AN IN-DEPTH EVALUATION OF OTOLITH ZONATION AND AN AGEING COMPARISON OF OTOLITHS, SCALES, AND PECTORAL FIN RAYS OF REDBAND TROUT (*Oncorhynchus mykiss gairdneri*) AND BLUEGILL (*Lepomis macrochirus*)

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The following individuals read and discussed the thesis submitted by student Dennis A. Daw, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

I dedicate this work to my loving family; specifically my wonderful wife Lanette, who has been very supportive and patient throughout this project. I believe she probably worked harder than I during this process. As she was left home caring for our two young boys and working full time, while I stomped around creeks and went for boat rides.

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ABSTRACT

Redband Trout *(Oncorhynchus mykiss gairdneri)*, a native subspecies of Rainbow Trout residing east of the Cascade Mountains, USA, are a popular sport fish in much of its range. Bluegills (Lepomis macrochirus) are one the most important recreational fishes in North America, and are also sought by anglers in many Idaho waters. There is extensive confusion surrounding the timing and interpretation of otolith zones, specifically, which zone represents fast growth and which represents slow growth. Further, otoliths are a lethal sampling method and regardless of population status, many fisheries biologists prefer to use no lethal sampling methods. To determine if the zonation confusion is a species-related difference, I calculated monthly growth rates and investigated otolith zonation for a cold-water (Redband Trout) and a warm-water (Bluegill) species, in two Southern Idaho streams and three ponds. I also compared the assigned age and precision of sagittal otoliths, pectoral fin rays, and scales for these two species. Redband Trout showed their fastest growth during the month of June, with continuation of growth through September. The opaque zone started to form in March; by June 100% of the Redband had formed an opaque outer edge on the otolith. As with Redband Trout, the fastest growth rates for Bluegill were during late spring and early summer. This fast growth coincided with the formation of the translucent zone, which was observable in 95% of Bluegill by the month of May. Similar to Redband Trout, 100% of Bluegill had begun forming their fast growth zone by June. Based on edge analysis the otolith zonation pattern for Bluegill is translucent-forming during summer months and opaque-forming in fall to spring. Conversely, the otolith zonation pattern for Redband Trout is opaque-forming during summer months and translucent-forming in winter. Our findings suggest that Redband Trout and Bluegill do indeed form opposite appearing otolith zones during their respective periods of fast somatic growth. Although we have limited data for other warm and cold-water species in our study waters, we observed similar patterns for other species in these two groups. In addition, the otoliths for the first two age classes of both species were validated as forming one annulus per year. These findings have implications for both experienced and novice biologists conducting ageing studies. Lacking water-specific validation, the annulus for temperate warm-water centrarchids should be considered the opaque zone. Conversely, the annulus for temperate cold-water trout should be considered the translucent zone. Otoliths of Redband Trout were the most precise at both locations, followed by fin rays with scales being the least precise. I found no difference in the assigned age of fin rays and otoliths at Mores Creek. However, I found a statistical difference between assigned ages of otoliths and fin rays, with fin rays producing lower age estimates, specifically on older fish at Harris Creek. Scales ages were less precise and had lower age estimates than that of otoliths or fin rays at both locations. Though, our findings showed a difference in assigned age, at Harris Creek, and precision at both locations. I feel that fin rays produced an acceptable age estimate for montane Redband Trout. These findings, along with those that have shown that fin ray removal did not affect growth and survival, leads us to suggest that fin rays may be an acceptable, non-lethal, ageing structure for Redband Trout in montane streams. We suggest this with caution and suggest further research be completed. Conversely, we do not recommend the use of scales, given the fact that scales are less precise and produced lower age estimates. Otoliths of Bluegill were found to be the most precise at both water bodies. Scale and fin ray age estimates differed in precision depending on water body. Scale age estimates were more precise at Atwood's Pond while fin ray age estimates were more precise at Bruneau Dunes Pond. Pairwise regression comparisons showed that scale age estimates significantly underestimate the age of fish when compared to that of otoliths, at both locations. There was not a significant difference between the assigned age of otoliths and fin rays at either location. We do not recommend the use of scales or fin rays as the primary aging structure for Bluegill. Although, we did not find assigned ages of otoliths and fin rays to differ, estimates of the latter demonstrated far less precision. The difference in precision concerns us. We suggest a study be undertaken to validate fin rays prior to them being used as a primary ageing structure for Bluegill in Idaho waters.

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PREFACE

Age is the basis for calculating many of the population dynamics used to manage fish populations, which make it vitally important to be able to accurately and precisely assign an age to a morphological structure. There have been numerous structures used to age fish. Interestingly, the most widely used ageing structure, otoliths, have a considerable amount of confusion surrounding them. Specifically which zone forms during fast somatic growth? Further, otoliths are a lethal sampling method, and many fisheries biologists, regardless of population status, prefer to use non-lethal ageing techniques. Throughout the course of this study we investigated the timing of otolith zone formation of a warm-water fish, Bluegill, and a cold-water fish, Redband Trout. These two species were chosen due to their recreational fishing popularity and because they are examples of the species at the heart of the confusion surrounding otolith zone formation. We also compared three structures, otoliths, pectoral fin rays and scales, to determine if the three structures produced similar age estimates for these two species.

Redband Trout, a native subspecies of Rainbow Trout in both high dessert and montane streams in the western USA, is a popular sport fish in much of its range. Montane stocks residing in larger river systems such as the Payette and Boise rivers, and their tributaries receive considerable angling pressure and thus good ageing structures are needed to produce dynamic rate functions for management.

Bluegill are one of the most popular recreational fishes in North America, and are found in 49 states and 6 Canadian providences. They are also sought after in many Idaho waters. The recreational and economic importance of Bluegill makes it vitally important to correctly manage this species.

The three ensuing chapters investigate the confusion surrounding otolith zonation and whether there is a quality non-lethal ageing method for these two important Idaho recreation sport fishes. To facilitate future publication of this work, each of the chapters was written as a stand-alone journal article, and for this reason are written in plural form. Given the similarity in field and laboratory methods there is some unavoidable repetition of writing style in the introduction and method sections.

Chapter one is a comparison of the zonation patterns of Bluegill and Redband Trout to determine if this difference is species specific. It has been suggested by many authors who study salmonids that the opaque zone is related to fast somatic growth. Conversely, most authors who study warm water species suggest the translucent zone forms during fast somatic growth. We calculated monthly growth rates and compared this to edge analysis; which allowed us to determine the time of year that fast somatic growth was occurring, and which otolith zone coincided with the highest rate of growth. Monthly samples also allowed us the opportunity to use marginal incremental analysis to validate otoliths as forming one annulus per year in the first two age classes, for both species in all five water bodies.

Though otoliths were validated in our study sites and have been shown to be quite precise and accurate; they are a lethal sampling method. Chapters two and three investigate whether there is a difference in precision between otoliths, pectoral fin rays and scales. This is accomplished for each species independently.

CHAPTER 1: OTOLITHS AND THE CONFUSION SURROUNDING THE INTERPRETATION OF ZONATION: A COMPARISON OF REDBAND TROUT (*Oncorhynchus mykiss gairdneri*) AND BLUEGILL (*Lepomis macrochirus*)

Abstract

There is extensive confusion surrounding the timing and interpretation of otolith zones, specifically, which zone represents fast growth and which represents slow growth. To determine if this confusion is a species-related difference, we calculated monthly growth rates and investigated otolith zonation for a cold-water (Redband Trout: *Oncorhynchus mykiss gairdneri*) and a warm-water (Bluegill: *Lepomis macrochirus*) species, in two Southern Idaho streams and three ponds. Redband Trout showed their fastest growth during the month of June, with continuation of growth through September. The opaque zone started to form in March; by June 100% of the Redband had formed an opaque outer edge on the otolith. As with Redband Trout, the fastest growth rates for Bluegill were during late spring and early summer. This fast growth coincided with the formation of the translucent zone, which was observable in 95% of Bluegill by the month of May. Similar to Redband Trout, 100% of Bluegill had begun forming their fast growth zone by June. Based on edge analysis the otolith zonation pattern for Bluegill is translucent-forming during summer months and opaque-forming in fall to spring. Conversely, the otolith zonation pattern for Redband Trout is opaque-forming during summer months and translucent-forming in winter. Our findings suggest that Redband Trout, and Bluegill do indeed form opposite appearing otolith zones during their

respective periods of fast somatic growth. Although we have limited data for other warm and cold-water species in our study waters, we observed similar patterns for other species in these two groups. In addition, the otoliths for the first two age classes of both species were validated as forming one annulus per year. These findings have implications for both experienced and novice biologists conducting ageing studies. Lacking water-specific validation, the annulus for temperate warm-water centrarchids should be considered the opaque zone. Conversely, the annulus for temperate cold-water trout should be considered the translucent zone.

Introduction

Fish are one of the most aged organisms with over a million, and most likely closer to two million, aged worldwide in 1999 (Campana and Thorrold 2001). There are two reasons for the widespread ageing of individual fish. First fish do not have a maximum size at maturity, and may grow and mature at different sizes and rates within different habitats (Casselman 1987; Weatherly and Gill 1987; Campana 2001). When fish exceed approximately four years of age there is no clear distinction of age at a specific size, and there is a considerable amount of length overlap between differing age classes (Casselman 1987). The second reason relates to the recreational and commercial exploitation of fish stocks. Extensive exploitation of fish makes it vitally important to understand key population dynamics; factors such as mortality, growth rate and recruitment; each of which requires knowledge of age. Without an understanding of these, and other demographic parameters individual populations may be at risk for overexploitation and population decline (Casselman 1987).

Of the structures used to age fish, otoliths are widely used in part to their accuracy, but also due to their precision and ease of removal (Casselman 1987; Secor et al. 1992). Otoliths grow by adding concentric rings of calcium carbonate and proteins. Small, seasonal differences in the concentration of proteins result in the rhythmic growth patterns (Alvarez et al. 2008). These patterns appear as translucent or opaque zones wherein the translucent band allows light to pass through while the opaque zone blocks light, which gives each zone a distinct appearance. The study of these zones has been termed zonation (Casselman 1987).

For many decades there has been, and remains, confusion as to the time of year when the different otolith zones form (Beckman and Wilson 1995; Schill 2009; Schill et al. 2010), and which zone represents fast growth and which represents slow growth (Pannella 1980). This conflict may be due, in part, to seasonal differences between warm and cold-water fish growth rates (Daniel J. Schill, Idaho Department of Fish and Game, personal communication). Though many warm and cold-water species coexist in the same water body, the distinguishing difference between the two groups is the optimal temperature at which each grows (Casselman 2002). Most cold-water biologists typically view the opaque zone as the fast growth zone (Jerald 1983; Schill et al. 2010), while warm-water fish researchers tend to view the translucent zone as the fast growth zone (Schramm 1989; Devries and Frie 1996; Hales and Belk 1992). It has been suggested that the differences are species-specific (Schramm 1989; Beckman and Wilson 1995).

Schill (2009) suggested such differences may not be real, and advanced the hypothesis that such differences are not family or species-specific. Such family-related differences simply could be due to illumination methods, sample preparation or reader interpretation (Beckman and Wilson1995; Campana 2001; Schill 2009). For example, the type of light used to view these zones can alter their appearance. Reflected light on a black background causes the opaque zone to appear light colored, if not white, and the translucent zone to appear dark. Transmitted light will cause the opposite appearance of the zones; i.e. opaque zones appear dark and translucent zones appear light (Casselman 1967). Pannella (1974) noted that "one is left to wonder whether some of the confusion around zones is semantic or observational." To address this issue, Pannella (1980) suggested that the otolith zonation for different species within a similar area should be studied concurrently.

Along with the confusion surrounding the meaning of the two otolith zones, uncertainty as to the timing of fish growth within individual populations may further cloud the issue. Generally it is believed that fish grow during the warm summer months, with a decrease, if not a cessation, of growth in the colder winter months (Gerking 1967; Weatherly and Gill 1987; Cada et al. 1987). This view is not unfounded and fish growth often occurs during the warmer summer months (Mortensen 1982; Neves et al. 1985; Rypel 2009). However, other studies found that some fish species have a bimodal growth pattern with most growth occurring in the spring/fall period (Brown 1945; Kaeding and Kaya 1978; Railsback and Rose 1999; Meeuwig et al. 2004, Schill et al. 2010). Reported differences among studies could reflect differences in species, or habitat, and could also be due in part to the temporal scale at which growth has been commonly investigated. In general, few studies of monthly growth patterns exist in the literature. The vast majority estimate annual growth and most authors make assumptions about seasonal growth.

Casselman (1987) and Campana (2001) recommended that ageing structures should be validated for each species, and further suggest validation for a given species residing in separate geographic regions. The three most widely used validation techniques are chemically marking the structure and doing a mark-and-recapture study, marginal incremental analysis (MIA), and by using known-age fish (Beamish and MacFarlane 1983; Campana 2001). Because of the difficulty of identifying the first annulus (Campana 2001), these authors also consider measurement of the distance from the focus-to-the-first annulus another feature of successful age validation.

The primary goal of this study was to evaluate whether species-specific difference could explain some of the confusion surrounding the interpretation of otoliths zones. To this end, in this study a single biologist compared the timing of fish growth and otolith zone formation for both a cold-water species, (steno-thermic species: Redband Trout *Oncorhynchus mykiss gairdneri*), and a warm-water species, (eu-thermic species: Bluegill *Lepomis macrochirus*), with the same illumination methods. The specific objectives of this study were: 1) determine what otolith zone forms during periods of fast somatic growth (translucent vs. opaque), and whether the zone differs between Bluegill and Redband Trout; 2) determine what time of year fast somatic growth occurs, and does this season differ between Bluegill and Redband Trout; 3) validate whether or not otoliths of Redband Trout and Bluegill form one annulus per year in our study waters.

Sample Site Description

We sampled fish from two habitat types: 2 lotic waters (Harris Creek and Mores Creek), and 3 lentic waters (Attwood's Pond, Crane Falls Lake and Bruneau Dunes State Park Pond, from here on out Bruneau Dunes Pond) (Figure 1:1). Harris Creek is a second order tributary to the Payette River, in Boise County, Idaho. Our sample site started at an elevation of 1130 m and ended at an elevation of 1250 m. We sampled approximately 2.5 km of stream throughout the study period. Harris Creek has a mean width of 2.5 m and a mean depth of 0.18 m during the summer and fall months. Spring runoff flows, can be highly variable, at Harris Creek and during this period has a mean depth of 0.91 m, and a mean width of 3.2 m.

Mores Creek is a second order tributary to the Boise River, in Boise County Idaho. Our sample site started at 1465 m in elevation and ended at an elevation of 1500 m. We sampled approximately 3.5 km of stream over the course of the study. During summer and fall base flows, Mores Creek has a mean width of 3.6 m and a mean depth of 0.53 m. During spring runoff, high flows, the mean width was 5.8 m with a mean depth of 1.1 m.

Atwood’s Pond is a privately owned pond that is located next to the Payette River in Payette County, Idaho. The pond is a reclaimed gravel pit and is fed by hyporheic water from the nearby Payette River. It has a maximum depth of 4.9 m and covers approximately 9.3 ha. The depth of Atwood’s Pond fluctuates with the fluctuation of the Payette River flows.

Crane Falls Lake, located in Owyhee County, Idaho, is a natural lake which formed following the construction of C.J. Strike dam and the ensuing irrigation of agriculture land on the plateau above (Jeff Dillon, Idaho Department of Fish and Game, personal communication). The lake is fed by hyporheic water from the Snake River. Crane Falls Lake has a mean depth of 5.5 m and covers approximately 45.5 ha, and remains at a relatively stable depth throughout the year.

Bruneau Dunes Pond is located inside of Bruneau Dunes State Park, in Owyhee County, Idaho. Bruneau Dunes Pond is a manmade pond that is filled by pumping water from the Snake River into the pond through the winter months, generally November-March. The mean depth is 4.2 m and covers approximately 15.8 ha. Water levels at Bruneau Dunes decreases slightly throughout the summer months and then increases during the winter months, which corresponds to the time of pumping.

Methods

We sampled the five water bodies for 15-17 months, depending on the water body. Atwood's pond and Harris Creek were both sampled from June 2011-Oct 2012. Crane Fall Lake, Bruneau Dunes State Park Pond, and Mores Creek were sampled from Aug 2011-Oct 2012. During the months of December and January the lentic waters were ice covered. The ice was too thick to allow boat access but not thick enough to safely allow us to angle through the ice. We did attempt to angle from docks and the bank to no avail. Due to these conditions we were unable to obtain a sample from Atwood's Pond and Crane Falls Lake during those two months. Bruneau Dunes pond was not sampled during December due to ice, however we were able to sample there in January. Water temperature was measured continually with, Onset USA tidbit, thermographs. Within the lotic systems the thermograph was attached to an easily distinguishable structure in the center of the thalweg. Within the lentic waters thermographs were attached .3 meters below a floating buoy, which was anchored to the bottom.

