or autumn at age 0, so as to minimize the loss of tagged fish during their first winter. We tagged age-1 Brown

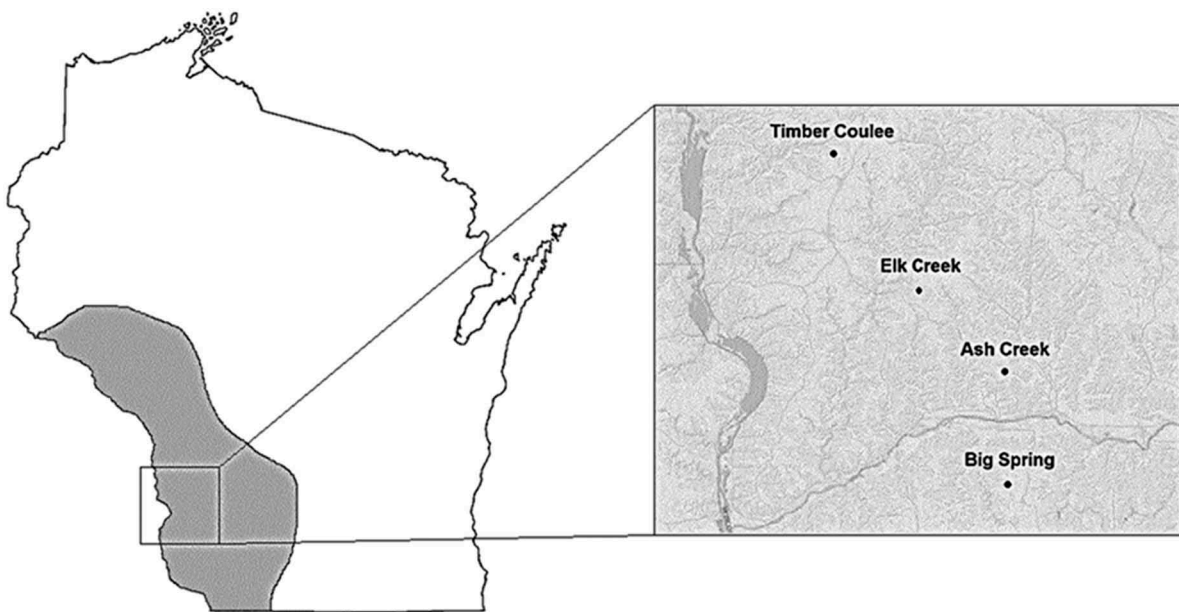


FIGURE 1. Map showing the locations of the four study streams in southwestern Wisconsin. The shaded region is the portion of the Driftless Area that is in Wisconsin.

