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# Validation of scales and otoliths for estimating age of redband trout in high desert streams of Idaho

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**Abstract** Studies validating aging structures for rainbow trout are sparse and none have been conducted for redband trout, a common western U.S. sub-species. Oxytetracycline mark-recapture methods (MR), marginal incremental analysis (MIA), and comparisons across multiple populations were used to evaluate the utility of two structures for aging redband trout in high desert streams. We assessed periodicity of annulus formation on scale and otolith samples from all age classes of trout residing in two streams, identified the location of the first annulus on otoliths, and compared age estimates and between-reader coefficient of variation on nine additional streams. The use of MIA successfully validated opaque zone periodicity for fish transitioning from age-0 to age-1, and from age 1 to age 2 in two streams. For fish at liberty 13 and 28 months in the same two streams, MR-derived age estimates from whole and sectioned otoliths were 94–100% accurate for fish from 2–9 years old. Scales were only 77% and 38% accurate for fish at liberty 13 and 28 months, respectively. Between-reader coefficient of variation (CV) for scales was high (11.5%), while CV for sectioned otoliths using whole otoliths as corroboratory structures averaged 2.3%. Scales were thus, an unacceptable aging structure for desert redband trout. Given the confusion in the literature, we suggest that more rigorous research should be conducted to define and explain otolith zone formation.

**Keywords** Otoliths · Redband trout · Validation · Marginal incremental analysis · Aging · Oxytetracycline

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## Introduction

In 2001, a multi-year study was begun to allow for a broad-based assessment of redband trout, *Oncorhynchus mykiss gairdneri*, status and life history in southern Idaho (Schill 2009). The importance of accurate age estimates in such efforts would seem self-evident, and studies purporting to validate aging approaches have been conducted since the middle of the 20th century (Cooper 1951; Brown and Holton 1953; McFadden 1959; Regier 1962). Accurate age estimates are critical since fish aging error is propagated through longevity, age at maturity, mortality rate, and survival rates (Mills and Beamish 1980; Campana 2001; Muir et al. 2008).

Validation of any aging structure as accurate requires that the zones counted as annuli be shown to form once each year. This can be accomplished directly by examining structures of known age fish (Heidinger and Clodfelter 1987; Buckmeier 2002; McBride et al. 2005), or more commonly by labeling structures with oxytetracycline (OTC) and examining them after a known period in a mark-recapture study (MR) to ascertain whether or not the number of annular marks coincides with the years at liberty (Laine et al. 1991; Murphy and Taylor 1991; Fowler and Short 1998). A second widely used, but indirect approach is marginal incremental analysis, or MIA; clear descriptions of which are provided by Casselman (1987) and Hyndes et al. (1992). The basic tenant of MIA is that the marginal increment will increase if measured throughout the year and should decrease markedly only once yearly upon formation of an annulus.

Campana (2001) considered a two step process necessary for a sound age validation study using fish of unknown age including: 1) verification that a defined annulus on an aging structure has annual periodicity, preferably across the entire age range of interest; and (2) identification of the first annulus location or growth increment on the aging structure. Step 2 can be accomplished by identifying the radius or diameter of an aging structure at the time of first annulus formation.

Despite hundreds of aging or age-reliant studies for rainbow trout, *Onchorhynchus mykiss*, in the primary and grey literature, few direct or indirect age validation studies have been completed. Alvord (1954) conducted a scale validation study for brown trout *Salmo trutta*, rainbow trout, and brook trout *Salvelinus fontinalis* in a Montana stream and concluded that his work substantiated the general validity of the scale method for aging wild trout in streams. However, in another study, scales were a poor structure for estimating rainbow trout ages in small southern Appalachian streams, while ground whole otoliths proved remarkably accurate (Hining et al. 2000). Because of highly variable life histories and life expectancies reported for rainbow trout across its range, more age validation studies for rainbow trout in other geographic areas are needed (Hining et al. 2000).

While validation by means of the above-described methods is desired, Kocovsky and Carline (2000) recommended comparing results from multiple aging structures when beginning research on unstudied fish stocks, and many other authors have used this comparative approach (e.g., Boxrucker 1984; Sharp and Bernard 1988; Stolkarski and Hartman 2008). Although such an effort does not constitute a true validation study, obtaining similar age estimates for multiple structures can provide additional confidence with aging results (Casselman 1987).

We are unaware of any studies evaluating age structures for redband trout in either desert or montane environments, and, in this study, sought to validate both scales and otoliths as aging structures for the entire range of age classes residing in two southern Idaho streams. Lethal sampling to develop a broadly applicable maturity model (Schill 2009) also afforded an opportunity to compare scale and otolith age estimates across a wide range of streams and growth conditions in southern Idaho. The specific study objectives were to: 1) use MIA to validate the periodicity of annulus formation on otoliths of redband trout in the first two age classes on two streams; 2) identify the physical location of the completed first annulus on otoliths from the same two waters; 3) validate the periodicity of annulus formation on scales and otoliths of age-2 and older trout using MR; and 4) compare age estimates and between-reader precision derived from otoliths and scales sampled in nine desert redband trout streams. The overarching goal of this study was to provide a solid aging foundation upon which subsequent estimates of growth, natural mortality and age-at-maturity could be based (Schill 2009).

## Methods

Redband trout were sampled from a total of 10 southern Idaho streams. The streams ranged in width from 1.2 to 2.9 m with specific conductivities of 33 to 315 $\mu S \cdot cm^{-1}$. For a description of the general study area including underlying geology, vegetation, fish populations, and a taxonomic description of redband trout (see Schill et al 2007; Schill 2009). Throughout this paper, we use the term translucent when referring to otolith zones formed during the slow growth periods and the term opaque to refer to the fast growth periods (Panella 1974; Casselman 1987). We sought to validate opaque zones as annuli (Beamish 1979; Devries and Frie 1996; Hining et al. 2000). Either zone can be considered an annulus and counted as long as the time of formation for the zone selected is understood and annual periodicity has been confirmed (Williams and Bedford 1973; Wilson et al. 1987).