Within the lentic waters we sampled primarily Bluegill, but also collected a small sample of Largemouth Bass (*Micropterus salmoides*), Pumpkinseed Sunfish (*Lepomis gibbosus*), Warmouth (*Lepomis gulosus*), Black Crappie (*Pomoxis nigromaculatus*), and

Yellow Perch (*Perca flavescens*). These other species were collected to asses if otolith zonation and timing is uniform across multiple warm water species.

Lentic sampling consisted of electrofishing with a Smith-Root electrofishing boat and, throughout the months of April-September, towing a 1 x 2 m floating neuston net of 1mm bar mesh. The purpose of the neuston net was to capture young-of-the-year larval Bluegill. Due to the inefficiencies of electrofishing during the winter (December-March), we also set 2, 13 mm treated black mesh with 0.9 x 1.8 m frame and a 22.9 m lead trap nets, and a 45 m X 1.8 m clear monofilament sinking experimental gill nets with 6 panels composed of 1.9, 2.5, 3.2, 3.8, 5.1, and 6.4 cm bar mesh in each pond; both net types were soaked for 24 hours. The change of sampling techniques was not an issue since we were not concerned with catch rate; our goal was to obtain a qualitative sample for age and growth examination. To avoid repeated electroshocking of the same fish, we sampled in a rotational clockwise pattern. This pattern allowed us to sample the entire pond over the course of a year. We never sampled a specific area more than once a year and we never sampled any location more than twice throughout the study period.

Streams were sampled using backpack electrofishing equipment, where we targeted Redband Trout. Incidental samples of Brook Trout (*Salvelinus fontinalis*), and Sculpin Cottus sp. were also collected at Mores Creek to determine if otolith zonation and timing was uniform across several cold water species. We avoided electroshocking the same fish repeatedly by sampling in a contiguous fashion upstream, which allowed us to sample a different location each month. Mores Creek, being higher in elevation, was predominantly covered in ice through the winter months which made acquiring a sample challenging. In the spring of the year during high flows both creeks were difficult to sample, but we were able to collect, at least some, fish from both waters every month of the year.

After sampling, fish were sacrificed with an overdose of peppermint oil, returned to the laboratory, and kept frozen until dissected. After defrosting each fish was weighed to the nearest tenth of a gram, measured to the nearest millimeter, and sex and maturity were determined using the approach of Downs et al. (1997). Larval fish weighing less than a tenth of a gram were weighed to the nearest hundredth of a gram. Sagittal otoliths were removed, cleaned of any soft tissue, dried, and stored in 1.5 ml centrifuge tubes.

Digital images of whole otoliths were taken using a Leica DC 500 camera mounted on a Leica DM400B compound microscope using 12-100x magnification, depending on size of the otolith. Images of dry whole otoliths were taken, the otolith was then submerged in water and images were taken again. This allowed us to compare and contrast the edge during edge analysis. We captured approximately 25,000 images throughout this study. The images were then analyzed using Image Pro Insight. The clearest images were used for edge analysis, marginal incremental analysis (MIA), and ageing.

Edge analysis consisted of determining whether the edge of each otolith was opaque, partially opaque, or translucent. We used a modified version of both Casselman (1987) and Yosef and Casselman (1995) methods. Instead of different symbols, otolith edge was qualified as O-opaque, T-translucent, and PO-partially opaque. Anything with 25% or less of the edge transitioning to a new zone was described as partially opaque, this description was used as the transition zone for both transition periods: i.e. translucent to opaque or opaque to translucent. The wet and dry images were both evaluated to assess the edge condition. Edge analysis was conducted by only one reader (Schill et al. 2010).

Edge analysis was used in conjunction with monthly instantaneous growth rate calculations to identify the otolith zone formed during the period of fastest somatic growth. Monthly instantaneous growth rate (G), based on weight, was calculated using the formula $(G) = (\ln(W_2) - \ln(W_1)) / (T_2 - T_1)$ (Busacker et al. 1990) where W_2 is the mean weight of an age class from month two, W_1 is the mean weight of the same age class from the previous month, and T_2-T_1 (the number days between sampling periods).

We used MIA (Maceina and Betsill 1987; Beckman et al. 1988; Hyndes et al. 1992) to validate age on the first two age classes. Young-of-the-year otoliths were measured, in microns, from the center of the otolith (focus) to the distal edge of the postrostrum. We continued this throughout the year until the point when a new annulus had formed. Measurements of the first age class from August 2011 were used to determine the mean focus to first annulus distance for Bluegill as this was the month that the zones transitioned. The measurements from September 2011 were used for this purpose in Redband Trout, as this was when a majority of the first age class had transitioned zones. Otoliths already containing one observable annulus were measured from the distal edge of the otolith to the edge of the last complete annulus. The monthly samples allowed us to follow the formation of the annulus throughout the year (Casselman 1987). These measurements were averaged and plotted for each month. To aid in our confidence of age and growth estimates, a sub sample of otoliths were aged by two readers, and the between-reader coefficient of variance (CV) was calculated (Chang 1982).

Results

Edge Analysis and Growth

Mean water temperature varied throughout the study period and between sites (Figure 1:2). We generally had larger sample sizes during the summer months than during winter months. Though, sample sizes varied throughout the study and between sites (Tables 1:1 and 1:2), we collected a total of 2,699 Bluegill and Redband Trout for this study. Atwood's Pond and Harris Creek produced the most complete data on older age classes due to the larger sample size each month (Tables 1:1 and 1:2).

Bluegill otoliths in Atwood's pond had 100% translucent edge during the first two months of sampling (June-July 2011). By August the percentage of Bluegill otoliths with translucent edge had dropped to a range of 16-63% depending on fish age (Figure 1:3). All Bluegill sampled in September had an opaque edge. The edge of the Bluegill otoliths remained opaque until May at which time the percentage of Bluegill with a translucent edge ranged from 50-95% depending on age. Bluegill otoliths in June of 2012 again showed 100% translucent edges. However, July results were different than the previous year. Only age one Bluegill had 100% translucent in July; the other age classes ranged from 37-50% translucent (Figure 1:3). Bluegill from Bruneau Dunes Pond had the same general pattern, except the translucent zone started forming in April and the outer edge of one year old Bluegill were 100% opaque by August (Figure 1:4). Bluegill from Crane Falls Lake had a similar pattern as the other two water bodies. However, Crane Falls Lake shows 15% of age two fish still having a translucent edge into September (Figure 1:5). Based on our edge analysis the annul otolith zonation pattern for Bluegill is translucent forming during spring to summer months and opaque-forming in fall to spring.

Instantaneous growth rates were calculated for the first three age classes. We did not calculate growth for older age classes due to small sample size. Age-one and age-two Bluegill from Atwood's pond showed the highest rate of growth during June and July in 2011. However the highest rate of growth for 2012 occurred during May (Figure 1:6). Age three Bluegill showed variable growth, but followed a similar pattern as that of the first two age classes: fast growth during spring and early summer. This same pattern was observed at all three warm water sample sites (Table 1.3). We graphically present growth rates and the transition of zone formation for age-one Bluegill from Atwood's pond to depict the timing of zone formation in relation to instantaneous growth. The highest rate of somatic growth for Atwood's Pond Bluegill occurred during the initiation of a translucent zone, during the month of May. However, growth at a lower rate appears to continue through the transition from translucent to opaque (Figure 1:7).

Our sample size was not sufficiently large enough to calculate growth for the other warm water species that were sampled. However, edge analysis indicated that the timing of translucent zone formation of all warm-water species we sampled was similar to that of Bluegill, i.e. translucent formed spring-summer and opaque fall-spring (Appendix).

All Redband Trout otoliths from Harris Creek had an opaque edge for the first two months of sampling (June-July 2011). The opaque edge proportion persisted at 85% or higher until October of 2011. Only the first three age classes had any opacity in November, and by December 100% of all otoliths had a translucent edge (Figure 1:8). All

Redband Trout from Harris Creek remained translucent until March 2012 at which point 37% and 44% of the Redband Trout in the first two age classes had an opaque edge. By June 2012 all otoliths had an opaque edge which remained through August 2012 in older age classes. Similar to 2011, the opaque zone transitioned into translucence from August to October in 2012 (Figure 1:8).

Mores Creek otolith zonation and timing was very similar to that of Harris Creek. We found that during August of 2011 100% of the otoliths had an opaque edge, by November only 50% of age one Redband Trout otoliths retained an opaque edge at Mores Creek (Figure 1:9). Like Harris Creek all otoliths showed a translucent edge in December 2011. The formation of the opaque zone started in May 2012, slightly later than in lower elevation Harris Creek, and it persisted in many fish until October 2012 at sampling cessation (Figure 1:9). Thus, the otolith zonation pattern for Redband Trout appears to be opaque forming during spring and summer months with the translucent zone forming during fall to winter months.

Redband Trout from Harris Creek showed the highest rate of somatic growth during late spring and early summer, specifically May-July (Figure 1:10). Growth slowed in late fall (October) and did not increase again until spring (May 2012). There was a spike of growth in January 2012 (Figure 1:10), however we do not believe this to be a true increase in growth but likely a function of small sample sizes of one and two fish (Table 1:2). A similar pattern of fast growth in the late spring/early summer months with a slowing during winter months was observed at Mores Creek (Table 1.4). Comparison of instantaneous growth rates and edge analysis allowed us to determine that the highest rate of somatic growth occurred during the formation of the opaque zone for Redband Trout in Harris Creek (Figure 1:11).

Although Brook Trout and Sculpin were also collected at Mores creek; we did not have a large enough sample to calculate growth for these two species. However, we did perform edge analysis on these species. The annual otolith zonation patterns for Brook Trout and Sculpin were similar to that of Redband Trout: in general spring-summer was opaque; fall-winter was translucent (Appendix).

Based on our edge analysis and the monthly instantaneous growth rate data we found that Bluegill and Redband Trout were both growing fastest during late spring and early summer; at virtually the same time of year. However, the otolith zone that is associated with this growth was reversed. Bluegill were growing fast, predominantly, during the formation of a translucent zone though growth appears to continue through the transition from translucent to opaque while Redband Trout were growing rapidly during the formation of an opaque zone. Redband Trout appear to more definitive with the formation of the opaque zone being tightly associated with somatic growth (Figure 1:11). Conversely, Bluegill appears to be more variable, with the translucent zone being formed during the highest rate of somatic growth. However, growth continues through the transition period (transition from translucent to opaque) and is still occurring during the formation of the opaque zone (Figure 1:7).

Marginal Incremental Analysis (MIA)

MIA was completed on the first two age classes of fish from each of the five sample sites. The marginal increment of Bluegill from Atwood's Pond continued to increase from June 2011until April 2012. At this point the marginal increment started to slightly decrease, and then declined drastically in May 2012 (Figure 1:12). This decrease coincides with the formation of the annulus which, in the case of Bluegill, is the completion of the opaque zone. The mean distance to the first annulus for Bluegill at Atwood's pond was 925µm (±41µm). The marginal increment of (age 0-1) Bluegill from Bruneau Dunes showed a similar pattern to that of Atwood's Pond (Figure 1.13). The mean distance to the first annulus for Bluegill at Bruneau Dunes Pond was 964 µm (± 40 µm). Crane Falls Lake Bluegill also formed one annulus per year with the marginal increment increasing from August 2011 through May 2012. There was a decrease in the marginal increment for age 1 Bluegill in October 2011, we believe this to be a sample size issue (n=2) (Table 1:1). The annulus was completely formed by April 2012 (Figure 1:14). The mean distance to the first annulus for Bluegill at Crane Falls Lake was 627µm (±78µm). We thus validated the use of whole otoliths for the first two age classes of Bluegill in all three of our study waters. The timing of annulus formation was slightly variable between the three water bodies. However, in all three water bodies the annulus was completed by June or earlier.

The marginal increment for Redband Trout from Harris Creek increased from June 2011 through March 2012 at which time it declined slightly. May of 2012 showed a drastic single decline (Figure 1:15) which signifies the formation of an annulus, which in the case of Redband Trout is the completion of the translucent zone. The mean distance to the first annulus for Redband Trout at Harris creek was 519μm (±27μm). The marginal increment of Redband Trout in Mores Creek increased from August 2011 through March of 2012. The annulus was completely formed by April, which can be seen by a very distinct drop in the marginal increment distance (Figure 1:16). The mean distance to the first annulus for Redband Trout at Mores Creek was 517μm (±46μm).

Discussion

We found that both species grew at similar times of year but growth was evidenced by opposing zone formation. Fast somatic growth of Redband Trout occurred during spring and summer months and was associated with the formation of an opaque zone. Bluegill grew rapidly during a similar time period but the highest rate of growth was related to the formation of the translucent zone, though growth, at a lower rate, continued through the transition from translucent to opaque in the fall of the year. We also found, via MIA, that Bluegill and Redband Trout formed one annulus per year on sagittal otoliths of age zero and one fish.

Bluegill formed the translucent zone during the highest rate of somatic growth, though growth, at a decreased rate, continued in the fall when the otolith edge was opaque. These results, though different from what the other studies have suggested (Beckman and Wilson 1995), it is not unprecedented as a number of researchers have concluded that warm-water species form the translucent zone during the highest rate of somatic growth (Schramm 1989; Taubert and Tranquilli 1982; Maceina and Betsill 1987; Blackwell and Kaufman 2012). Further it has been shown in laboratory studies that Bluegill will grow in variety of temperatures 25-32 C˚ (Lemke 1977, Beitinger and Magnuson 1979). The Bluegill in the present study showed the highest rate of growth during May-July depending on the water body. These months had a mean temperature of 20-28 C˚ depending on month and water body. The time of year we found Bluegill to be growing is also consistent with what has been previously reported, which suggests Bluegill have a “spurt of growth in the spring” followed by a slowing of growth through the summer and fall (Gerking 1966). Given our data and that of others we believe that other warm-water species form the translucent zone during the season of highest somatic growth.

In contrast, Redband Trout formed an opaque zone during the highest rate of somatic growth, which also occurred during spring and summer. The zonation pattern of spring-summer opaque and fall-winter translucent held true for Brook Trout and Sculpin. These findings agree with the majority of studies on otolith zone formation. Most research shows the highest rate of somatic growth occurs during the formation of the opaque zone (Beckman and Wilson 1995), though there is some variation in the timing of the opaque zone between years (Williams et al. 2005) and with latitude (Hoie et al. 2009). However, it should be noted that the two previously mentioned studies were determined by studies in salt water environments.

Our findings hopefully explain some of the literature confusion surrounding which zones are forming during fast somatic growth. We found that Bluegill, a centrarchidae, is forming the translucent zone during periods of fast somatic growth. This is in agreement with research that suggested the translucent zone formed during fast growth in centrarchids (Taubert and Tranquilli 1982; Maceina and Betsill 1987; Schramm 1989; Beckman and Wilson 1995). Centrarchids do not appear to be the only assemblages with a translucent zone forming during fast somatic growth. Several studies have suggested translucent zones form during fast somatic growth in both fresh and saltwater fishes: Yellow Perch (Blackwell and Kaufman 2012), Red Drum (*Sciaenops ocellatus*) (Fuiman and Hoff 1995), Sole (*Sole a vulgaris*), and Brill (*sole a rhombus*) (Arneri et al. 2001).

MIA for Redband Trout showed that the first two age classes formed one annulus per year. This validates the use of otoliths as a reliable ageing structure for the first two age classes of Redband Trout residing in montane streams. During the course of the sampling period we sampled fish up to five years of age, and found that all age classes sampled formed the same zone at relatively the same time of year, although sample sizes for older fish were too small to conduct MIA. Some researchers have warned against making the assumption that validating younger fish does not necessarily validate the same ageing structure for older fish (Beamish and McFarlane 1983, Campana 2001). However, our results and other Redband Trout age validation studies (Hining et al. 2000; Schill et al. 2010) suggest that whole and sectioned otoliths can be used to age older Redband Trout. In addition, Beckman and Wilson (1995) used edge analysis as an otolith validation method for adults.

MIA for Bluegill also showed that the first two age classes formed one annulus per year in their sagittal otoliths. Though it has been suggested that latitude may cause fluctuations in annulus formation (Beckman and Wilson 1995; Hoie et al. 2009); our results for Bluegill show the annulus, opaque zone, forms at nearly the same time of year as Bluegill in Florida (Mantini et al. 1992). Although MIA was used to validate only the first two age classes we did perform edge analysis on all ages sampled. Edge analysis showed that all age classes have a similar seasonal pattern of otolith zonation, which can be used as a validation method (Beckman and Wilson 1995). Given this data we suspect that Bluegill in these waters are forming one annulus per year for older age classes, and believe ages of older fish can be used, albeit with caution.