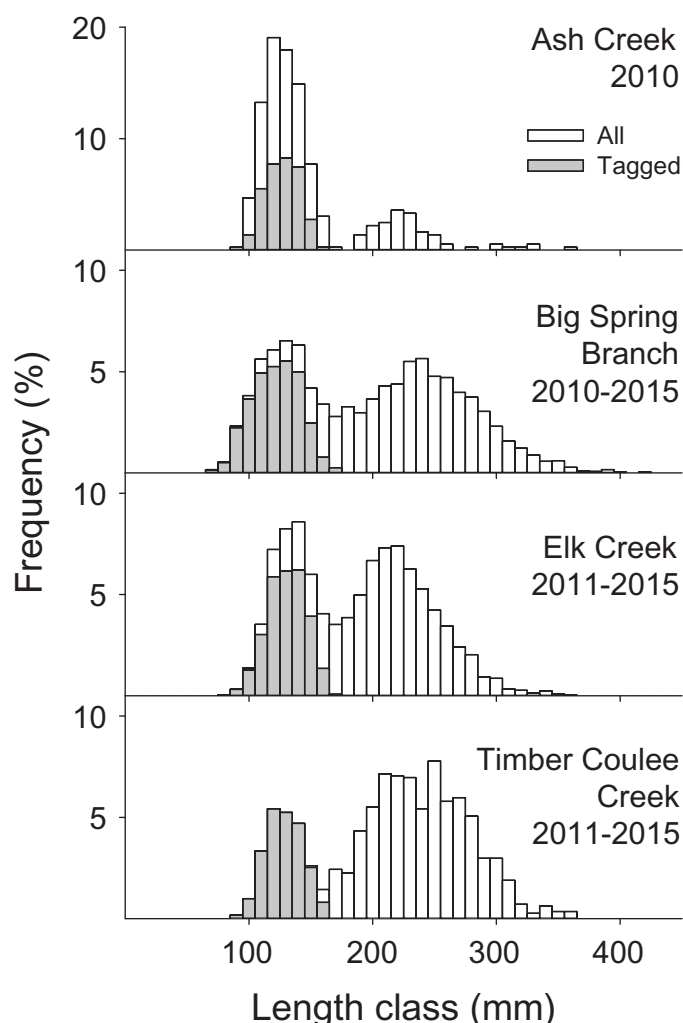


FIGURE 2. Length frequency distributions of Brown Trout caught in April in Ash Creek (2010; $n = 362$; 122 tagged), Big Spring Branch (2010–2015 combined; $n = 3,414$; 1,053 tagged), Elk Creek (2011–2015 combined; $n = 6,077$; 1,721 tagged), and Timber Coulee Creek (2011–2015 combined; $n = 1,105$; 257 tagged). The shaded bars show length frequency distributions of age-1 Brown Trout that were tagged. An additional 1,019 age-1 Brown Trout collected in targeted sampling were tagged and not included in these length frequency distributions.

Trout with coded wire tags (Northwest Marine Technology) in Ash Creek in 2010, in Big Spring Branch in 2010–2015, in Elk Creek in 2011–2015, and in Timber Coulee Creek in 2011–2015. Coded wire tags were injected into the head using a handheld coded wire tag injector (Northwest Marine Technology), and batch or individual coded tags were used to identify cohort year in recaptured fish. We used three approaches to verify that we were tagging age-1 Brown Trout: (1) we constructed length frequency histograms by stream to identify age-1 Brown Trout by length (Devries and Frie 1996; Figure 2), (2) we inspected otoliths from a sample of Brown Trout collected at the time of tagging in 2010 and 2011 to verify that a single annulus was present, and (3) we

verified the presence of a single annulus on otoliths in Brown Trout recaptured in autumn that were tagged 6 months earlier in spring. We checked all captured Brown Trout for coded wire tags in spring and autumn surveys of the four study streams in 2010–2015 to verify the presence of tagged fish surviving and remaining in the study areas from year to year.

Tags and sagittal otoliths were collected from recaptured Brown Trout in Ash Creek in April and June 2011 and 2012, in Big Spring Branch in April 2014 and April and October 2015, in Elk Creek in October 2015, and in Timber Coulee Creek in October 2012 and 2015. Coded wire tags were read to identify cohort, and otoliths were removed and prepared for aging analysis. One otolith from each fish was mounted in Epo-Kwik fast-cure epoxy and transversely sectioned using a low-speed diamond blade saw set at 0.55 mm (South Bay Technology, Model 650 Low Speed Diamond Saw Wheel; 4-in \times 0.012-in standard grit high-concentration blade). Sectioned otoliths were affixed to glass microscope slides, sanded with 1,000 grit sandpaper, and if necessary polished using a 5.0-micron silicon carbide disk to achieve maximum clarity. We applied immersion oil to the otoliths and obtained digital images (20 \times to 40 \times magnification) using transmitted light and a Nikon Digital Sight DS-Fi1 camera mounted to a Nikon SMZ1000 compound microscope and Nikon Elements D imaging software.

Each otolith was independently examined by three readers to estimate each fish's age. All readers had prior experience aging fish using a variety of structures, the majority being otoliths. Each reader independently assigned an age to each fish without knowledge of the tagging date, recapture date, or fish length. Complete agreement occurred when all readers assigned the same age, partial agreement occurred when at least two readers assigned the same age, and no agreement occurred when all readers assigned different ages. If a discrepancy in age assignment existed among readers, the three readers reviewed the otolith together and came to a consensus on age assignment (i.e., consensus age).

We used age-bias plots similar to those presented in Campana et al. (1995) to examine age estimate bias between readers and between the consensus age and known age of the fish. We quantified the precision of age estimates using a coefficient of variation ($CV = 100 \cdot SD/mean$). The coefficient of variation was calculated for each individual fish based on age estimates from the three readers, and means and standard errors of the CVs were calculated by known age and for all ages combined. We also calculated percent reader agreement for each fish within each stream (Isermann et al. 2010; Faust et al. 2013; Oele et al. 2015).