MIA validation—age 0 to age 2

Because of high mortality observed following OTC marking and tagging reported for small juvenile rainbow (Hining et al. 2000), we employed MIA (Maceina et al. 1987; Beckman et al. 1988; Hyndes et al. 1992) for validation of annulus periodicity for otoliths collected from the first two cohorts of redband trout. On 9 August and 11 September 2002 we began monthly collection of juvenile redband trout with a backpack electrofishing unit from 1.7 km reaches of Jump Creek and Little Jacks Creek, respectively. We sought to return to both streams monthly on the same day of the month over the next year with a target of ±2 days and sample size goal of 10 fish for the two smallest age classes present each month. Within the two stream reaches, sampling was conducted upstream of the prior months location to minimize the likelihood that fish being collected had been shocked previously. All fish collected were killed via overdose with clove oil and processed the next day.

In the laboratory, both sagittal otoliths were removed using the method of Schneidervin and Hubert (1986), cleaned of any soft tissue, and stored dry in vials. Digital images (40× and 100× magnification) were obtained using a Leica DC 500 camera mounted on a Leica DM4000B compound microscope for each pair of otoliths, submerged in water and illuminated with oblique reflected light. The clearest image was chosen for interpretation. Completed opaque zones were counted as annuli (Beamish 1979; Devries and Frie 1996; Hining et al. 2000). Fish age and cohort (hatch year) were assessed by two readers with no knowledge of fish length, and differences were subsequently reconciled to improve accuracy (Buckmeier 2002).

Incremental growth was measured for the 2001 and 2002 cohorts. For age-0 trout with no completed opaque zone, the distance from the focus to the otolith margin was measured (Campana 2001). For each fish, measurements were made to the nearest μm in a consistent posterior plane (Fig. 1a) using Image Pro Plus digitizing software (Media Cybernetics 2007). For otoliths with one or two opaque annuli apparent, the distance from the distal edge of the outermost annulus to the otolith margin was measured in the same plane as above. Resulting increments widths

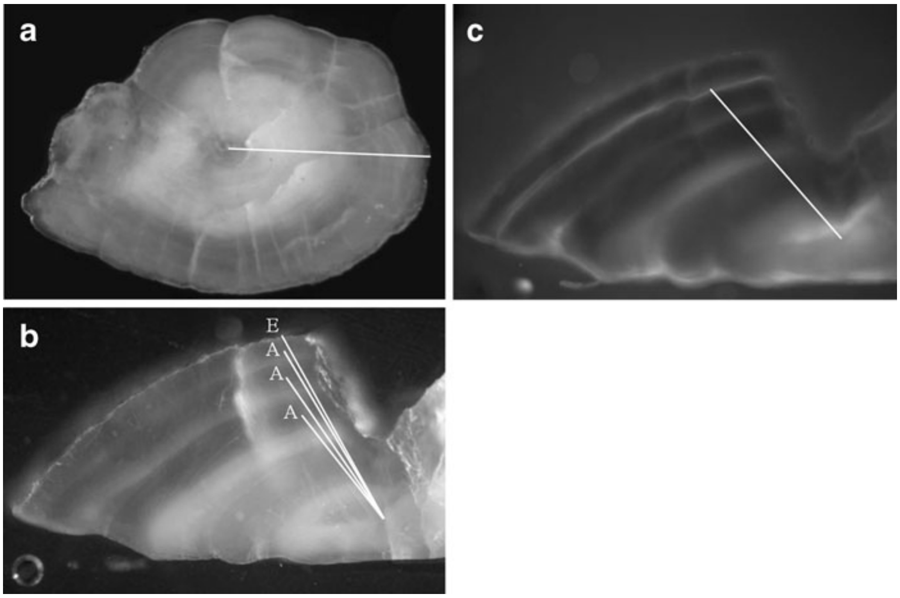


**Fig. 1** Redband trout otoliths showing measurements made (nearest μm) from the nucleus to the proximal edge of several structure locations using digitizing software. **a** MIA-distance to the otolith margin in the posterior plane of an Age 0, 68 mm fish, **b** Mark-Recapture-distance to the outermost three annuli (A) and the otolith section edge (E) under oblique reflected light at 100× magnification, and **c** Mark-Recapture-distance to OTC mark under UV light at 100× magnification

were plotted monthly for the two cohorts and examined to verify that the marginal increment declined markedly only once during the year.

The location of the first opaque annulus on otoliths from the two populations was estimated using fish one year older than those used in the MIA validation (Beckman and Wilson 1995; Campana 2001). For both streams, this measurement was made for fish from the 2001 cohort during September when the start of a new opaque zone at the margin first enabled exact location of the first opaque annulus just completed.

MR validation—age 2 and older

To validate ages estimates of these age classes, redband trout over 90 mm total length (TL) were collected on 8 October, 2002 from Jump Creek ($n$= 296) and on 9 October, 2002 from Little Jacks Creek ($n$=300) using a backpack electrofisher generating pulsed DC. In both streams, fish were netted, placed in 19 L buckets, anesthetized with clove oil, weighed to the nearest gram (g) and measured to the nearest mm TL. Scales were removed from the left side of fish between the lateral line and the dorsal fin (Devries and Frie 1996). Fish were then tagged with Passive Integrated Transponder (PIT) tags via intraperitoneal injection using standard techniques (Prentice et al. 1990) and subsequently injected with 50 $mg{\cdot}kg^{-1}$ of OTC (Hall 1991; Hining et al. 2000) inter-muscularly (Murphy and Taylor 1991; MacLellan and Fargo 1995) in the thickest part of the dorsal musculature. The adipose fin was clipped to enable rapid identification of tagged fish during subsequent sampling. Marked fish were released in the vicinity of their original collection site.