Given the difference between warm and cold-water fish otoliths we have reported in the current study, and disparate findings of other studies (Taubert and Tranquill 1982; Maceina and Betsill 1987; Schramm 1989; Beckman and Wilson 1995, Fuiman and Hoff 1995, Arneri et al. 2001, Blackwell and Kaufman 2012), we feel it is vitally important to understand the timing and zonation patterns for otoliths in other fresh water fish. Ageing a fish by starting to count the wrong zone as the annulus could cause an error of up to one year of age (Williams and Bedford 1973; Campana 2001). On a long lived species this perhaps may not be a crucial issue. However, Campana (2001) suggests that an error of even one year on younger fish would introduce an unacceptable error in age determination, and the ensuing growth estimate. For example, when calculating the growth of a short lived fish, (e.g. an age 3 Black Crappie), the growth estimate would be off by one third. Therefore, we agree with the recommendation of Casselman (1987), that validation of ageing structures on all species in all geographic locations is desirable, and agree with Williams and Bedford (1973) that knowledge of zone timing is also crucial.

If such efforts are not possible, then some guidelines for ageing warm vs. cold-water fish within the temperate freshwater regions of North America are needed. Based on the present research and the literature (Taubert and Tranquill 1982; Maceina and Betsill 1987; Schramm 1989; Beckman and Wilson 1995), warm-water fish form the translucent zone during the highest rate of somatic growth which is spring and early summer. Lacking water-specific data we suggest, the opaque zone, which forms during slow somatic growth (usually fall-spring), should be considered the annulus for these freshwater fish. Conversely, cold-water species form the opaque zone during the highest rate of somatic growth, which typically forms during spring to summer. The translucent zone, formed during periods of slow somatic growth in fall-winter, should be considered the annulus for these species. We also agree with Casselman (1987) and others (Campana 2001) in that the most difficult aspect of ageing a fish is determining where the first annulus begins. The mean focus-to-first-annulus distance that we reported for Bluegill and Redband Trout are good references to assist other South Idaho biologists determine the location of the first annulus on these species. The focus-to-first-annulus we reported for Crane Falls Lake was considerably smaller than that of Atwood’s Pond or Bruneau Dunes Pond. We believe this is due to the minimal growth rates observed at that location. This leads us to suggest using this guideline with caution, especially within stunted populations. Further, we do not recommend using this guideline in broadly different habitats or other geographic locations.

Many agencies do not have time or resources to do such extensive research into the otoliths they are using to estimate age. However, we strongly urge that more research be completed on this subject, especially studies to evaluate which species grow during opaque zone formation and which grow during translucent zone formation. Based on the species we have found in the literature (Taubert and Tranquill 1982; Maceina and Betsill 1987; Schramm 1989; Beckman and Wilson 1995, Fuiman and Hoff 1995, Arneri et al. 2001, Blackwell and Kaufman 2012), the split is highly variable and extends not only freshwater but also saltwater species. We encourage similar follow up zonation studies by other researchers on more species in more geographic locations, as originally suggested by Pannella (1980).

A second consideration for future research is to determine the mechanism behind the apparent difference in otolith zone formation. Knowing that the growth zones are different for different species leads to the question why? There has been much research on the microchemistry of otoliths, most of which has tried to correlate temperature and zonation as well as using otoliths to reconstruct life history (Kalish 1992; Campana 1999; Elsdon et al. 2004). Microchemistry of the otolith does not necessarily match that of the environmental conditions (Kalish 1989; Kalish 1992; Campana 1999; Campana and Thorrold 2001). The endolymphatic fluid, which is where the otolith is formed, is highly controlled by the endocrine system (Kalish 1989; Campana 1999; Alvarez et al. 2008). Mosegraad et al. (1988) showed that growth may be decoupled from otolith zonation and suggested the zones are actually a product of metabolic rates. This and our findings which show that Bluegill grow through the transition of zones, may hint that the mechanisms behind the warm-water: cold-water zone reversal we reported in the present study could be driven by the endocrine system and metabolic rates. Further, it was observed that the zonation patterns of otoliths and fin rays of Bluegill appeared reversed (Chapter 3). However this phenomenon did not seem apparent in Redband Trout (Chapter 2). This leads us to suggest that otoliths are highly variable in the timing of zone formation, which has been documented in the literature (Taubert and Tranquill 1982; Maceina and Betsill 1987; Schramm 1989; Beckman and Wilson 1995, Fuiman and Hoff 1995, Arneri et al. 2001, Schill 2009, Schill et al. 2010, Blackwell and Kaufman 2012). We further suggest that though environmental conditions affect whole otolith chemistry, the endolymphatic fluid and hormones may be controlling the timing of zone formation and an otoliths unique mode of calcification (Payan et al. 1997; Campana 1999; Campana and Thorrold 2001). Future research should investigate how metabolic rates and what hormones are effecting otolith zone formation. Further, chemical analysis should be undertaken to determine the difference between the timing and composition of the different zones on species that show a reversal of the otoliths zones, e.g. Redband Trout and Bluegill.

Conclusions

A primary goal of this study was to determine which zone was forming relative to the time of year Redband Trout and Bluegill were growing. We found that both species are growing rapidly at relatively the same time of year but this growth results in different otolith zones. Assuming this finding is correct, and can be corroborated by others, it has important implications for both experienced and novice biologist conducting ageing studies. Lacking water-specific validation, the annulus for temperate warm-water centrarchids should be considered the opaque zone. Conversely, the annulus for temperate cold-water trout should be considered the translucent zone.

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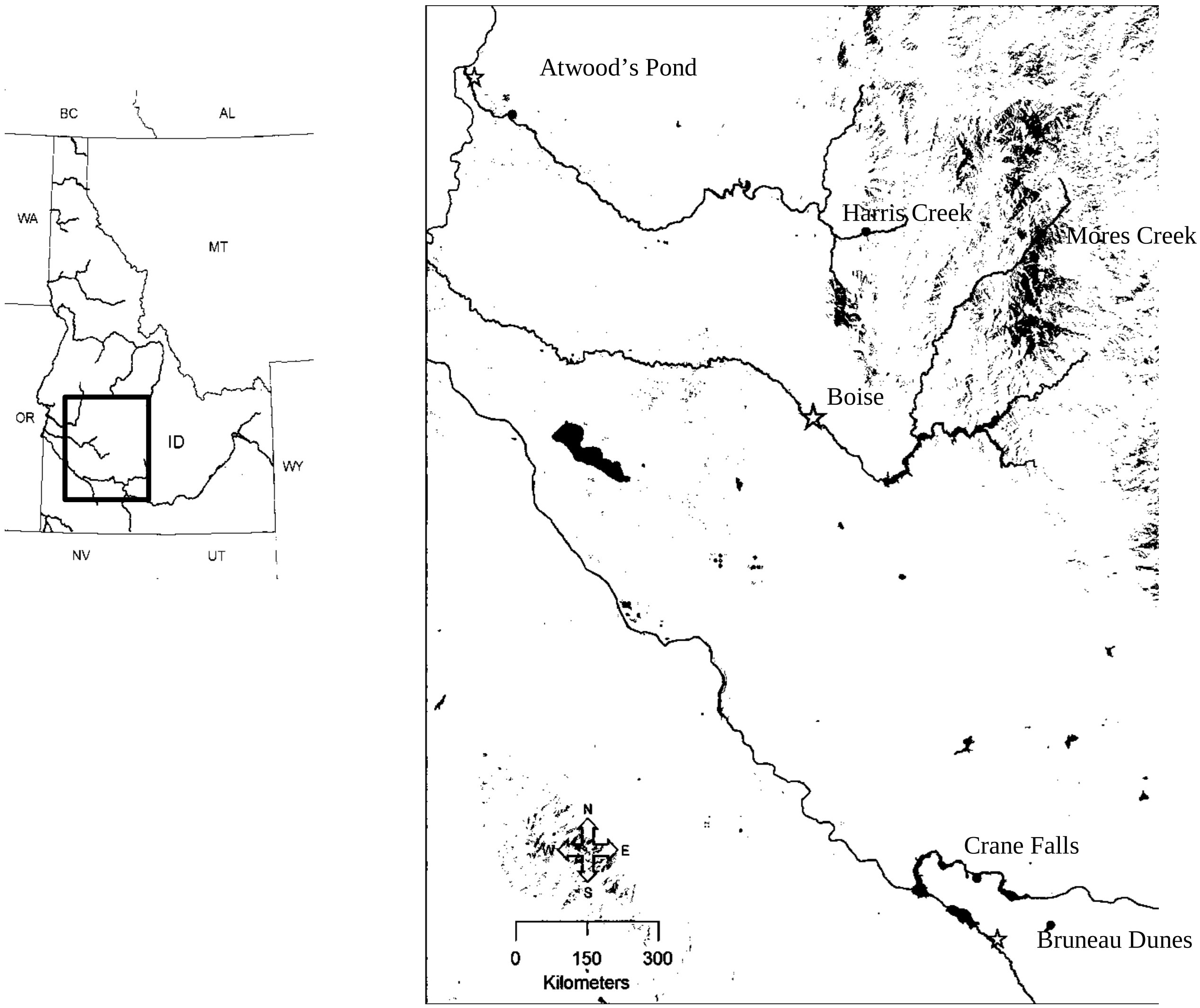

Figure 1:1: Map depicting the sampling locations within Southwestern Idaho

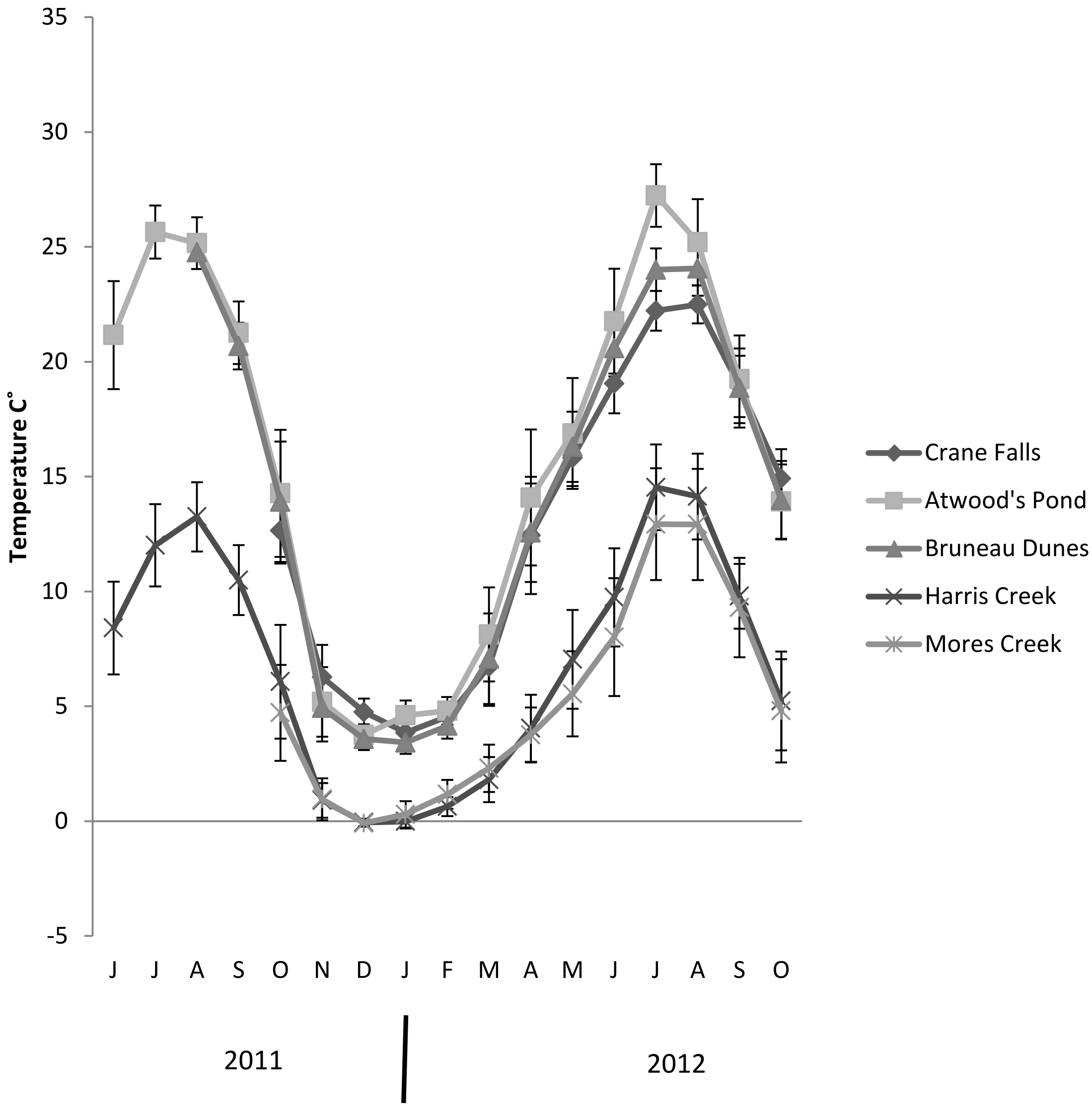

Figure 1:2: Monthly temperature (C°) for each sample site (Mean ± 1 SD).

Table 1:1: **Bluegill Sample size of all age classes that were sampled from all three of the Bluegill sample sites. Dashes represent a sample size of zero for that age and month. Crane Falls Lake and Bruneau Dunes Pond were not sampled in June and July of 2011. Ponds were not sampled in December and January due to ice. Sample period is from June 2011 through October 2012.**

	Atwood's Pond						Crane Falls Lake										Bruneau Dunes Pond								
cohort	0	1	2	3	4	6	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8
Month																									
June	-	20	19	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
July	15	20	25	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
August	18	35	36	2	-	-	9	6	10	35	3	3	1	-	-	-	18	23	17	2	1	-	1	-	-
September	16	31	20	2	-	-	10	4	16	30	5	2	-	-	-	-	14	11	6		-	-	-	-	-
October	6	3	12	8	-	-	2	7	17	5	-	-	-	-	-	-	16	3	1	1	1	-	-	-	-
November	16	29	-	-	-	-	3	5	13	10	4	4	3	1	4	-	8	11	15	1	1	-	-	-	-
December	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
January	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	4	-	-	-
February	11	7	7	2	-	1	-	2	6	-	2	3	2		4	1	-	-	4	3	2	1	-	-	1
March	6	17	3	1	-	1	-	-	9	4	3	9	3	2	2	-	-	-	1	1	-	2	2	-	-
April	5	18	18	5	1	-	6	8	1	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-
May	14	20	8	4	1	-	3	12	11	15	2	4	1	1	-	-	-	-	2	1	1	-	-	-	-
June	8	27	18	3	1	-	-	12	13	4	1	2	1	3	-	-	1	18	6	1	-	-	-	1	-
July	15	23	8	2	-	-	6	14	27	13	2	1	-	-	-	-	13	8	-	-	-	-	-	-	-
August	23	23	5	1	-	1	13	10	18	10	-	-	-	-	-	-	-	5	1	-	-	-	-	-	-
September	26	22	6	2	-	-	1	15	19	7	1	-	-	-	-	-	6	12	3	1	-	-	-	-	-
October	21	14	1	1	-	1	8	2	17	13	1	-	1	1	-	-	1	26	4	1	-	1	-	-	

Table 1:2: Monthly sample size of Redband Trout from Harris Creek and Mores Creek. Dashes represent a sample size of zero for that age class and month. Samples are from June 2011 through October 2012. Mores Creek was not sampled in June and July 2011.

	Harris Creek						Mores Creek				
Age	0	1	2	3	4	5	0	1	2	3	4
Month											
June	-	20	18	7	8	2	-	-	-	-	-
July	-	15	11	7	4	2	-	-	-	-	-
August	10	41	21	8	3	-	3	20	16	13	1
September	15	20	13	3	4	-	9	17	8	6	1
October	2	5	18	3	2	-	3	13	15	7	1
November	11	9	4	2	2	3	2	7	5	4	1
December	4	5	5	2	-	-	-	2	1	-	-
January	1	2	-	-	-	-	-	1	1	-	-
February	11	15	2	4	1	-	-	1	1	-	-
March	9	8	2	1	3	-	3	1	2	-	-
April	-	4	-	1	-	-	1	1	-	-	-
May	8	2	4	1	-	-	-	1	-	-	-
June	20	17	11	2	1	-	-	4	2	-	-
July	19	14	8	4	-	-	5	2	7	2	1
August	19	13	11	2	-	-	18	10	5	1	1
September	23	15	13	-	1	-	26	10	5	5	-
October	23	13	8	1	-	-	13	12	5	1	-

Table 1.3: Monthly instantaneous growth rates for the first three age classes from Atwood's Pond, Bruneau Dunes Pond, and Crane Falls Lake. $\ln(W_2)-\ln(W_1)/(T_2-T_1)$. Asterisks indicate a sample size of less than 3. Minus sign represents no sample. Empty cells are representation of the inability to perform the calculation due to samples not being continuous. Samples are from June 2011 through October 2012. Bruneau Dunes and Crane Falls were not sampled in June and July 2011.