RESULTS

We tagged 4,172 age-1 Brown Trout in the four study streams over the course of the study. We routinely recaptured and released coded-wire-tagged Brown Trout in the years following their original release, verifying that tagged fish

were surviving and were observable from year to year at older ages. Otoliths were collected from 252 coded-wire-tagged Brown Trout; 206 came from fish recaptured in October 2015 and 46 came from fish recaptured at other times in 2011–2015. Three fish were removed from the aging analysis because of poor otolith quality. Otoliths from 249 of the 252 Brown Trout were used for validating age.

Length frequency distributions of Brown Trout caught during spring in each study stream indicated distinct peaks associated with age-1 fish (Figure 2). Otoliths from 18 Brown Trout (mean TL = 147 mm; SD = 14 mm; range = 121–170 mm) collected during spring at the time of tagging each exhibited a single annulus indicating they were age 1. All three readers identified a single annulus on 17 of the fish; one fish was identified as age 1 by two readers and age 2 by one reader, with a consensus that the fish was age 1. The mean CV was 2.4% (SE = 2.4). Otoliths from 129 of 132 tagged Brown Trout recaptured in the same year they were tagged as known age-1 fish exhibited a single annulus (Table 1). Three of the 132 fish were identified as age 1 by one reader but misidentified by consensus as older fish (Table 1).

Ages were validated for 249 known-age Brown Trout, including 18 from Ash Creek, 66 from Big Spring Branch, 153 from Elk Creek, and 12 from Timber Coulee Creek. Total length of recaptured Brown Trout from which otoliths were collected ranged from 136 to 375 mm (mean TL = 211 mm; SD = 45 mm) (Figure 3). Consensus age matched the known age of fish 93% of the time. Complete agreement among readers was 74%, partial agreement was 98%, no agreement occurred for 2% of the otoliths, and mean CV was 9.4% across all Brown Trout ages 1–5. For otoliths with partial agreement on age, the age originally agreed upon by two of the three readers was selected as the consensus age 79% of the time. Complete agreement ranged from 61% to 100% by age, partial agreement between readers ranged from 97% to 100% by age, and consensus age agreement with known age ranged from 81% to 100% by age (Table 2).

Age-bias plots of age estimated from one reader against another did not deviate significantly from the equivalence line (Figure 4). However, there was an increase in variation in age estimates between reader 1 and readers 2 and 3 (but not between readers 2 and 3) for older-aged Brown Trout

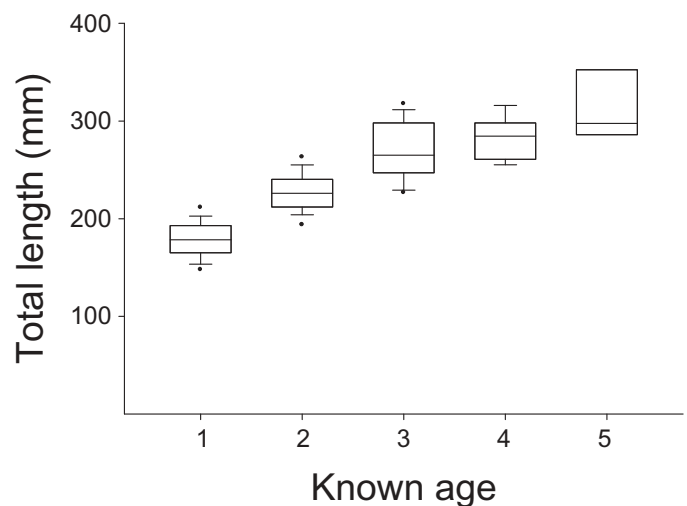


FIGURE 3. Box plots of Brown Trout length at the time of recapture in the four study streams ($n = 249$). The lines within the boxes indicate the medians, the lower and upper boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers indicate the 10th and 90th percentiles, and the black dots indicate the 5th and 95th percentiles.

(Figure 4). There was also no significant deviation from the equivalence line between known age and consensus age in the age-bias plot for Brown Trout ages 1–5 (Figure 5).