Recapture electrofishing was first conducted on 3 November 2003, approximately 13 months after initial tagging. All PIT-tagged fish recaptured as indicated by a portable PIT-tag detector were killed with an overdose of clove oil, and frozen for subsequent laboratory analysis. We returned to Little Jacks Creek and Jump Creek on 25 March 2005, to collect additional PIT-tagged fish that had been at liberty for nearly 2.4 years.

In the laboratory, fish from both recapture events were defrosted and remaining age structures were collected. Otoliths were removed as above and stored dry in darkness to prevent OTC degradation. Scales were collected from the right side of each fish opposite the side where scales were collected earlier during the marking effort.

Scales

For both streams, we compared age estimates for scales collected from the right and left sides of the fish at time of PIT-tagging and recapture to determine if the number of additional annuli observed coincided with the years individual fish were at liberty (Laine et al. 1991; Hining et al. 2000). Individual scales were placed between acetate slides and read on a microfiche reader (Devries and Frie 1996; Hining et al. 2000). Scale annuli at time of initial capture were counted by one reader, without a priori knowledge of fish length, based on the presence of crossing over, crowding of circuli, and width of individual circuli (Lagler 1952; Devries and Frie 1996). A second reader aged the fish independently and any fish with an annulus count that differed between readers was reconciled to a single age estimate (Clayton and Maceina 1999; Buckmeier 2002). Scale samples for the same fish at time of recapture were coded and randomly shuffled to eliminate reader knowledge of prior results. Ages at recapture were then estimated as above. Fish with 100% unreadable scales due to regeneration on either sampling occasion were excluded from further analysis. For fish recaptured in November 2003, we calculated the percentage that had formed one complete annulus since initial capture (Laine et al. 1991; Hining et al. 2000). Based on MIA scale data (D. Schill, unpublished data) we would not expect fish recaptured on 25 March 2005, to have formed a complete annulus. Thus, we evaluated the accuracy of annulus counts for these fish by determining what percentage had two more complete annuli visible than the original scale sample.

Whole otoliths

The percentage of fish that had completely formed the correct number of opaque annuli outside the mark on whole otoliths given the number of months they were at liberty was assessed (Hining et al. 2000). Whole otoliths were prepared, illuminated, interpreted, and ages reconciled as above. The otoliths were then covered with Type B immersion oil and examined for OTC marks at 40× and 100× magnification by illuminating them with a 100 W EL6000 Halide UV light system, a 450–490-nm excitation filter, a 510-nm dichromatic mirror, and a 515-nm barrier filter. Opaque zones and OTC marks were readily discernable under UV light. The number of annuli completed outside the mark was recorded and the percentage of fish that had been at liberty 13 months and had completed one opaque annulus outside the mark was calculated for all age classes of fall 2003 recaptured trout (Hining et al. 2000). The percentage of fish that had been at liberty just over 28 months that had completed two opaque annuli outside the mark was assessed for trout recaptured in March 2005.

Sectioned otoliths

We subsequently sought to validate age estimates from sectioned otoliths using more stringent validation procedures (Francis et al. 1992; Francis 1995; Morrison et al. 1998). Otoliths from recaptured fish in both streams were embedded in epoxy resin and sectioned transversely (0.60 mm width) through the nucleus along the dorso-ventral plane (Chilton and Beamish 1982). Sections were covered with Type B immersion oil and digital images of the most readable section were obtained using digital imaging equipment as above under reflected light (40× and 100×) and UV (100×). To facilitate annuli and OTC mark measurements, a line from the focus to the otolith section margin along a 45° axis relative to the dorso-ventral plane was digitally placed on a copy of both the reflected light and UV 100× images at identical locations using Image Pro Plus software (Media Cybernetics 2007).

The reflected light and UV images were then decoupled to prevent unintentional reader bias in annulus counts outside the OTC mark (see Francis et al. 1992). Otolith images were given to an individual not associated with the study, who generated a random number series, renamed the individual fish images, and returned them to the authors for age interpretation. Images from fall 2003 and spring 2005 recaptures were also mixed to eliminate a priori reader expectation about edge condition and subsequent age estimate (Morrison et al. 1998).

Reflected light section images were subsequently displayed on a 64×41 mm monitor, interpreted, and distance among various structures measured. Completed opaque zones were counted, reconciled as above, and recorded. The distal edges of the three outermost reconciled annulus locations (if available) were electronically marked on 100× images on the 45° line using Image Pro Plus (Fig. 1b). Following age estimation using completed opaque zones, the section edge was also digitally tagged on the same line. Distances from the otolith focus to each marked annulus and to the margin were digitally measured to the nearest μm.

Randomized UV section images were then displayed on the same monitor as above and evaluated. A single reader recorded the presence or absence of an OTC mark. For images with a visible mark, the distance from focus to the distal mark edge was measured to the nearest μm (Fig. 1c). Following measurements, all data for individual fish were subsequently merged. The number of completed opaque zones on section images with measured distances outside that of the UV mark was noted and recorded (Murphy et al. 1998; Brown et al. 2004).

Additional population comparisons

We compared otolith and scale age estimates for 481 individual redband trout collected from nine populations of desert redband trout between 20 March and 23 April 2001. Whole otolith and section preparation, lighting, imaging, interpretation and reconciliation procedures were as above. Age estimates were assigned viewing sectioned otolith images as the primary structure and whole otolith images as corroboratory structures. Completed opaque zones were counted as annuli with opacity at the edge evaluated, assuming fish had a 1 January nominal birthday (Tesch 1971; Devries and Frie 1996). Scales from these fish were mounted between glass slides and displayed on microfiche, evaluated by two readers with reconciliation procedures (Buckmeier 2002), annulus counts, and plus growth edge interpretation as described above for otoliths.