	Atwood's Pond			Crane Falls Lake			Bruneau Dunes Pond		
Month	Age 0	Age 1	Age 2	Age 0	Age 1	Age 2	Age 0	Age 1	Age 2
June		0.016	0.01	-	-	-	-	-	-
July	0.033	0.01	-0.001	-	-	-	-	-	-
August	0.028	0.004	0.006	0.01	0.005	0.001	0.048	0.021	0.009
September	-0.005	-0.007	0.013	0.004	0.009	-0.015	-0.023	0.008	0
October	0.025	-0.001	-	0.010*	-0.015	0.007	0.02	-0.017	0.003*
November	-	-	-	-	-	-	-	-	-
December	-	-	-	-	-	-	-	-	-
January	-	-	-	-	-	-	-	-	-0.005*
February	-0.002	-0.008	-0.023 *	-		0.002	-	-	0.012*
March	-0.015	0.009	0.022*	-	0.001	-0.008	-	-	-
April	0.037	0.002	0	0.004	0.007	0.027*	-	*	-
May	0.011	0.017	0.007	-	0.022	0.008	-	-	-0.008
June	0.016	0.007	0.002	-	0.001	0.008	-0.029*	-0.001	-
July	0.014	0.006	0.001*	0.006	0.01	0.002	-	0.002	-
August	0.008	0.006	0.014*	-0.012	-0.008	-0.009	0.003	0.01	0.003*
September	-0.002	0.003	0.019*	0.021*	-0.004*	0.01	-0.003*	0.007*	-0.004

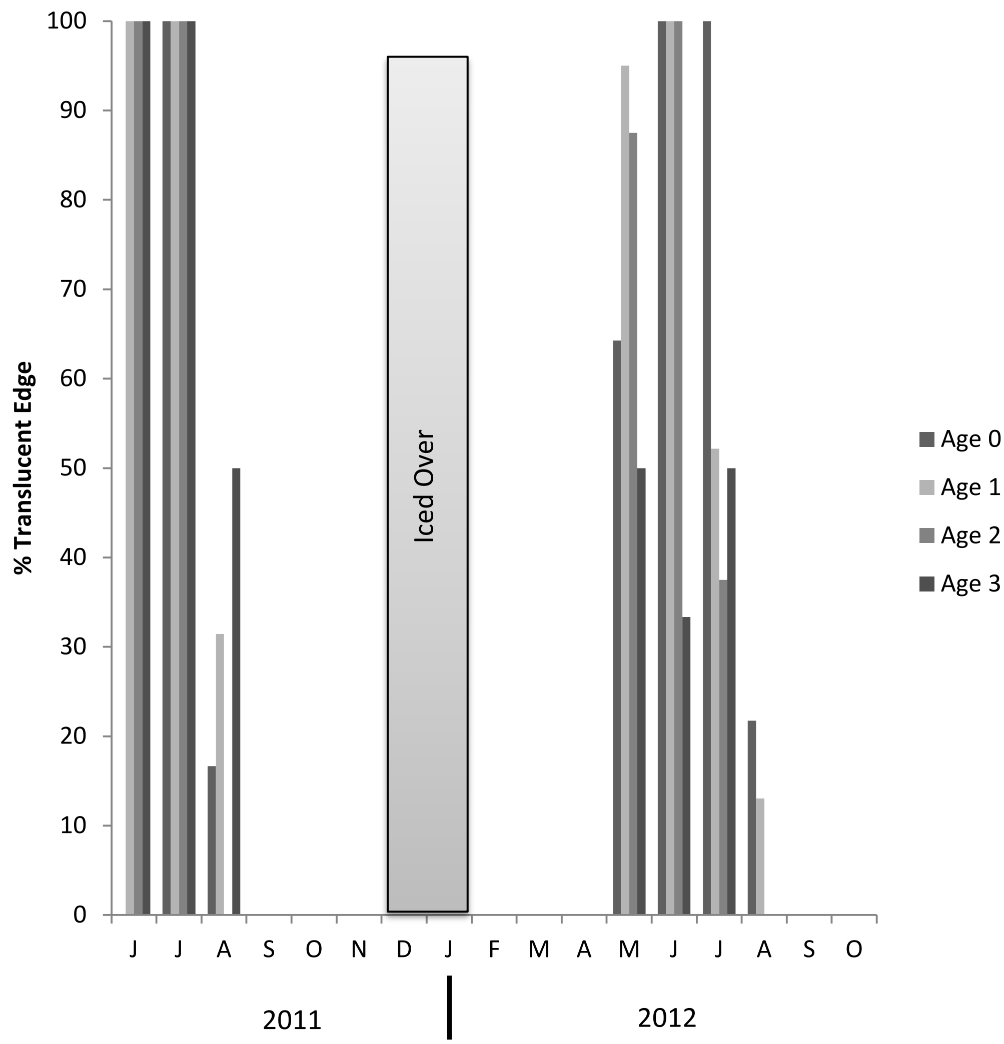

Figure 1:3: The percentage of Bluegill each month from Atwood's pond that had a translucent otolith edge. The edge was determined to be opaque, partially opaque or translucent. Areas without data represent those months where 100% of otoliths have an opaque edge, except January and December when we were unable to sample due to ice.

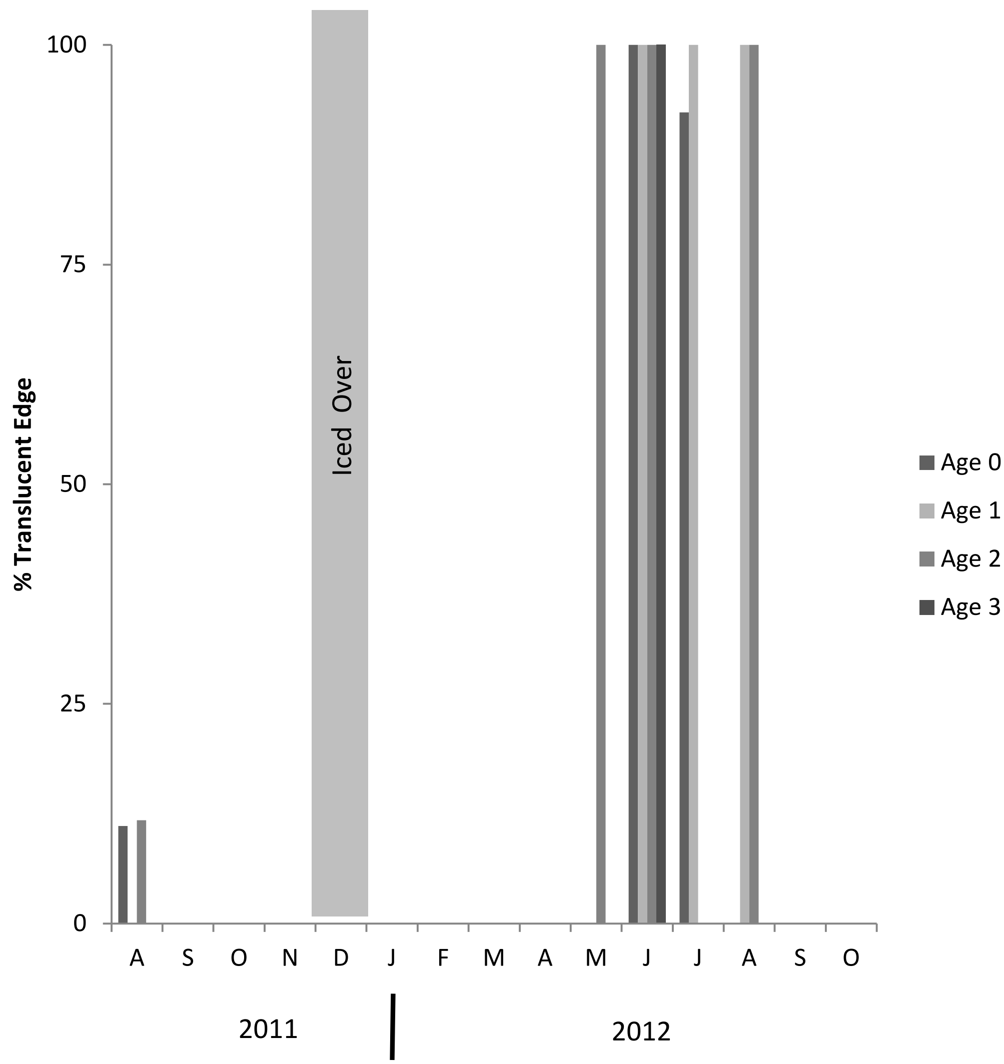

Figure 1:4: The percentage of Bluegill each month from Bruneau Dunes pond that had a translucent otolith edge. The edge was determined to be opaque, partially opaque or translucent. Areas without data represent those months where 100% of otoliths have an opaque edge, except December when we were unable to sample due to ice.

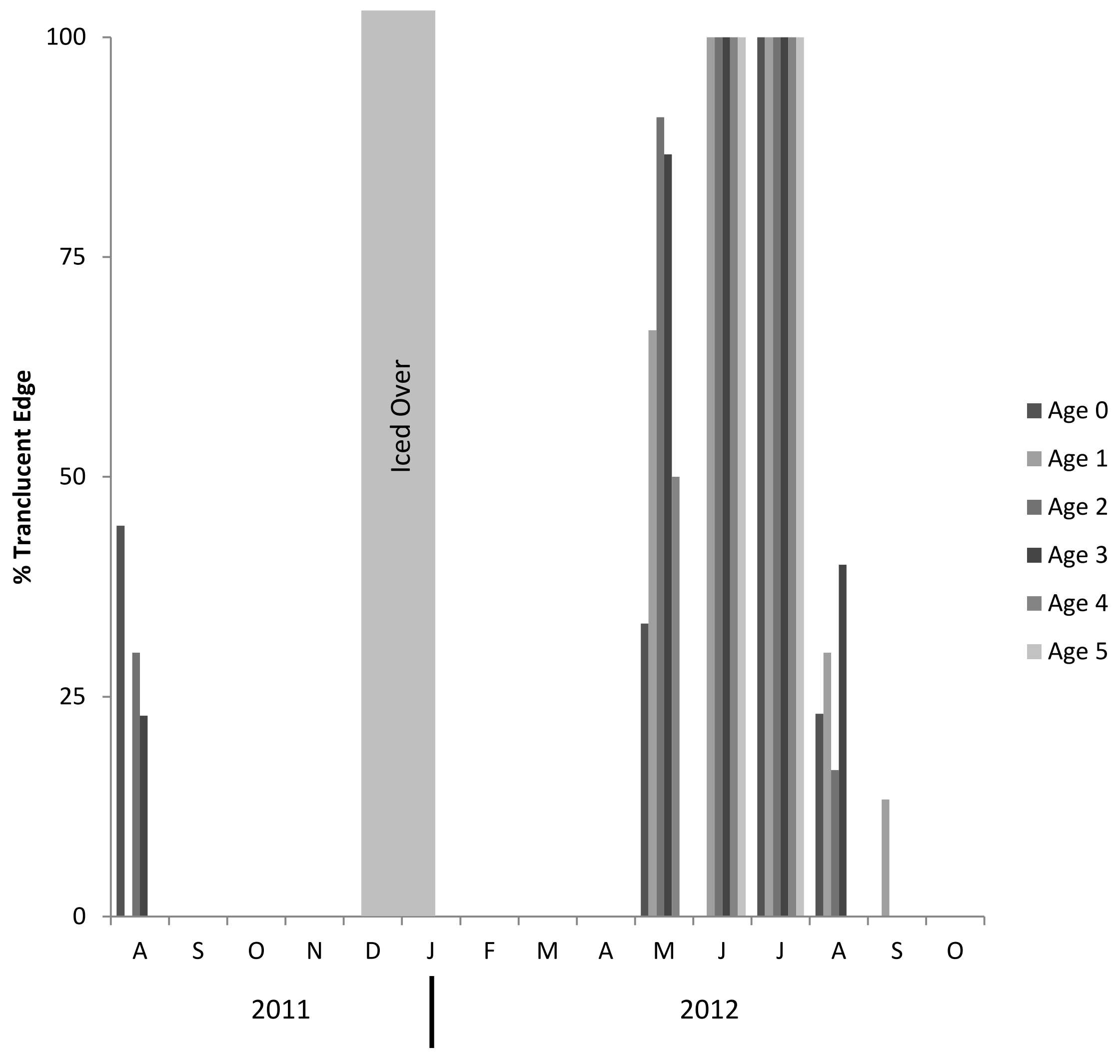

Figure 1:5: The percentage of Bluegill each month from Crane Falls Lake that had a translucent otolith edge. The edge was determined to be opaque, partially opaque or translucent. Areas without data represent those months where 100% of otoliths have an opaque edge, except January and December when we were unable to sample due to ice.

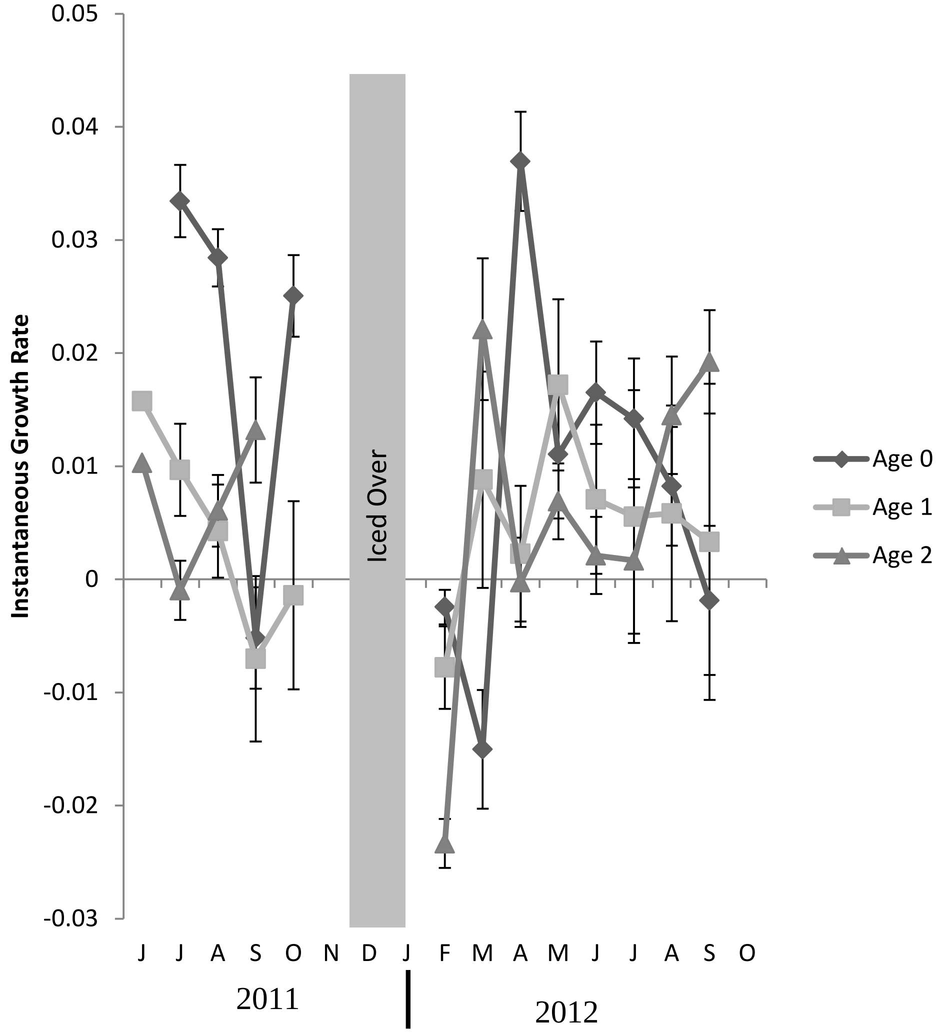

Figure 1:6: Instantaneous growth rate for the first three age classes of Bluegill from Atwood's pond (mean ± 1 SE). The equation (G) = ($\ln(W_2)-\ln(W_1))/(T_2-T_1)$ was used for calculating instantaneous growth rate.