DISCUSSION

Accuracy in this study was highest when age assignments from the three readers were aggregated and readers worked together to determine a consensus age. Consensus ages for the 249 Brown Trout were 93% accurate, compared with 86–90% accurate age assignments by individual readers. These levels of accuracy, both individually among readers and in aggregate, exceeded the level of 80% recommended by Maceina et al. (2007) as a minimum level of quality for standard fishery assessments. Independent age assignments were in complete agreement by the three readers 74% of the time, indicating that some disagreement existed among readers for 26% of the otoliths in this study. The value of having multiple readers working together to address discrepancies in aging can be illustrated by our results for age estimation of known age-1 fish. Of the 132 known age-1 Brown Trout, reader assignment of age 1 ranged from 114 to 123 fish, reader assignment of age 2 ranged from 7 to 17 fish, and reader assignment of age 3 ranged from 1 to 4 fish. However, when working together to resolve discrepancies and assign a consensus age, readers assigned age 1 to 129 fish and only incorrectly assigned 3 fish as age 2 (Table 1). We recognize that the use of three readers may not be feasible for all management agencies due to increased staff time and costs. However, we found value in terms of improved accuracy by using three independent readers and having those readers work together to arrive at consensus

TABLE 1. Number of age assignments by three independent readers and consensus age assignments based on otoliths from Brown Trout known to be age 1.

Group	Age 1	Age 2	Age 3
Known age	132	0	0
Reader 1	114	17	1
Reader 2	121	7	4
Reader 3	123	8	1
Consensus	129	3	0

TABLE 2. Summary statistics of reader agreement for Brown Trout otolith age estimates (complete agreement = all three readers assigned the same age; partial agreement = at least two readers assigned the same age). Mean CV was calculated from CVs of individual fish ($CV = 100 \cdot SD/mean$). Consensus age was the age agreed upon by the three readers, either by all independently assigning the same age or agreeing upon an age when age estimates differed.

Age	<i>n</i>	Complete agreement (%)	Partial agreement (%)	Mean \pm SE CV (%)	Consensus age agreement with known age (%)
1	132	77	98	10.2 \pm 1.6	98
2	70	73	99	8.9 \pm 1.8	89
3	31	61	97	10.2 \pm 2.7	81
4	10	70	100	4.9 \pm 2.5	90
5	6	100	100	0.0 \pm 0.0	100
All ages	249	74	98	9.4 \pm 1.1	93

ages whenever partial agreement occurred. Therefore, we recommend the use of multiple independent readers whenever possible as this can reduce aging error and increase confidence in the application of aging data.

Measures of precision provide a useful means of quantifying the reproducibility of determining fish age from structures such as otoliths, as well as comparing skill levels among readers and in general describing the relative ease of using the structure for age estimation (Campana 2001). Our mean CV of 9.4% was greater than CVs for otoliths published for other salmonids such as Redband Trout *Oncorhynchus mykiss gairdneri* (2.3% in Schill et al. 2010), Brook Trout (7.45% in Stolarski and Hartman 2008), and Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* (8.40% in Kruse et al. 1997). The most frequently reported CV for otoliths in a summary of 117 previously published studies was 5%, but precision may vary not only by reader but also by the species of interest and the nature of the aging structure (Campana 2001). A CV of 5% may serve as a reference point for many fish species of moderate longevity and reading complexity (Campana 2001), but more analyses of salmonid aging precision may be needed to provide better context for attainable precision in such studies.

Variation in aging precision among readers can perhaps be improved by training readers using a reference collection of structures from known-age fish or by adjusting structure preparation, imaging, and interpretation procedures. For example, Schill et al. (2010) attributed their low CV to the combined use of sections as a primary structure and whole otoliths as a corroboratory structure. Readers in our study interpreted age from digital images of otoliths and were therefore inherently limited by the quality of the captured image and the inability to adjust focus during reading. Any improvements in image quality and what the reader is viewing may help improve precision among readers. This can include adjusting the magnification to achieve greater overall size of the otolith and adjusting the lighting or using an alternate illumination method, such as a fiber optic light, to achieve maximum contrast between transparent and opaque bands, as well as taking multiple images during sanding or polishing to make sure every annuli is accounted for (Hall 1991). We also recommend that reference sets of sectioned otoliths from

known-age Brown Trout be developed to train readers in Brown Trout age estimation.