We compared agreement between the two aging structures graphically with an age bias plot including a 45° line denoting perfect agreement (Campana and Nielsen 1985; Kocovsky and Carline 2000). The between-reader coefficient of variation (Chang 1982) was calculated for age estimates prior to reconciliation for both otolith and scale age estimates from each stream.

## Results

### MIA validation—age 0 to age 2

Results of the MIA confirmed annual periodicity of opaque zone formation in desert redband trout as they transitioned to from age 0 to age-1 and from age 1 to age 2 on both streams. The measured marginal increment of the 2001 and 2002 cohorts in Little Jacks Creek generally increased from September 2002 to June 2003, and decreased markedly only once (Fig. 2a). A single decline was also observed on Jump Creek, but annulus formation was well underway by mid-May and completed by July (Fig. 2b).

Estimates of the first annulus location for otoliths were similar for each stream. The estimated radius of whole otoliths at the first annulus as measured in the 2001 cohort during September was 952 μm in Little Jacks Creek ($n$=7). The estimate for Jump Creek, as measured in October in the same cohort year was 927 μm ($n$=7).

### MR validation—age 2 and older

Seventy-one PIT tagged redband trout were recaptured from Little Jacks Creek during the 2003 fall recapture effort and 9 additional fish were collected during March 2005. On Jump Creek, a total of 27 tagged trout were recaptured during both recapture periods, only one of which was captured in spring 2005. The upper size range of recaptured fish encompassed all but the nine largest fish (in length) originally marked in the two streams combined.

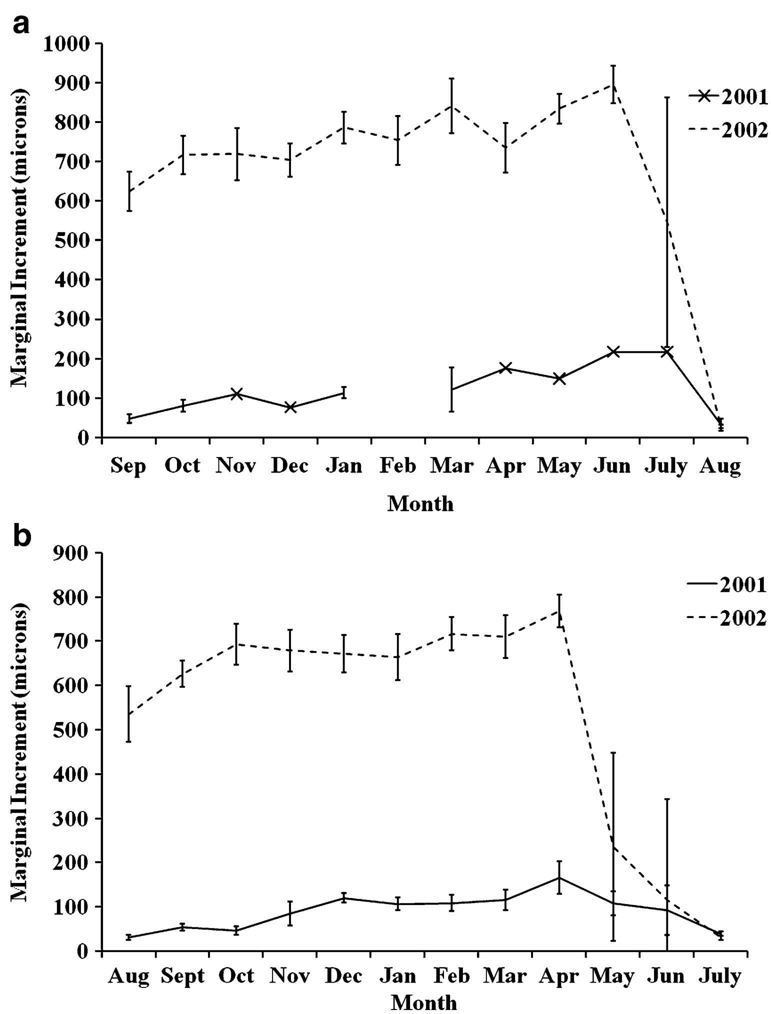


**Fig. 2** Mean otolith marginal increments for the 2001 and 2002 redband trout cohorts (see text for measurement descriptions) collected in **a** Little Jacks and **b** Jump creek, 2002–2003. Bars are 95% confidence bounds, x indicates $n \leq 2$

### Scales

Less than half of all the redband trout recaptured from the two study streams 13 months after initial capture had scales with an additional annulus apparent. Results for the smallest size class of tagged fish were similar in the two streams with 83–89% of the fish estimated to be age-2 at recapture forming an annulus in the prior 13 months. Only 56–60% of those estimated to be age-3 at recapture formed a third scale annulus in the same time period (Table 1). Results for age-4 fish in Little Jacks Creek were similar to those of the prior year class; however, no fish estimated to be age-4 at recapture in Jump Creek or age-5 or older fish in Little Jacks Creek formed an additional scale annulus while at liberty. Only 38% ($n$=8) of age 4–6 fish recaptured in March 2005 having readable scales and at liberty for 28 months formed the expected two additional annuli. Regeneration of scales was uncommon on the short-lived Jump Creek population but common on Little Jacks Creek where 36% of the samples could not be compared between mark and recapture time periods.