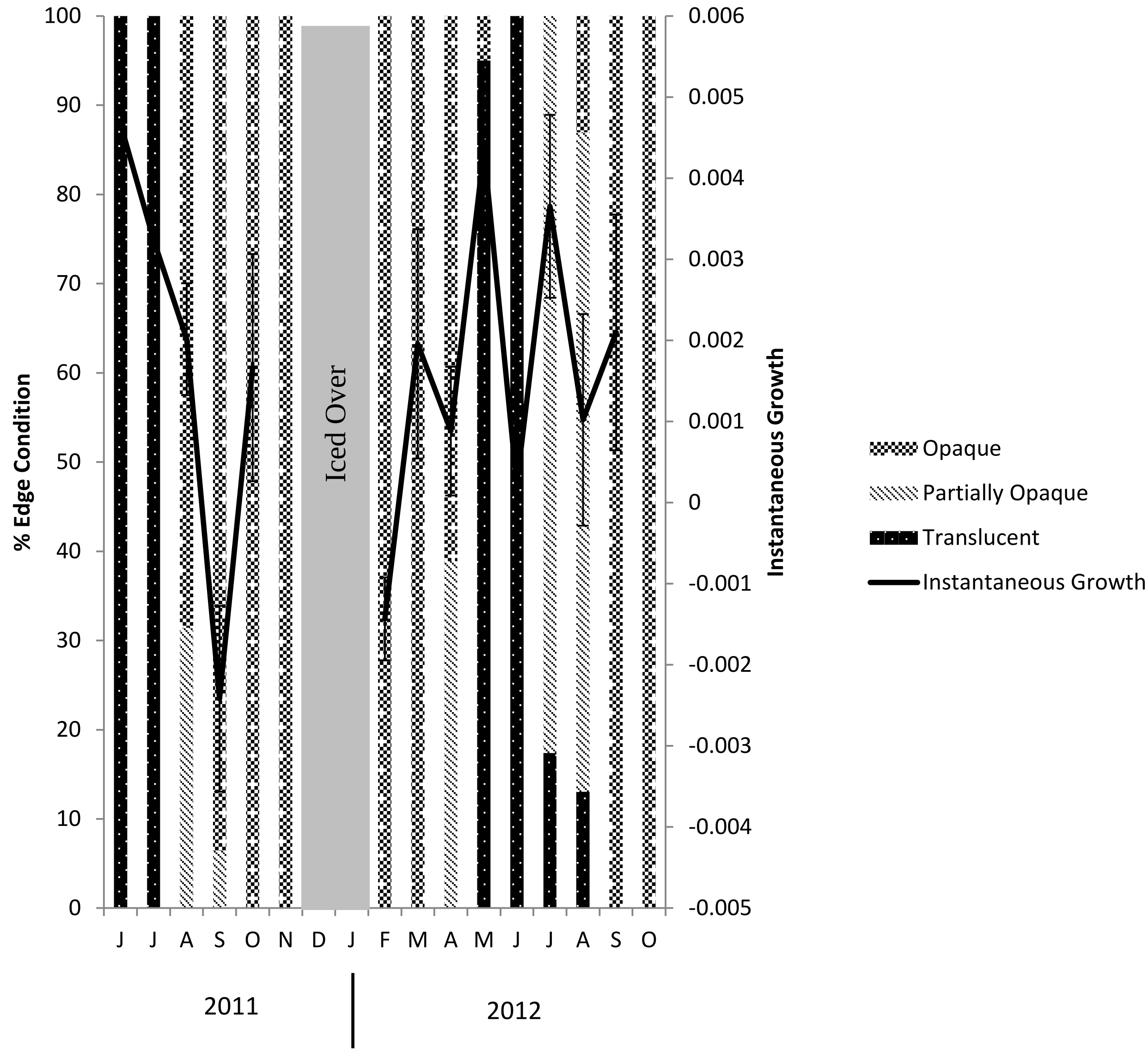

Figure 1:7: Age one Bluegill from Atwood's pond with instantaneous growth depicted with the monthly cycle, starting in June of 2011, of zonation. The black dotted bars are translucent the gray dotted bars are opaque and the hash marked bars are partially opaque, which is based on edge analysis. The black line and right Y axis are instantaneous growth rate (mean ± 1 SE). The highest percentage of fish have translucent zone forming during the spring and summer months. This is also the time of highest somatic growth.

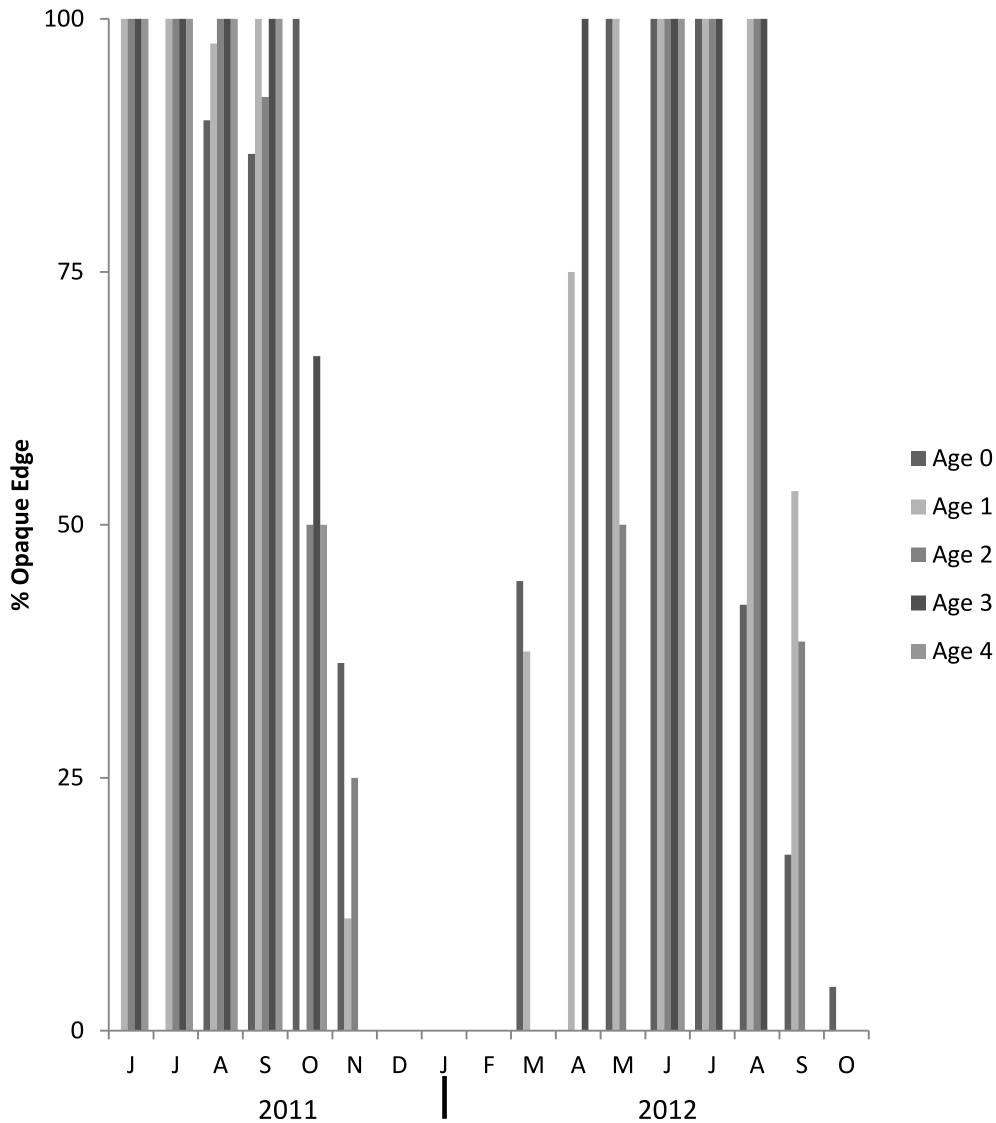

Figure 1:8: The percentage of Redband Trout each month from Harris Creek that had an opaque otolith edge. The edge was determined to be opaque, partially opaque or translucent. Areas without data represent those months where 100% of otoliths have a translucent edge.

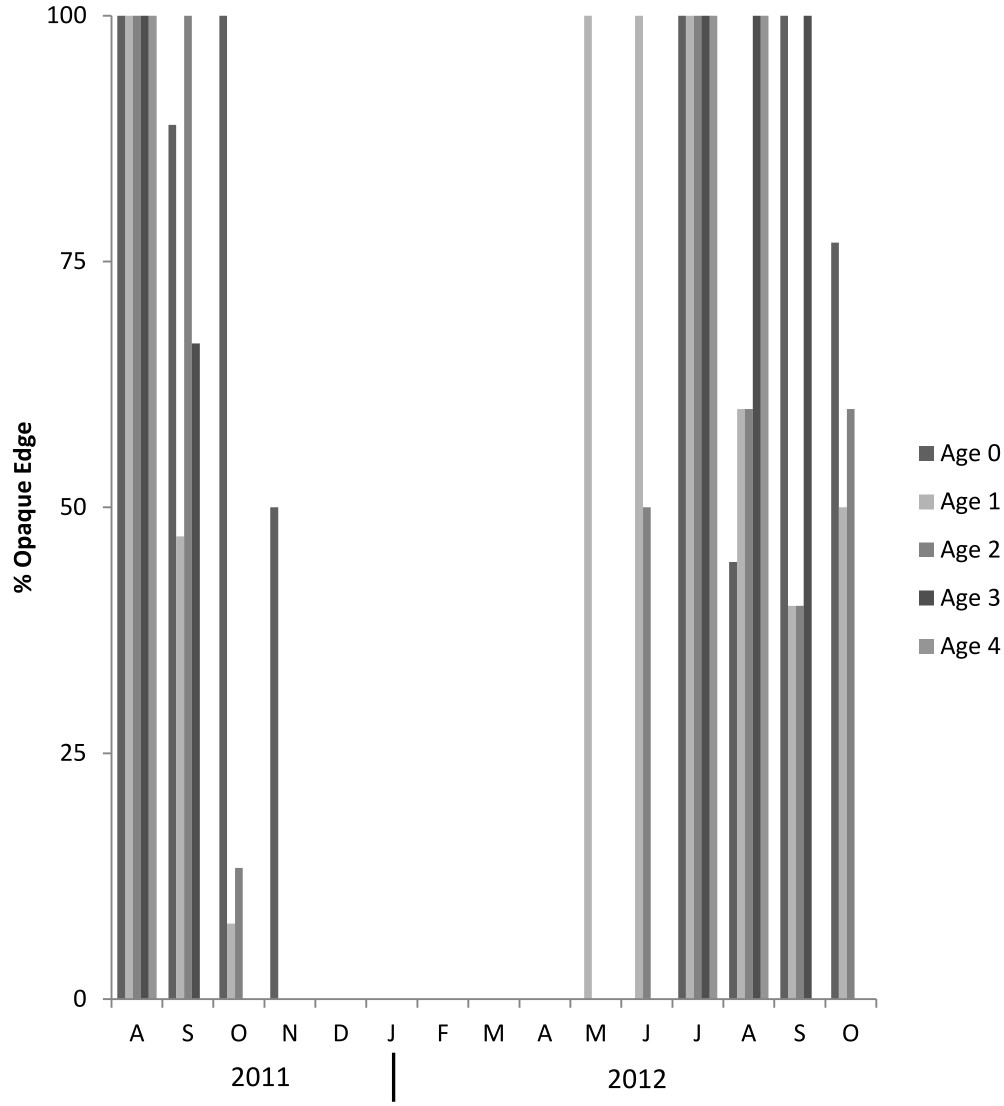

Figure 1:9: The percentage of Redband Trout each month from Mores Creek that had an opaque otolith edge. The edge was determined to be opaque, partially opaque or translucent. Areas without data represent those months where 100% of otoliths have a translucent edge.

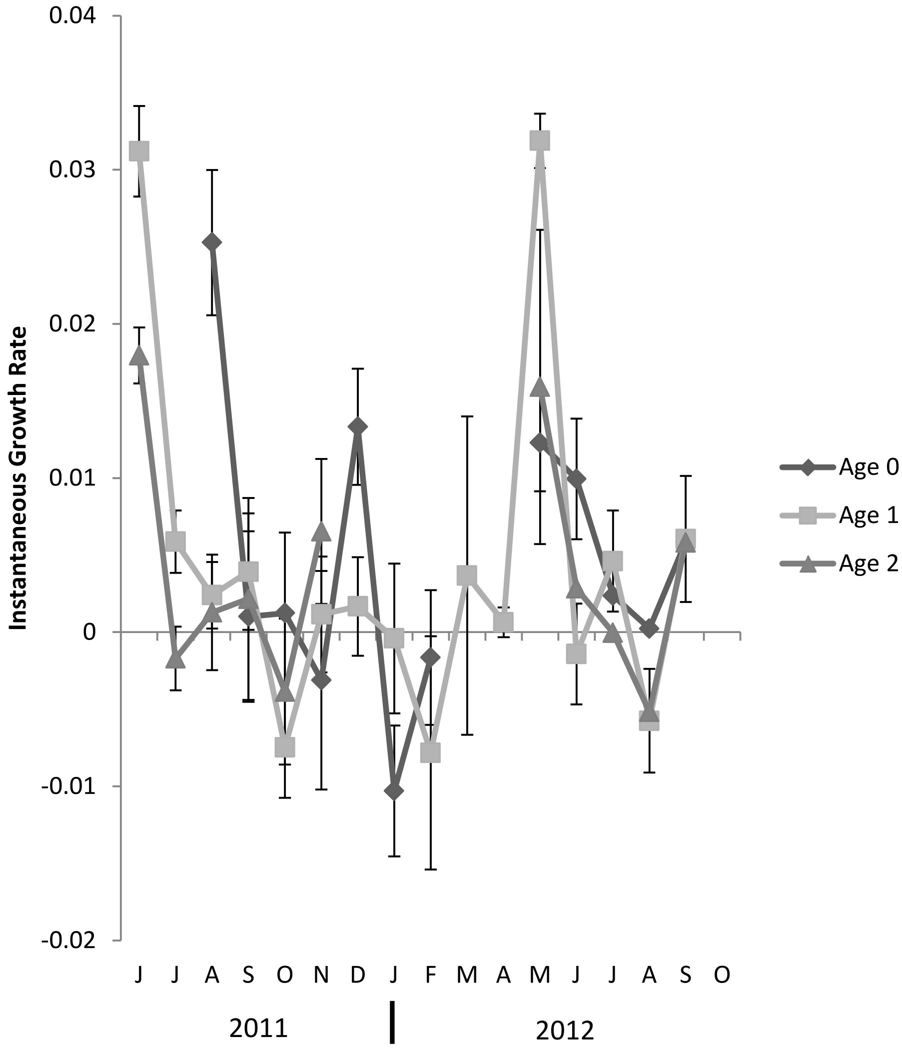

Figure 1:10: Instantaneous growth rates for the first two age classes from Harris Creek (mean ± 1 SE). The equation G= (ln(W2)-ln(W1))/(T2-T1) was used to calculate instantaneous growth rates.

Table 1.4: Monthly instantaneous growth rates for the first three age classes from Harris Creek and Mores Creek. ln(W2)-ln(W1)/(T2-T1) Asterisks indicate a sample size of less than 3. Minus sign represents no sample. Empty cells are representation of the inability to perform the calculation due to samples not being continuous. Samples are from June 2011 through October 2012.