Fisheries agencies rely on accurate estimates of fish age based on validated aging methods to make effective management decisions. Validation studies are often based on the release of tagged, known-age fish. While some age validation studies rely on the release of known-age and marked fish obtained from hatcheries (Secor et al. 1995; Buckmeier et al. 2002; Buckmeier and Howells 2003), our interest was in validating age estimation of wild fish. We tagged known age-1 Brown Trout from wild populations where age was reasonably approximated by size (Campana 2001). Brown Trout spawn in autumn and emerge in spring, and age-0 fish are identifiable based on size through their first summer and autumn. By the following spring, these fish are now age 1 and have formed a first annulus, and fish in this cohort are still identifiable based on size as depicted in length frequency histograms. We tagged fish at age 1 during spring in order to avoid the loss of tagged fish during their first winter, when natural mortality for salmonids can be high (Elliott 1994; Cunjak et al. 1998; Mitro and Zale 2002).

A potential issue in identifying age-1 Brown Trout based on size is the possibility of tagging an age-2 fish as an age-1 fish. Age-1 and age-2 Brown Trout size ranges may overlap during spring, but the occurrence of many age-2 fish in a group of age-1 fish defined by size is likely uncommon. We perhaps could have further limited or eliminated the possibility of tagging an age-2 fish as an age-1 fish by lowering the maximum length of what we would consider an age-1 fish. But such an approach may have precluded early emerging or fast-growing fish (i.e., large age-1 fish) from the samples. There was little evidence that we mistakenly tagged age-2 Brown Trout as age 1. There were 3 of 132 Brown Trout (2.3%) collected in autumn as known age-1 fish that were assigned a consensus age of 2, but we cannot be certain whether they were age 1 and misidentified as age 2 or age 2 and correctly identified as age 2. In each case there was disagreement among the readers as to the assigned age and one of three readers assigned the age as 1. Also, 2 of 70 Brown Trout (2.9%) identified by coded wire tags as known age 2 were assigned age 3 by each of the three readers. Here it is likely