### Whole otoliths

In contrast to scales, all fish with readable whole otoliths had the expected number of completed opaque annuli formed during time at liberty. Of the 97 tagged redband trout recaptured in both streams during fall 2003, 94 contained visible OTC marks on whole otoliths. Whole otolith images from three of these fish were unreadable under reflected light. Of the remaining 91 fish from both streams, 100% had formed an additional annulus outside the OTC mark during the 13 months at liberty. Ages of these fish at recapture ranged from 2 to 9 years of age (Table 1). A total of 10 fish on both streams in age classes 4 to 6 was recaptured 28 months after marking; 100% had visible OTC marks and two opaque annuli outside the mark on whole otoliths.

### Sectioned otoliths

Results from sectioned otoliths were similar to those reported above for whole otoliths. We successfully sectioned otoliths from 88 redband trout recaptured in both streams during fall 2003; all had a visible OTC mark. Four of the section images viewed under reflected light were unreadable. On Jump Creek, otolith sections from all 22 fish collected in November 2003 had an opaque annulus measured outside the mark, while the lone fish recollected in March 2005 had formed two additional annuli (Table 2). Estimated age of fish at recapture for Jump Creek ranged from 2 to 4. There were 62 recaptured fish from Little Jacks Creek with sectioned otoliths measurements, a sample size we deemed large enough to preclude presentation here of individual measurement results. These fish ranged in estimated age at recapture from 2 to 9 years of age and 94% had an additional complete opaque annulus measured outside the OTC mark during the 13 months at liberty (Table 1). A single fish estimated to be Age 6 at recapture was aged incorrectly. In that instance, an extra opaque zone was incorrectly identified on the image outside the OTC mark. A total of eight fish on both streams in age classes 4 to 6 were recaptured 28 months after marking; all had visible OTC marks and two opaque annuli measured outside the mark.

Age estimates derived from whole and sectioned otoliths were identical for most mark-recapture fish on the two streams but observed discrepancies were not random about a 45° equivalence line. For 84 fish, 93% of the age estimates were identical while a discrepancy was observed for six fish. Of the six differences, whole otolith surfaces had one more annulus counted than sections in five instances, and two more annuli in one instance.

Of redband trout recaptured with visible OTC marks that were injected on 8–9 October, 2002, all had marks located within the opaque zone in both whole otolith and section view. In nearly all cases the mark was located just within the zone, shortly after opaque material had first formed.

### Additional population comparisons

Age-bias plots indicate that age estimates for older fish derived from scales were lower than estimates from otoliths in the majority of redband trout populations in this study. Scale age estimates declined below the 1:1 line of agreement in two thirds of the populations, typically by the age of three or four (Fig. 3). Examination of scales and otoliths provided virtually the same age estimates across all size ranges in two populations, Dive Creek and McMullan Creek.

**Table 1** Comparison by structure of the number of marked redband trout recaptured in Jump and Little Jacks Creeks on 13 November 2003, at liberty 13 months that had completed one additional annulus. Ages are the estimated ages at recapture

| Jump Creek | | | | Little Jacks Creek | | | |
|---|---|---|---|---|---|---|---|
| Age | No. of recaptures | Additional annulus | | Age | No. of recaptures | Additional annulus | |
| | | Number | Percent | | | Number | Percent |
| Scales— | | | | | | | |
| 1 | 0 | | | 1 | 0 | | |
| 2 | 19 | 17 | 89% | 2 | 12 | 10 | 83% |
| 3 | 5 | 3 | 60% | 3 | 16 | 9 | 56% |
| 4 | 1 | 0 | 0% | 4 | 14 | 9 | 64% |
| 5 | 0 | | | 5 | 1 | 0 | 0% |
| 6 | 0 | | | 6 | 1 | 0 | 0% |
| Regenerated | 1 | | | Regenerated | 27 | | |
| Total | 26 | 20 | 77% | Total | 71 | 28 | 39% |
| Whole otoliths— | | | | | | | |
| 1 | 0 | | | 1 | 0 | | |
| 2 | 16 | 16 | 100% | 2 | 17 | 17 | 100% |
| 3 | 5 | 5 | 100% | 3 | 5 | 5 | 100% |
| 4 | 1 | 1 | 100% | 4 | 32 | 32 | 100% |
| 5 | 0 | | | 5 | 4 | 4 | 100% |
| 6 | 0 | | | 6 | 7 | 7 | 100% |
| 7 | 0 | | | 7 | 2 | 2 | 100% |
| 8 | 0 | | | 8 | 1 | 1 | 100% |
| 9 | 0 | | | 9 | 1 | 1 | 100% |
| No mark | 3 | | | No mark | 0 | | |
| Unreadable | 1 | | | Unreadable | 2 | | |
| Total | 26 | 22 | 100% | Total | 71 | 69 | 100% |
| Sectioned otoliths— | | | | | | | |
| 1 | 0 | | | 1 | 1 | 1 | 100% |
| 2 | 18 | 18 | 100% | 2 | 15 | 15 | 100% |
| 3 | 3 | 3 | 100% | 3 | 6 | 6 | 100% |
| 4 | 1 | 1 | 100% | 4 | 29 | 29 | 100% |
| 5 | 0 | | | 5 | 3 | 3 | 100% |
| 6 | 0 | | | 6 | 6 | 5 | 83% |
| 7 | 0 | | | 7 | 1 | 1 | 100% |
| 8 | 0 | | | 8 | 0 | 0 | |
| 9 | 0 | | | 9 | 1 | 1 | 100% |
| Unreadable | 1 | | | Unreadable | 3 | | |
| Total | 23 | 22 | 100% | Total | 65 | 61 | 94% |

The Sinker Creek samples produced anomalous results with use of scales producing older age estimates than otoliths in the oldest age group.

There were consistent, sizeable differences in reader precision between scales and otoliths across populations (Table 3). Between-reader variability between scale age estimates ranged from 5.3 to 19.2%, averaging 11.5%. Between-reader variability between otolith age estimates ranged from 0.0 to 6.1% and averaged 2.3%.