	Harris Creek			Mores Creek		
	Age 0	Age 1	Age 2	Age 0	Age 1	Age 2
June	-	0.031	0.018	-	-	-
July	-	0.006	-0.002	-	-	-
August	0.025	0.002	0.001	0.041	0.009	0.009
September	0.001	0.004	0.002	0.009	0.001	-0.013
October	0.001*	-0.007	-0.004	0.033	-0.013	0.008
November	-0.003	0.001	0.007	*	-0.009	0.012
December	0.013	0.002	-	-	0.027*	-0.015*
January	-0.010*	-0.000*	-	-	0.018*	-0.003*
February	-0.002	-0.008	0.019*	-0.001	-0.019*	0.005*
March	-	0.004	*	-0.041*	-0.008*	*
April	-	0.001	-	-	-0.025*	-
May	0.0123	0.032	0.016	-	0.028*	-
June	0.0099	-0.001	0.003	0.018	0.006	0.018
July	0.0024	0.005	0.000	0.031	0.018	-0.011
August	0.0002	-0.006	-0.005	0.004	0.012	-0.006
September	0.0059	0.006	0.006	0.016	-0.003	0.019

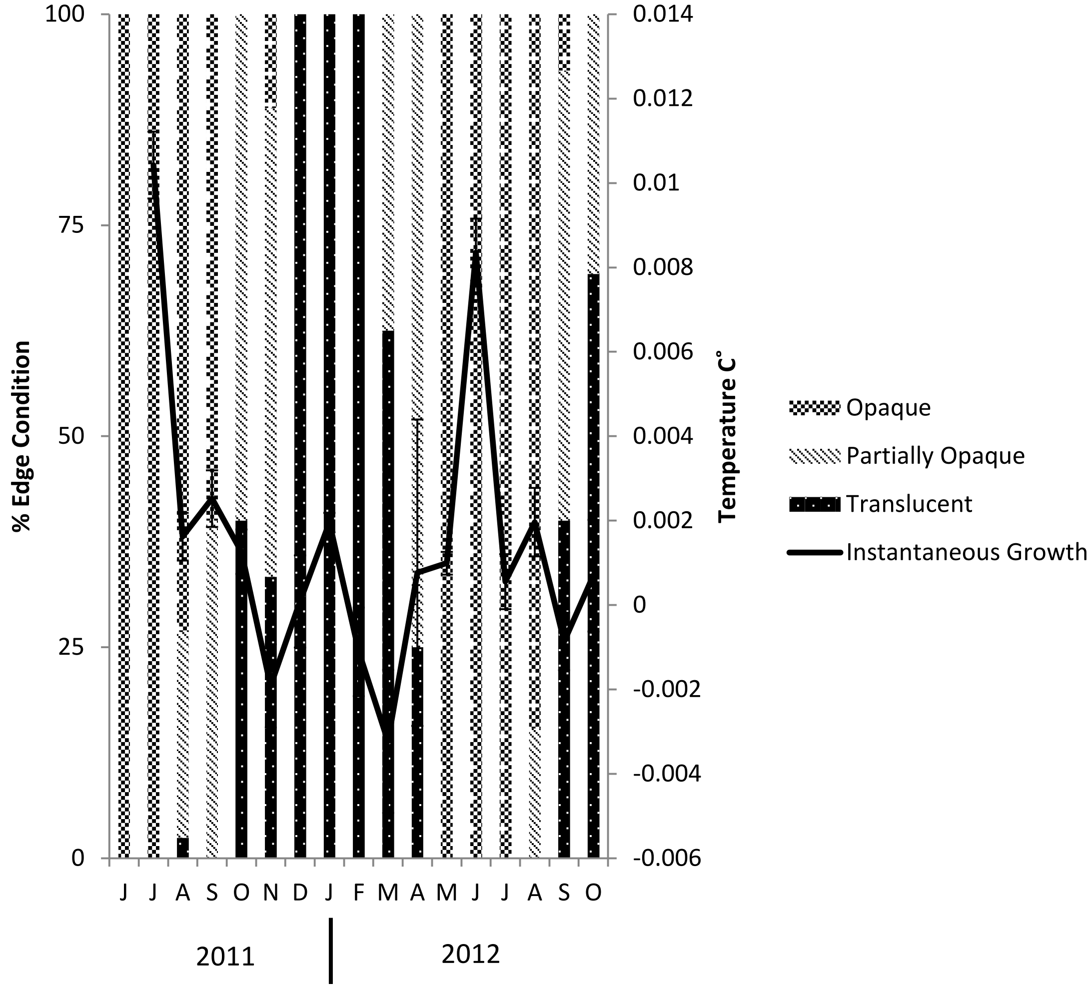

Figure 1:11: Age one Redband Trout from Harris Creek. The black dotted bars are the % of otoliths each month that have a translucent edge. The grey dotted bars are % of otoliths with an opaque edge per month and hash marked bars are the % of otoliths with a partially opaque edge. The black line and right Y axis are instantaneous growth rate (mean ± 1 SE). The highest somatic growth is occurring during an opaque zone.

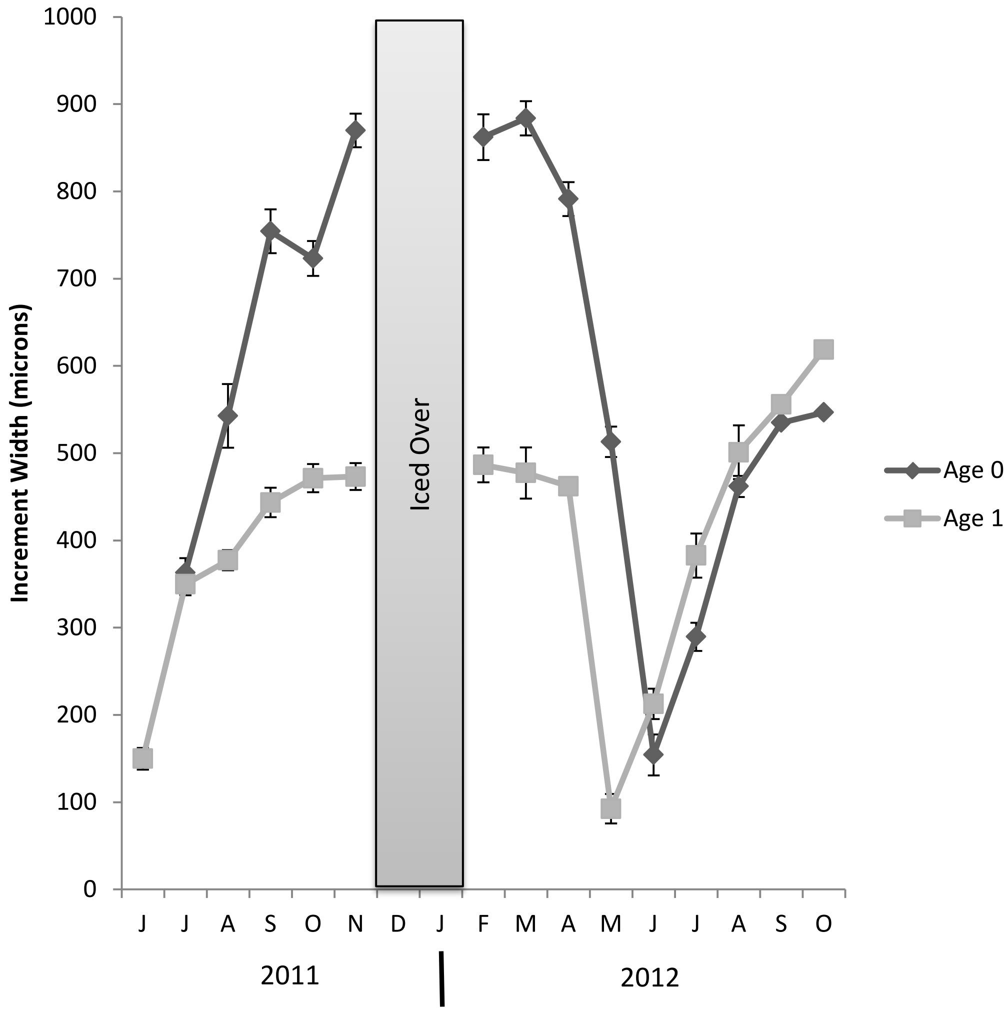

Figure 1:12: Marginal incremental analysis for Atwood's Pond Age 0 and 1 Bluegill (mean ± 1 SE). The rapid decrease indicates the formation of a new annulus.

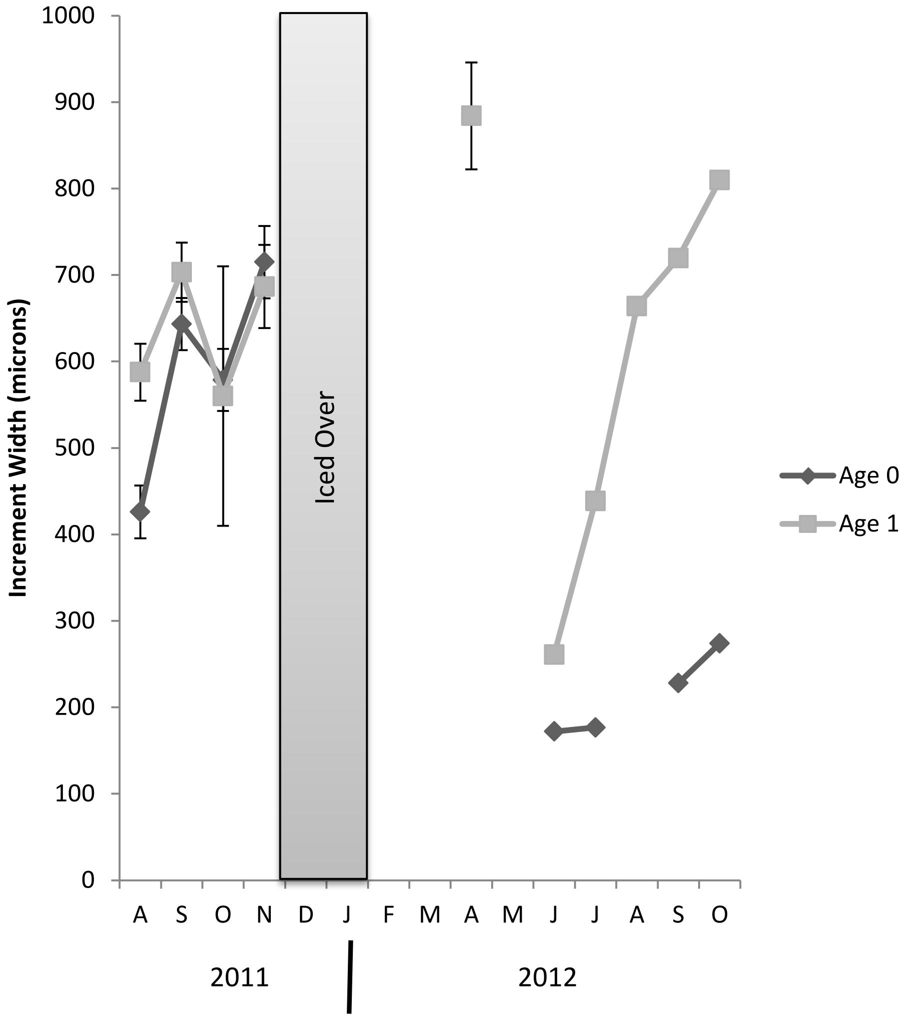

Figure 1:13: Marginal incremental analysis for Bruneau Dunes Pond Age 0 and 1 Bluegill (mean ± 1 SE). The rapid decrease indicates the formation of a new annulus.

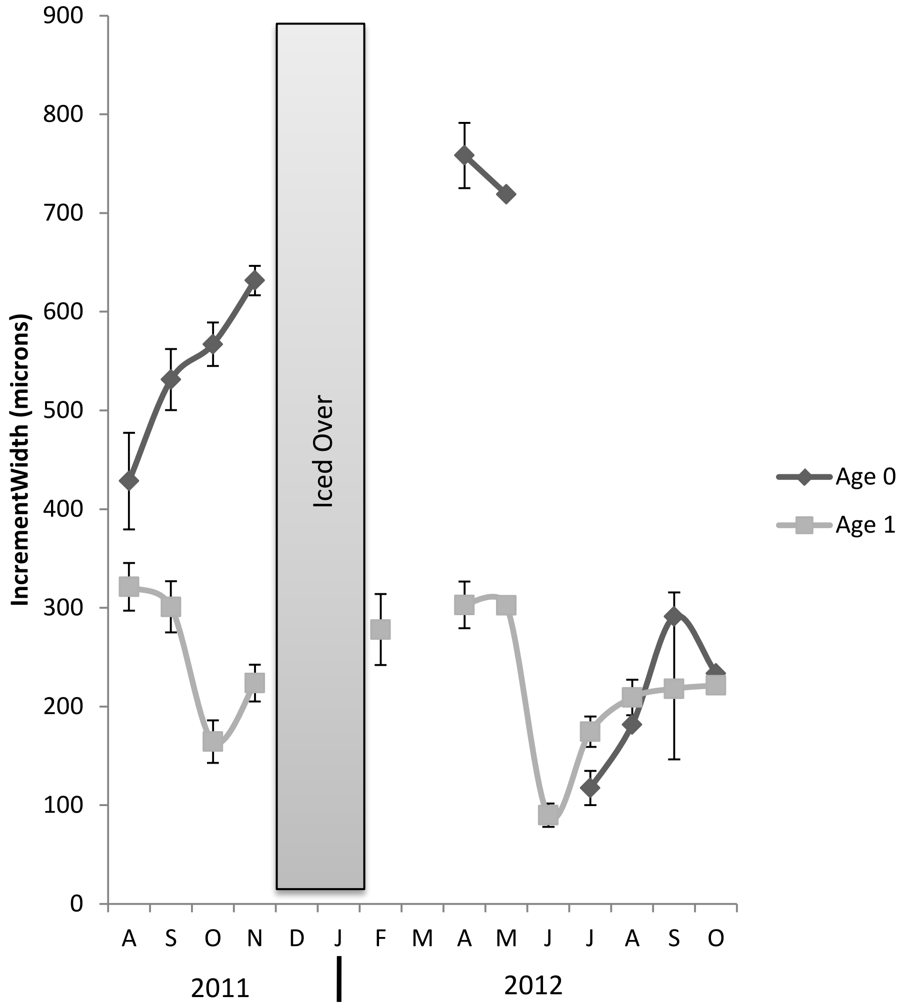

Figure 1:14: Marginal incremental analysis for Crane Falls Lake Age 0 and 1 Bluegill (mean ± 1 SE). The rapid decrease indicates the formation of a new annulus.

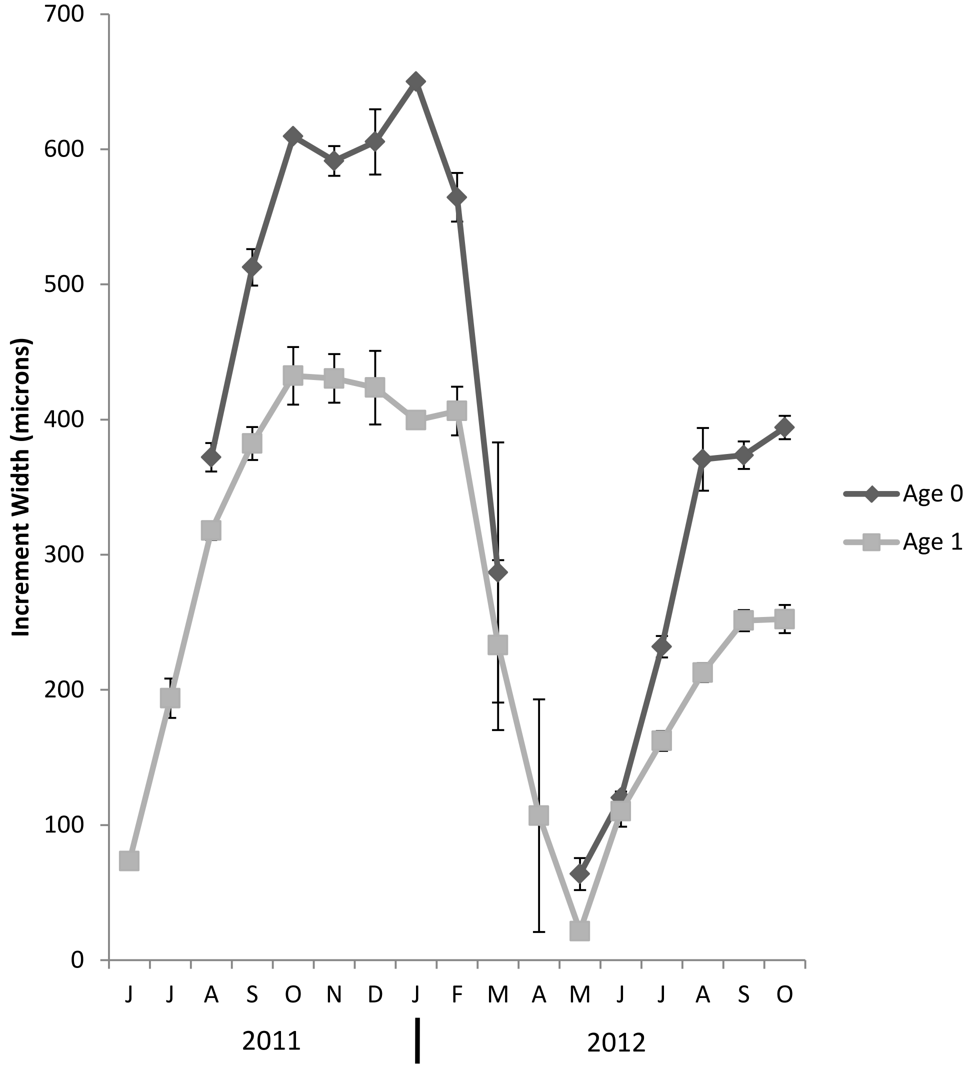

Figure 1:15: Marginal incremental analysis for Harris Creek Age 0 and 1 Redband Trout (mean ± 1 SE). The rapid decrease indicates the formation of a new annulus.