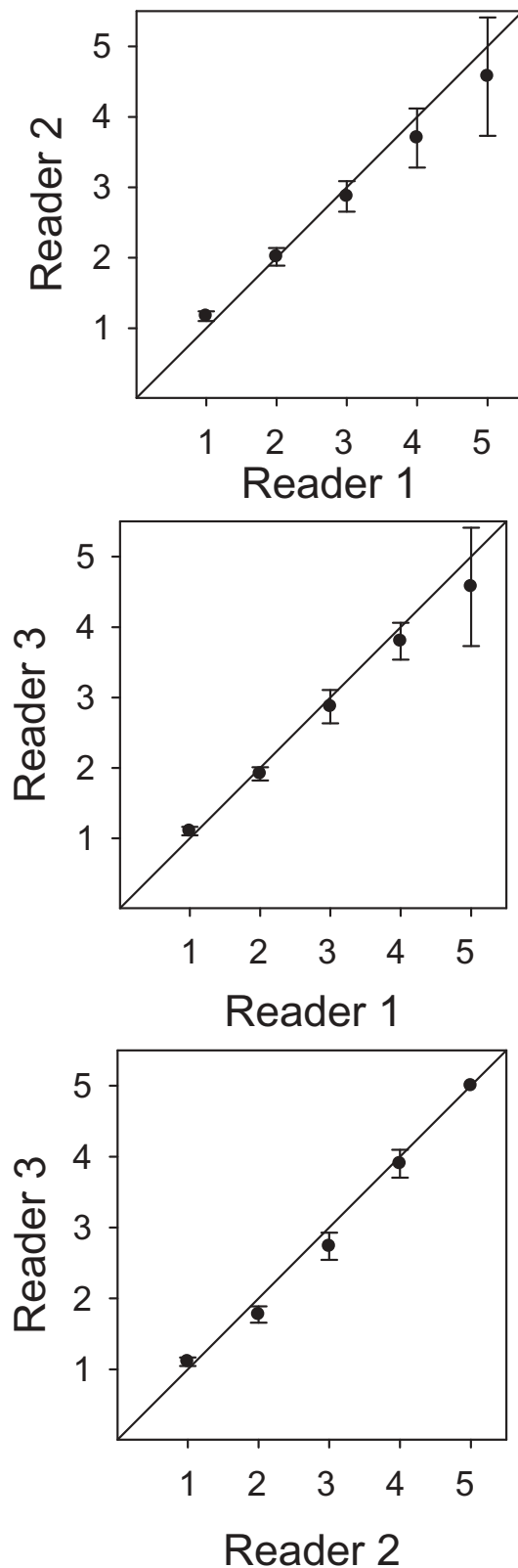


FIGURE 4. Age-bias plots comparing otolith age estimates between readers. Error bars indicate 95% confidence intervals.

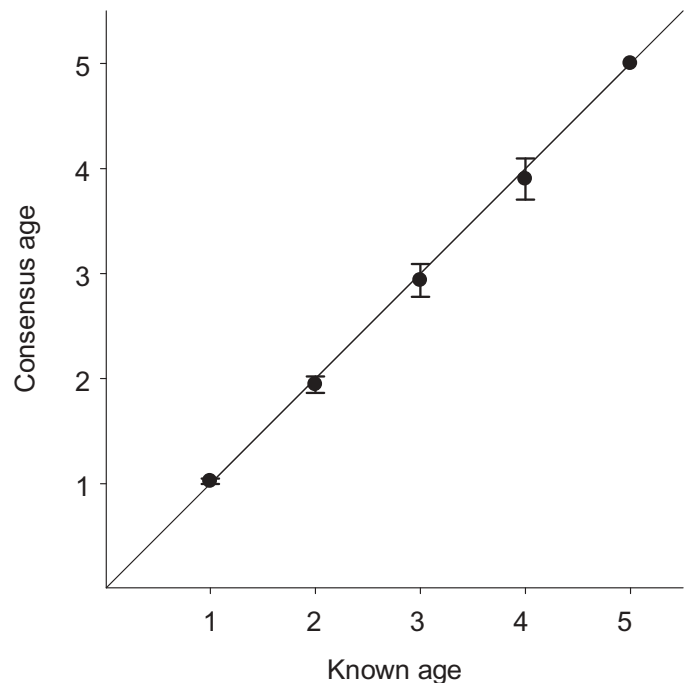


FIGURE 5. Age-bias plot comparing known ages of Brown Trout ages 1 through 5 to consensus ages agreed upon by the three readers. Error bars indicate 95% confidence intervals. The sample size in each age-class is as follows: 132 age-1, 68 age-2, 31 age-3, 10 age-4, and 6 age-5 Brown Trout.

that these two fish were tagged at age 2, as otoliths from each fish clearly showed three annuli. Overall, however, more than 97% of fish tagged as age 1 were likely in fact age-1 fish at the time of tagging.

We validated the use of otoliths for estimating the age of Brown Trout ages 1–5 in Driftless Area streams in Wisconsin. Brown Trout likely live to older ages in these streams, and older fish in stream populations are inherently less common than younger fish (e.g., we recaptured 132 age-1 versus 6 age-5 Brown Trout). In Elk Creek, for example, the recapture of Brown Trout tagged with visible implants of fluorescent elastomer (Northwest Marine Technology) identified Brown Trout that were a minimum of 8–10 years old based on the number of years between tagging and recapture (M. G. Mitro, unpublished data). Validation of ages for older Brown Trout will require more intensive tagging and sampling efforts to establish sufficient numbers of older, tagged known-age fish.

The knowledge of fish age has been stressed as an important need for the management of exploited populations. Prior work on the validation of otoliths as a suitable structure for determining the age of Brown Trout has been limited. Dodson et al. (2013) validated daily growth increments on sagittal otoliths between hatching and 1 week after emergence. Aymes et al. (2016) validated first annulus and subsequent annuli formation in otoliths of Brown Trout in a 25-month study of a Kerguelen Islands stream population. Our study builds upon this work by validating

otoliths for estimating the age of Brown Trout ages 1–5 in Driftless Area streams in Wisconsin. We conclude that otoliths provide a reliable method for estimating the age of Brown Trout. We recommend the development and use of reference structures from known-age Brown Trout to help train readers and improve reading precision, and we recommend using multiple independent readers to improve aging accuracy.

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