**Table 2** Recapture date, age and otolith section measurement data for redband trout caught via electrofishing in Jump Creek, OTC marked, and released on 8 October 2002. Otolith measurements were made from the section nucleus to the proximal edge of the OTC band and all subsequent completed opaque bands to the edge along a 45° line ventral to the sulcus acusticus

| Tagged | Recaptured | | Months at liberty | Estimated age (yr) | Distance from otolith nucleus (mm) | | | |
|---|---|---|---|---|---|---|---|---|
| Total length (mm) | Date | Total length (mm) | | | OTC mark | Annulus | Annulus | Otolith edge |
| 164 | 4 Nov | 167 | 13 | 2 | 0.501 | – | 0.591 | 0.650 |
| 109 | 4 Nov | 132 | 13 | 2 | 0.490 | – | 0.537 | 0.597 |
| 108 | 4 Nov | 138 | 13 | 2 | 0.419 | – | 0.480 | 0.518 |
| 100 | 4 Nov | 130 | 13 | 2 | 0.390 | – | 0.446 | 0.475 |
| 101 | 4 Nov | 128 | 13 | 2 | 0.426 | – | 0.492 | 0.526 |
| 119 | 4 Nov | 138 | 13 | 3 | 0.546 | – | 0.586 | 0.615 |
| 115 | 4 Nov | 172 | 13 | 2 | 0.489 | – | 0.591 | 0.638 |
| 121 | 4 Nov | 154 | 13 | 2 | 0.492 | – | 0.567 | 0.607 |
| 110 | 4 Nov | 147 | 13 | 2 | 0.465 | – | 0.522 | 0.573 |
| 151 | 4 Nov | 189 | 13 | 4 | 0.663 | – | 0.697 | 0.743 |
| 146 | 4 Nov | 170 | 13 | 3 | 0.499 | – | 0.550 | 0.589 |
| 150 | 4 Nov | 167 | 13 | 3 | 0.540 | – | 0.581 | 0.619 |
| 97 | 4 Nov | 135 | 13 | 2 | 0.476 | – | 0.564 | 0.615 |
| 118 | 4 Nov | 165 | 13 | 2 | 0.575 | – | 0.639 | 0.702 |
| 100 | 4 Nov | 116 | 13 | 2 | 0.377 | – | 0.438 | 0.467 |
| 100 | 4 Nov | 122 | 13 | 2 | 0.453 | – | 0.521 | 0.551 |
| 108 | 4 Nov | 132 | 13 | 2 | 0.475 | – | 0.543 | 0.568 |
| 114 | 4 Nov | 156 | 13 | 2 | 0.462 | – | 0.540 | 0.584 |
| 103 | 4 Nov | 131 | 13 | 2 | 0.411 | – | 0.447 | 0.489 |
| 110 | 4 Nov | 148 | 13 | 2 | 0.515 | – | 0.590 | 0.648 |
| 106 | 4 Nov | 153 | 13 | 2 | 0.433 | – | 0.530 | 0.588 |
| 110 | 4 Nov | 146 | 13 | 2 | 0.538 | – | 0.624 | 0.650 |
| 110 | 25 Mar | 190 | 28 | 3 | 0.475 | 0.596 | 0.707 | 0.770 |

## Discussion

The first task in a sound validation study is understanding what the zones in a given aging structure mean (Francis 1995). We found the concurrent use of both OTC labeling and MIA useful in understanding otolith zonation in redband trout. Trout in both streams were injected with OTC on 8–9 October and the subsequent location of this OTC mark near the inside or proximal edge of the opaque band for all age classes upon recapture suggested that these fish had just begun laying down opaque material associated with somatic growth prior to early October marking. This observation corresponded well with results of the MIA analysis which documented marginal increment growth in Sept/October in both streams, followed by opaque annulus completion, and strong translucent zone formation during mid-summer (Fig. 2a, b). Such a growth pattern, with near or complete summer growth cessation, is not new for salmonids (Swift 1961; Kaeding and Kaya 1978; Ensign et al. 1990; Railsback and Rose 1999) and has been termed inverted seasonal growth (Cada et al. 1987).

Because the mark-recapture approach actually validates annulus periodicity rather than absolute age, the identification or location of the first growth increment on an aging structure should be a mandatory adjunct in such efforts since, without a correctly defined starting point, all age determinations will be wrong by a constant amount (Campana 2001). We addressed this issue in the present study to varying degrees for the two aging structures being evaluated. The location of the first annulus on whole otoliths for