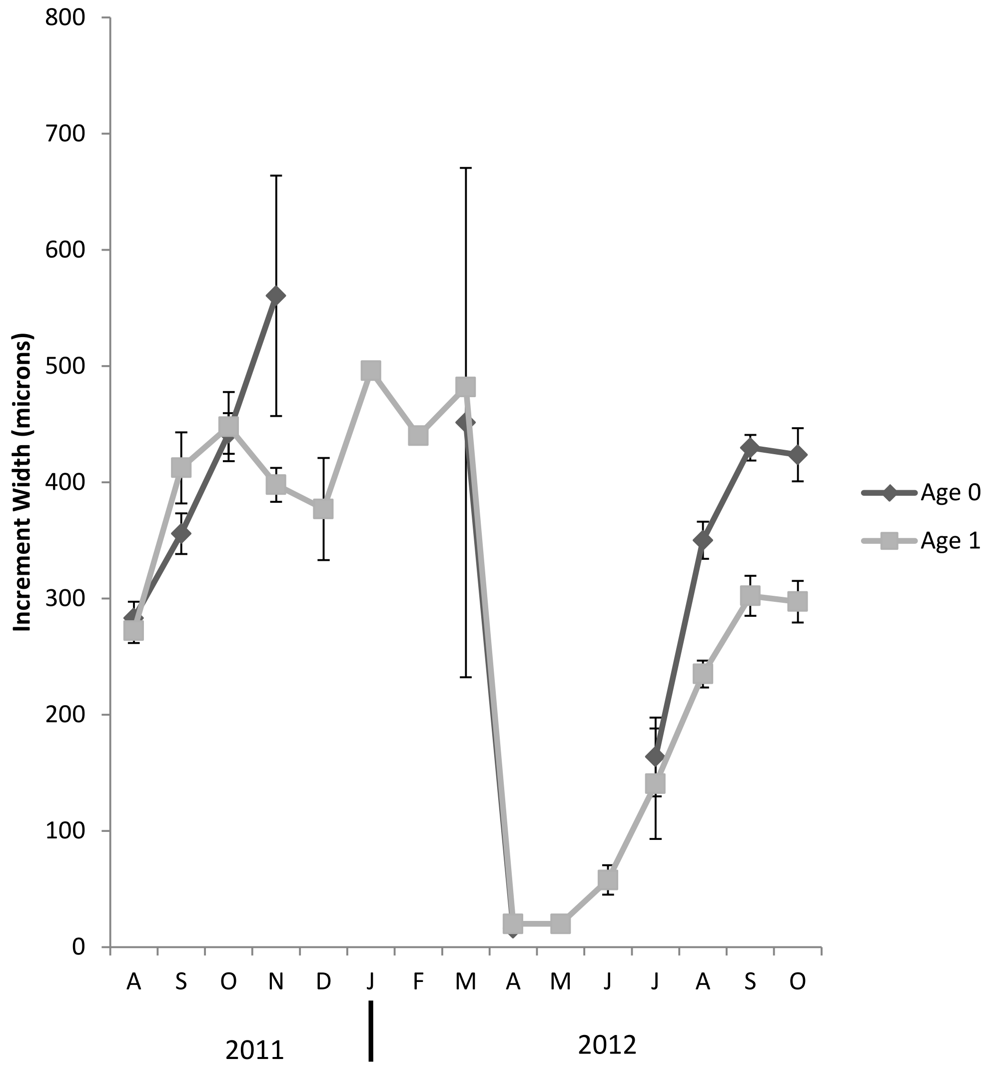

Figure 1:16: Marginal incremental analysis for Mores Creek Age 0 and 1 Redband Trout (mean ± 1 SE). The rapid decrease indicates the formation of a new annulus.

CHAPTER 2: A COMPARISON OF ASSIGNED AGE AND PRECISION OF SCALES, PECTORAL FIN RAYS, AND OTOLITHS OF MONTANE REDBAND TROUT (*Oncorhynchus mykiss gairdneri*)

Abstract

Redband Trout, a native subspecies of Rainbow Trout residing east of the Cascade Mountains, USA, are a popular sport fish in much of its range. We sampled two montane streams in southwestern Idaho, to collect Redband Trout of different age classes from young-of-the-year to the oldest age classes, and compared estimates of age and associated precision for sagittal otoliths, pectoral fin rays, and scales. We found no difference in the assigned age of fin rays and otoliths at Mores Creek; however fin rays ages were less precise than that of otoliths at both locations. Further we found a statical difference between assigned ages of otoliths and fin rays, with fin rays producing lower age estimates, specifically on older fish. Scales ages were less precise and had lower age estimates than that of otoliths or fin rays at both locations. Though, our findings showed a difference in assigned age, at Harris Creek, and precision at both locations. We feel that fin rays produced an acceptable age estimate for montane Redband Trout. These findings, along with those that have shown that fin ray removal did not affect growth and survival, leads us to suggest that fin rays may be an acceptable, non-lethal, ageing structure for Redband Trout in montane streams. We suggest this with caution and suggest further research be completed. Conversely, we do not recommend the use of scales, given the fact that scales are less precise and produced lower age estimates.

Introduction

Fish in different habitats grow and mature at different rates, making it difficult to generalize about fish age at a given size (Campana 2001). This is problematic given that age is the basis for many of the population dynamics used to manage fish populations. Therefore, it becomes important to be able to precisely assign an age based on a morphological structure (Campana 2001) and measure associated periodic growth increments in them. Although the same approach can be applied to a wide variety of living organisms, fish are one of the most frequently aged organisms in the world (Campana 2001).

Given the immense number of fish aged it should come as no surprise that there is a large range of morphological structures used. These include: vertebrae (Brown and Gruber 1988), opercular bones (Baker and Timmons 1991), cleithra (Casselman 1990), scales (Gerking 1966, Schill et al. 2010), fin rays (Herbst and Marsden 2011), spines (Turner 1980), and otoliths (Hales and Belk 1992; Soupir et al. 1997; Campana and Thorrold 2001; Schill et al. 2010). Of these, internal calcified bone appear to be the most accurate aging structures, with otoliths the most widely used due to the ease of dissection (Casselman 1987, Secor et al.1992). Two other commonly used structures are scales and fin rays (Maceina et al. 2007).

Each of the latter three morphological structures have strengths and weaknesses that appear to fluctuate across species and locale. Scales, which are non-lethal and easy to remove, tend to be inaccurate, imprecise, and underestimate the age of fish, especially in the older year classes (Soupir et al. 1997; Campana 2001; Metcalf and Swearer 2005; Schill et al. 2010; Herbst and Marsden 2011). The under-estimation in older fish is due to the circuli being too closely spaced together to distinguish annuli (Beamish and MacFarlane 1983; Casselman 1987), scale loss and regeneration (Cooper 1951; Bereiter-Hahn and Zylberberg 1993), or resorption of old or damaged scales (Persson et al. 1995). However, for some species, scales can be as precise as otoliths (e.g. Black Crappie *Pomoxis nigromaculatus,* Kruse et al. 1993; White Bass *Morone chrysops,* Soupir et al. 1997).

Age estimates with otoliths have been shown to be accurate, precise, and they are easy to remove using several approaches (Secor et al.1992). However, the use of otoliths as an ageing structure is a lethal sampling method (Metcalf and Swearer 2005). Due to this lethality, otoliths make a less desirable choice for species of concern, especially if other accurate options are available. In addition, for endangered species a non-lethal sampling method could allow for a larger sample size; which in turn could lead to improved results and coinciding management plan (Metcalf and Swearer 2005).

Fin Rays are a non-lethal sampling method that has been gaining interest and support (Koch et al. 2008; Herbst and Marsden 2011). Fin ray removal seems to have no significant impact on growth or mortality rates (Zymonas and McMahon 2006). However, fin rays have mixed reviews in precision and accuracy. Fin ray age estimates have been shown to be more precise than that of otoliths (Walsh et al. 2008), as well as being less precise than that of scales (Maraldo and MacCrimmon 1979). Further, periodic sampling has shown that fin rays do not always form an annulus each year (Buckmeier et al. 2012)

Otoliths, fin rays and scales have been used to age a variety of salmonid species (Maceina et al. 2007). Otoliths have been shown to be accurate and precise for ageing high dessert populations of Redband Trout (*Oncorhynchus mykiss gairdneri*), while scales seriously underestimated their age (Schill et al. 2010). However, the high mid-winter water temperatures reported by Schill resulted in an unusual growth pattern for the high desert population, and these results may not apply to montane stocks residing in much colder water. Although fin rays have not been examined as an ageing structure on Redband Trout, they have been shown to be accurate and precise on other salmonid species (e.g. Chinook Salmon (*Oncorhynchus tshawytscha*), Copeland et al. 2007, and Bull Trout (*Salvelinus confluentus*), Zymonas and McMahon 2009). Conversely, other authors have found it difficult to detect annuli, and as a result, the age estimates for some salmonids using fin rays have sometimes been underestimated (e.g. Brook Trout (*Salvelinus fontinalis*), Stolarski and Hartman 2008, and Dolly Vardin (*Salvelinus malma*), Stolarski and Sutton 2013).

Redband Trout a native subspecies of Rainbow Trout in both high dessert and montane streams in the western USA is a popular sport fish in much of its range (Behnke 1992; Meyer et al. 2014). Angler exploitation has been documented to be low in high desert populations (Schill et al. 2007). However, montane stocks residing in larger river systems such as the Payette and Boise rivers, and their tributaries receive considerably more angling pressure and thus good ageing structures are needed to produce dynamic rate functions for management (Daniel J. Schill, Idaho Department of Fish and Game, personal communication). Regardless of population status many salmonid biologists prefer to use non-lethal sampling (Maceina et al. 2007).

Therefore, it would be beneficial to find a non-lethal, quality, ageing structure for montane Redband Trout. Comparing otoliths, which have been partially validated in our sample sites (Chapter 1), to non-lethal ageing structures will allow us to determine which of these structures, if any, are as precise as otoliths. We are unaware of any studies that have attempted to find a high quality, non-lethal, ageing method for montane stocks of Redband Trout.

The purpose of this study were three fold: 1) determine if otoliths, pectoral fin rays and scales produce similar age estimate for Redband Trout in montane streams, 2) compare the precision of otoliths, pectoral fin rays and otoliths of Redband Trout, and 3) determine if there is a precise non-lethal ageing structure for Redband Trout.

Methods

We sampled two montane Redband Trout streams, Harris Creek and Mores Creek, over a 17 month period. Harris Creek is a second order tributary to the Payette River, in Boise County, Idaho. Our sample site started at an elevation of 1130 m and ended at an elevation of 1250 m. We sampled approximately 2.5 km of stream throughout the study period. Harris Creek has a mean width of 2.5 m and a mean depth of 0.18 m during the summer and fall months. Spring runoff can be quite variable at Harris Creek and the mean depth is 0.91 m. The mean width during spring flows is 3.2 m.

Mores Creek is a second order tributary to the Boise River, in Boise County, Idaho. Our sample site started at an elevation of 1465 m and ended at an elevation of 1500 m. We sampled approximately 3.5 km of stream over the course of the study. During summer and fall base flows, Mores Creek has a mean width of 3.6 m and a mean depth of 0.53 m. During spring runoff, high flows, the mean width is 5.8 m with a mean depth of 1.1 m.

We used backpack electrofishing equipment to sample Redband Trout. Fish were sacrificed with an overdose of peppermint oil, returned to the laboratory, and kept frozen until dissection. In the laboratory, each fish was defrosted, measured to the nearest millimeter, weighed to the nearest tenth of a gram and sex and maturity were determined using the general method of Downs et al. (1997). We removed scales, sagittal otoliths, and both pectoral fin rays. The fish used in this study were a sub-sample from a larger otolith validation study (Chapter 1). The fish were chosen, non-randomly, based on otolith age from throughout the 17 month sampling period, with a goal of seven fish (arbitrarily set) per age class from each sampling location. For some of the older age classes we were unable to meet our target sample size of seven fish (Table 2:1). We also took a tissue sample from the adipose fin to determine if the fish in our sample sites had any introgression with hatchery released Rainbow Trout. These samples were analyzed by the Eagle Fish Genetics Laboratory, Idaho Department of Fish and Game.

In the laboratory scales were removed from the right side of the body midway between the dorsal fin and the lateral line (Quist et al. 2013), and then stored in coin envelopes to dry. Pectoral fin rays were removed at the point where the pectoral fin articulates with the pectoral girdle (Koch et al. 2008), and then stored in coin envelopes to dry. We also removed both sagittal otoliths with the use of the guillotine method (Secor et al. 1992). Otoliths were cleaned of soft tissue, dried, and stored in 1.5 ml centrifuge tubes.

Scales were mounted between two microscope slides and then digitally imaged at 12-40X depending on the size of the scale, using a Leica DC 500 camera mounted on a

Leica DM 400B compound scope. Scales were aged by two readers, independent of one another, and without knowledge of length, or assigned age of other structures.

The three leading fin rays were embedded in Buehler epothin, a clear epoxy, and sectioned with a Buehler low speed Isomet saw. Samples were sectioned serially (0.6 mm thickness 4 sections) starting at the proximal edge, and digitally imaged at 100x magnification using the same equipment as above. Fin rays were aged by two readers independent of one another, and without knowledge of length or the age assigned of the other structures. Both readers were trained but inexperienced at ageing fin rays.

Whole otoliths were placed in a depression slide, sulcus groove down, submerged in water and digitally imaged, with the same equipment as above, at 25-40X magnification, depending on size. Otoliths were aged by two readers independent of one another, without knowledge of length, or assigned age of other structures.

To determine if there was a difference in the precision of the age estimate between the different structures; we calculated exact percent agreement (PA0), within one year percent agreement (PA1) (Beamish and Fournier 1981), and between reader coefficient of variance (CV) (Chang 1982) for all three structures. We then compiled age bias plots to visually discern the variation (Campana et al. 1995).

Pair wise comparisons using linear regression were used to determine if assigned age differed significantly between structures. This was accomplished by building age plots and using simple regression to compare the slope of the regression line to a slope of one (Isermann et al. 2003). We used otoliths as our standard for comparison with the other structures; because otoliths tend to be very precise and accurate for salmonids (Hining et al. 2000; Zymonas and McMahon 2009; Schill et al. 2010), further otoliths have been validated for two age classes of Redband Trout in our sample sites (Chapter 1) as well as for all ages up to nine in high dessert population within Idaho (Schill et al. 2010).

Results

We found that otoliths had the highest exact percent agreement, and the lowest between readers coefficient of variation, followed by fin rays. Scales had the lowest exact percent agreement and the highest between readers coefficient of variation (Table 2:2). However, all three structures had 100% within one year percent agreement. This pattern appeared at both sampling locations. The age bias plots allowed us to visually discern any bias in the assigned ages between readers and between structures. We found that the between reader precision was remarkably high for otoliths and fin rays, and reasonable for scales, though there was a positive trend of an increase in variation as age increased for both sample sites (Figures 2:1-2:2).

Pairwise comparisons for Harris Creek structures showed that scales underestimated age when compared to otoliths ($F_{(0.05)\ 1,\ 39}$ = 43.777 p =$7.23\text{x}10^{-08}$), and fin rays ($F_{(0.05)\ 1,\ 39}$ = 22.983 p=$2.39\text{x}10\text{-}^{05}$). In addition, we found that fin rays underestimated age when compared to otoliths ($F_{(0.05)\ 1,\ 39}$ = 2.1569 p=0.0171) (Figure 2:3). Pairwise comparisons from Mores Creek structures showed similar results with scales underestimating age when compared to otoliths ($F_{(0.05)\ 1,\ 32}$ = 15.844 p=0.0003) and fin rays ($F_{(0.05)\ 1,\ 32}$= 29.129, p=$6.26\text{x}10^{-06}$). However, fin ray assigned age did not differ statically from that of otoliths ($F_{(0.05)\ 1,\ 32}$= 0.675, p=0.4175) (Figure 2:4).

Genetic analysis showed that Harris Creek had a negligible amount of introgression with hatchery Rainbow Trout (1.6-2.1%) while Mores Creek had low levels of introgression (16.3-18.4%), unpublished data (Matthew Campbell, Idaho Department of Fish and Game, personal communication). Thus our results apply to two pure or nearly pure montane Redband Trout stocks.