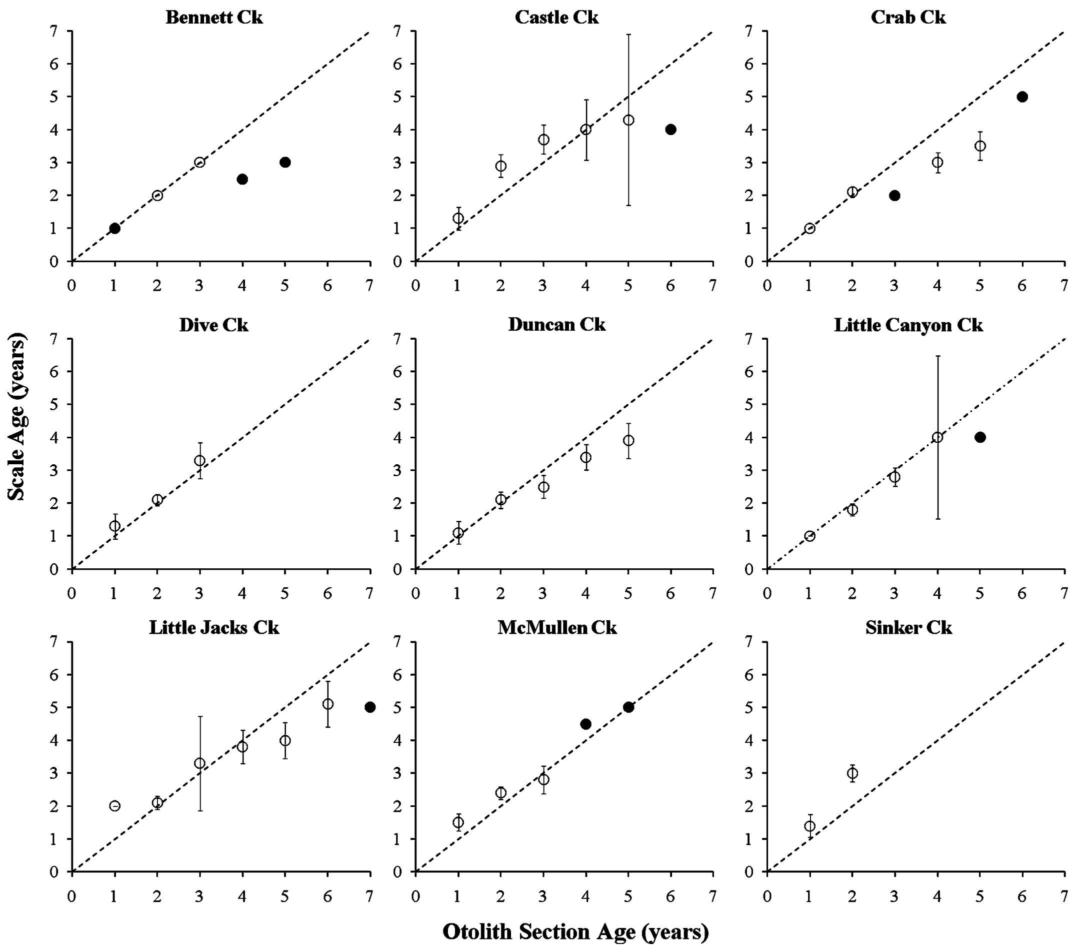


**Fig. 3** Age Bias plots for reconciled age estimates from scale and otolith images of desert redband trout collected in nine southern Idaho populations. The dashed line is the 45° equivalence slope. Otolith section images used as the primary aging structure with whole otolith images available for corroborating structure if desired. *Bars* are 95% confidence bounds, *black dots* indicate $n \leq 2$

the two populations being studied with mark-recapture methods was identified via measurements to the completed first annulus of age-1 redband trout (Campana 2001). This radius distance in the aging plane (925 to 952 μm) was, and is, a useful general starting point for locating the first annulus on whole otoliths of desert redband trout in Idaho. The procedure was not done on scales, but their performance in terms of periodicity accuracy was so poor across all age class that the need for such information was moot. We used the observed date of first opaque zone completion for whole otoliths to identify the same location on otolith sections by examining images of both structures for the same fish simultaneously on screen. The point of first annulus completion was quite distinct on sectioned otoliths, and became the best reference mark available for starting annulus counts for any of the three aging preparations evaluated.

Francis (1995) noted that there is confusion regarding what actually constitutes periodicity confirmation in an aging structure (validation) and has also questioned the objectivity of past validation studies (Francis et al. 1992). Of particular concern is the possibility that authors attempting to validate their aging techniques via mark-recapture may unconsciously search more closely for annuli outside of OTC marks or on the second scale sample unless proper blinding protocols are followed (Francis et al. 1992). The mark-recapture scale samples, sectioned otolith UV images of OTC marks, and paired reflected light images used for aging sections were randomly shuffled to prevent such unintentional bias. This procedure was not used for whole otolith validation. In that case, we followed

**Table 3** Comparison of between-reader coefficient of variation (CV) for scales and otoliths collected from redband trout in nine southern Idaho streams, March-April 2001

| Stream | n | Scales | n | Otoliths[a] |
|---|---|---|---|---|
| Bennett Ck | 32 | 5.3% | 32 | 1.5% |
| Castle Ck | 67 | 16.6% | 69 | 0.0% |
| Crab Ck | 45 | 11.0% | 45 | 3.3% |
| Dive Ck | 40 | 9.5% | 42 | 0.0% |
| Duncan Ck | 75 | 10.9% | 70 | 2.6% |
| Little Canyon Ck | 41 | 11.4% | 41 | 0.0% |
| Little Jacks Ck | 69 | 6.1% | 69 | 3.0% |
| McMullen Ck | 57 | 13.2% | 57 | 6.1% |
| Sinker Ck | 55 | 19.2% | 55 | 4.1% |
| Totals and means | 481 | 11.5% | 480 | 2.3% |

[a] Otolith section images used as the primary aging structure with whole otolith images available as corroborating structure when desired

the procedure of Hining et al. 2000, an approach that cannot be improved upon as the otoliths were subsequently sectioned. For otolith sections, the position of annuli in relation to OTC marks was evaluated by a more impartial measurement process rather than directly counting annuli outside the mark (Murphy and Taylor 1991; Murphy et al. 1998). The validation approach used for otolith sections in this study was the last, and most rigorous design of those employed for the three aging methods examined, and is recommended for those planning future validation studies.

Scales proved an inaccurate, imprecise aging structure for desert redband trout, a finding consistent with the past validation efforts for other rainbow trout sub-species. Although Alvord (1954) concluded that his results substantiated general validity of scales for aging wild trout, his results actually showed that scales were poor aging structures for rainbow trout 2 years of age or older. Age estimates derived from scales of rainbow trout were not considered satisfactory in Lake Lyndon, New Zealand, due to scale erosion and erroneous age estimates for known-age fish (Percival and Burnet 1963). Scales documented fish up to age-3, whereas otoliths identified fish up to age-8 in 12 East Tennessee streams (Cooper 2003). Scales in the same study were only 50% accurate for known age fish at age-3, while otoliths were 100% accurate at age-3. In two small Appalachian streams, rainbow trout formed an additional annulus on consecutive year scale samples on only 70–79% of samples for 2-year-old trout, and accuracy declined markedly thereafter (Hining et al. 2000). Accuracy results for Jump Creek and Little Jacks Creek redband trout were quite similar to those of Hining and colleagues, with scale regeneration another major limitation in the latter population. Also, in comparisons across nine additional populations in the present study, between-reader CV for scales was over 3-fold greater than for otoliths. Multiple-reader precision for otoliths has also been previously reported as superior for otoliths relative to scales (Hining et al. 2000). Chilton and Beamish (1982) have noted that "scales should always be used with the clear understanding that beyond the age of maturity, the age of fish may be under-estimated". Based on the present study, earlier validation efforts specifically conducted on rainbow trout, and the general aging literature, we question whether scales should be used as aging structures for non-anadromous rainbow trout.

This study validates age estimates derived from counts of opaque zones in both whole and sectioned otoliths in redband trout from southern Idaho. MR-derived age estimates from whole and sectioned otoliths were 94–100% accurate for fish from 2–9 years old in two streams while the use of MIA successfully validated opaque zone periodicity for fish transitioning from age-0 to age-1. However, agreement in age estimates between these two otolith preparations for the same fish was not perfect (93%), a finding consistent with the literature. Slightly lower rates of agreement between ground and sectioned otoliths have been reported in previous OTC studies of salmonids (80 and 87%) (Hall 1991; Hining et al. 2000). In subsequent age-growth studies of desert redband trout, Schill (2009) opted to rely on both whole and sectioned otolith images for two reasons. The first annulus was much easier to see in otolith sections for older trout. Second, when using sections as the primary structure and whole otoliths as a corroboratory structure, between-reader variation (CV) for these two structures was quite low (2.3%), compared to the 5% reported most frequently for otoliths from 117 previously published studies (Campana 2001).

It is possible that results from the mark-recapture portion of the study were affected by marking and handling stress (Alvord 1954). However, Hining et al. (2000) found that a marking/tagging procedure similarly invasive to ours and injection of the same

OTC concentration had no measurable effect on condition factor in small stream-dwelling rainbow trout in the Appalachians. We assumed this to also be the case in the present effort for a different rainbow trout subspecies comprised of similar-sized individuals.

Study limitations aside, perhaps the biggest impediment to completion of this study was the conflicting information regarding the timing and meaning of otolith zone formation encountered in the literature, studies consulted to address our own initial confusion associated with observation of unexpected opaque zone formation during the fall to spring period in desert redband trout. Panella (1980) has stated “it is obvious that the opaque zone is the zone of active growth”. However, translucent zone formation has also been associated with rapid somatic growth (Buxton and Clarke 1989; Lecompte-Finiger 1992). The author with perhaps the broadest experience in zonation studies (J. Casselman) has reported that opaque zones are associated with fast growth and translucent zones mark the slow somatic growth period for temperate zone species (Casselman 1983; Casselman 1987) and tropical species in Africa (Yosef and Casselman 1995; Admassu and Casselman 2000). Conversely, it has also been concluded that opaque zones are formed during periods of slow growth and translucent zones during rapid growth in warmwater bluegill *Lepomis macrochirus* (Schramm 1989; Hales and Belk 1992).

Such differences may be species-related (Schramm 1989; Beckman and Wilson 1995), but it is also possible that such differences are due to differences in illumination, sample preparation, or reader interpretation (Beckman and Wilson 1995; Campana 2001). For example, our analysis on a sub-species of rainbow trout suggest that opaque zone formation results from fast somatic growth, while the exact opposite interpretation was reported for another rainbow trout subspecies in two Appalachian streams (Hining et al. 2000). However, in both studies, the opaque zone began to form in early fall as indicated by an OTC time mark.

It is apparent that such confusion regarding otolith zones has been pervasive for over a third of a century. Panella (1974) discusses the issue under the heading “Terminology of Growth”, where he states that “one is left to wonder whether some of this confusion is semantic or observational”. A decade later Casselman (1983) lamented a similar state of affairs regarding both vague terminology and the confusion surrounding otolith zone formation. In the two subsequent decades, Beckman and Wilson (1995) and Campana (2001) continued this same dialogue. Perhaps nowhere have the seeds of confusion in otolith interpretation been sown more deeply than in the case of the two American Fisheries Society sponsored books, largely intended for students, entitled Fisheries Techniques, first and second editions (Nielsen and Johnson 1983; Murphy and Willis 1996). In the first edition, Jearld (1983) states that the translucent zone of otoliths denotes the period of slow growth. In the revised edition, Devries and Frie (1996) state exactly the opposite, noting that the opaque zone denotes the period of slow growth, failing to mention the prior opinion of Jearld (1983).

We expect little progress to be made in sorting out otolith zone questions unless a change in approach is employed. Panella (1980) urged study of zonation in otoliths from different species living in the same or different habitats as a way to identify otolith features that are genetically or physiologically controlled versus those that are environmentally controlled. A quarter century later, only a few authors (Fowler and Doherty 1992; Beckman and Wilson 1995; Brouwer and Griffiths 2004) appear to have attempted such comparisons, although the latter two efforts involved qualitative reviews of prior studies. Beckman and Wilson (1995) also suggested studying multiple populations of the same species across a broad geographic range to address the conflicting results reported in their review article. To our knowledge, no such effort has been undertaken. We urge that more rigorous research be conducted to define and explain otolith zone formation.

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