Discussion

Ageing structures for montane Redband Trout demonstrate a difference in precision, and possibly accuracy, though we did not directly evaluate accuracy during this study. We found that age estimates from otoliths are the most precise followed by fin rays and then scales, and assuming otoliths provide accurate age estimates, scales appear to significantly underestimate the age of older fish. The Redband Trout we collected had a maximum age of five (otolith assigned age), and scale age estimates generally began to fall below those of otoliths and fin rays by age three. These findings agree with Schill et al. (2010) and Hining et al. (2000) who both found that scale age estimates were lower than that of otoliths by age two in high desert Redband Trout, and Rainbow Trout in Appalachian streams, respectively. Underestimates of even one year in the short lived populations we studied could heavily influence an estimate of growth, survival and age at maturity (Campana 2001). While otoliths have been validated for all age classes of Redband Trout in high dessert streams (Schill et al. 2010) and ages up to two in montane streams (Chapter 1); scales have been shown to be unreliable even in young ages in our study sites (Figures 2.3 and 2.4) as well as in high desert Redband Trout (Schill et al. 2010). We therefore do not recommend their use for this subspecies.

No prior authors have attempted to validate fin rays for Redband Trout and the present study should not be misconstrued as a true validation either. Further, the inconsistent agreement between the age estimates of otoliths, which have been previously validated, and fin rays (Figures 2.3 and 2.4) do not allow for complete confidence in our age estimates for fin rays. Our findings also show that age estimates from fin rays are less precise (about 50% less) as that for otoliths based on CV (Table 2.2), a finding that conflicts with other ageing studies on salmonid species (Zymonas and McMahon 2009). However, the decreased between-reader precision and underestimation could be a result of both readers in the present study having had considerable experience ageing fish with otoliths, but none using fin rays. This may be evidenced by the appearance that one year was consistently added to fin rays at Mores Creek (Figure 2.4), though this pattern was not observed at Harris Creek (Figure 2.3).

The ability to non-lethally age a species would be a great addition to the tools managers have at their disposal when evaluating fisheries. Age estimates from otoliths appear to produce the highest quality age estimates but require lethal sampling. Thus, based on our results and the literature they would appear to be the best choice for medium to large populations. Scales do not require lethal sampling, but performed poorly during this study and others on Redband Trout and their close relatives Rainbow Trout (Hining et al 2000; Schill et al. 2010). We therefore do not recommend their use, even for small populations. Though we found age estimates of fin rays and otoliths to differ; fin rays, both in terms of non-lethal sampling and producing a reasonably precise age estimate, may be a suitable ageing structure. However, additional work is needed before fin rays be considered a preferred aging structure for the sub-species.

Confidence in the age assigned to a structure is an important aspect of any technique. Therefore, like Spiegel et al. (2010) we suggest giving each structure aged a confidence rating in the future. This would allow researchers to more objectively describe how challenging a structure was to age.

Conclusions

Based on precision and results of our studies, otoliths appear to be the superior ageing structure of the three compared. Our findings along with those of Zymonas and McMahon (2009) that fin ray removal did not affect growth and survival, lead us to suggest that fin rays could be an acceptable, non-lethal, ageing structure for Redband Trout in montane streams. Additional research which formally validates fin rays as forming one annulus per year for Redband Trout should be conducted prior to fin rays being routinely used as a primary ageing structure. Due to age estimates of scales being less precise, and producing lower age estimates relative to that of otoliths, which have been validated for Redband Trout (Schill et al. 2010; Chapter 1); we do not recommend the use of scales as an ageing method for Redband Trout.

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Table 2:1: Sample size for each age class for Harris Creek and Mores Creek. Age is based on assigned otolith age.

Age	Mores Creek	Harris Creek
0	4	7
1	7	7
2	7	7
3	7	7
4	7	7
5	2	6

Table 2:2: The exact percent agreement (PA-0), within one year Percent agreement (PA-1), and the between reader coefficient of variance (CV) for Redband Trout from Harris Creek and Mores Creek. Assigned age is based on the pairwise regression analysis, and different letters represent statical differences in the between structure comparison of assigned age.

			Harris Creek		
Structure	**PA 0**	**PA 1**	**CV**	**Precision**	**Assigned Age**
Otoliths	93%	100%	0.71	A	A
Fin Rays	90%	100%	1.32	A	B
Scales	80%	100%	3.71	A	C
			Mores Creek		
Otoliths	97%	100%	0.42	A	A
Fin Rays	94%	100%	1.18	A	A
Scales	76%	100%	6.88	B	B

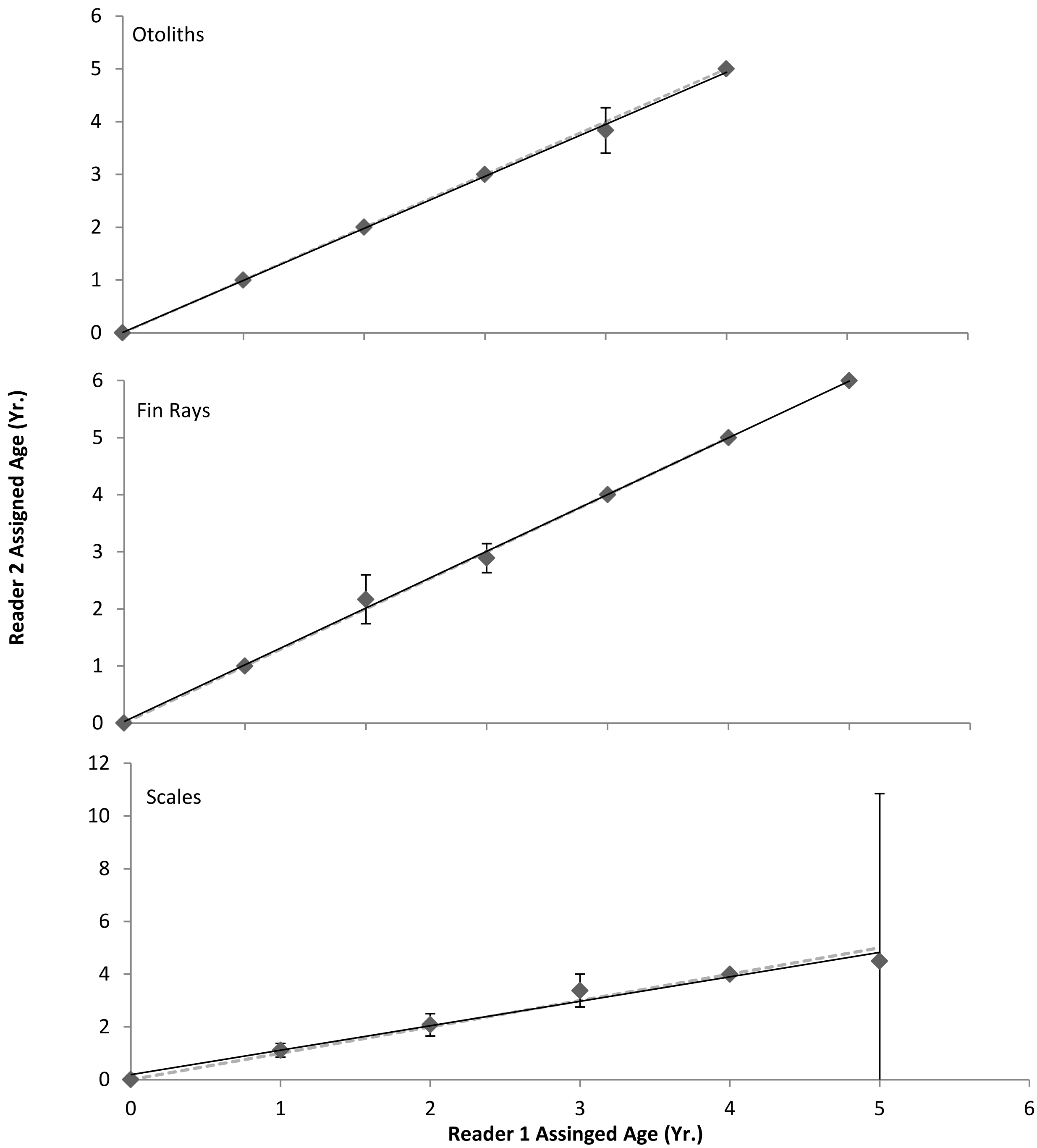

Figure 2:1: Age comparison for Mores Creek Redband trout, comparing the precision between two readers in estimating age using each structure. Diamonds equals means ± (95% CI) between brackets. Dashed line is hypothetical perfect agreement between readers; solid line is actual agreement between readers.

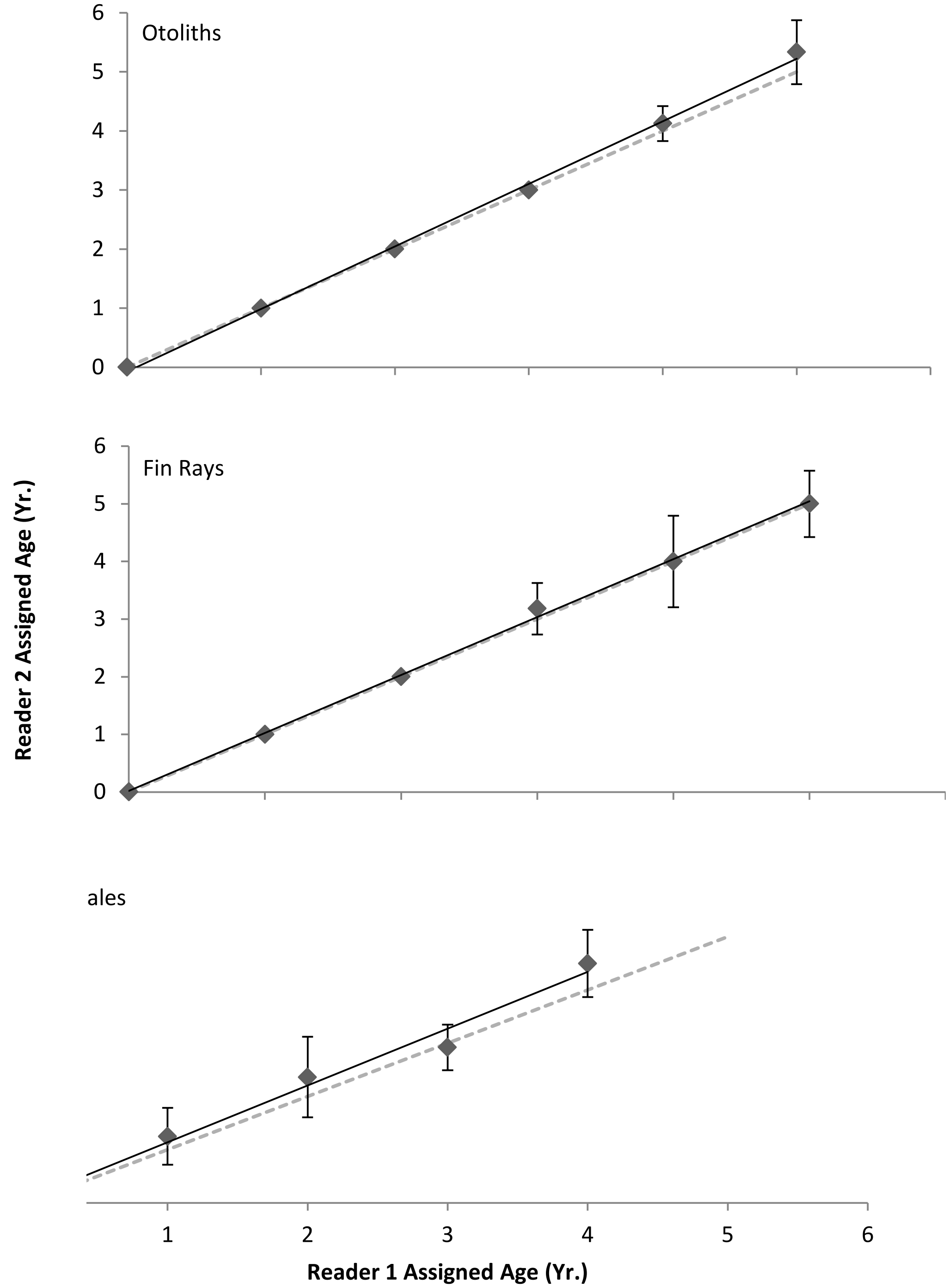

Figure 2:2: Age comparison for Harris Creek Redband trout, comparing the precision between two readers in estimating age using each structure. Diamonds equals means ± (95% CI) between brackets. Dashed line is hypothetical perfect agreement between readers; solid line is actual agreement between readers.

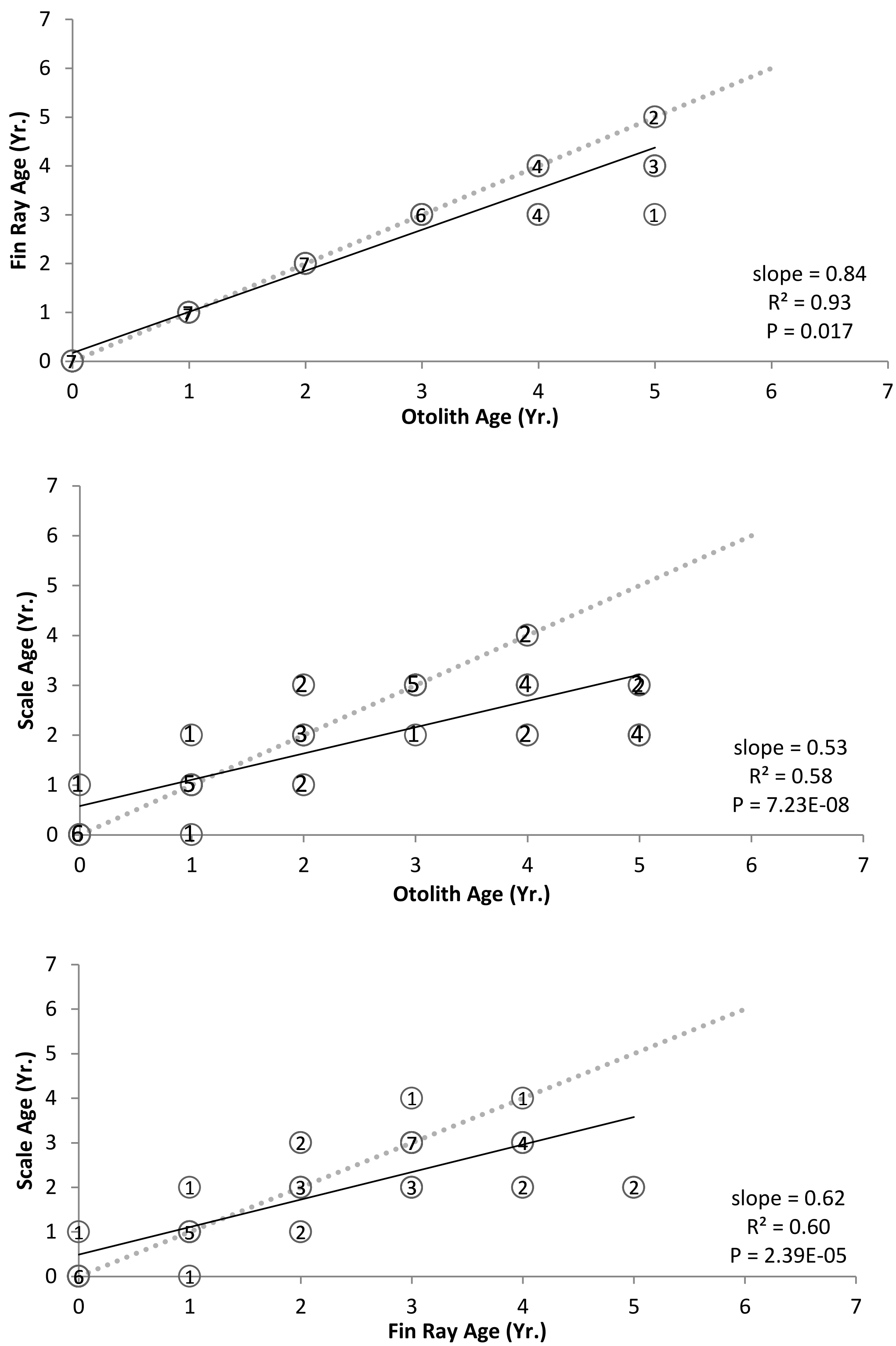

Figure 2:3: Pairwise regression comparisons of otoliths, fin rays and scales from Harris Creek. Solid line is the regression line for the age comparison. Dashed line is a hypothetical 1:1 relationship. P value represents a regression compared to a slope of one. Numbers in data points = n.

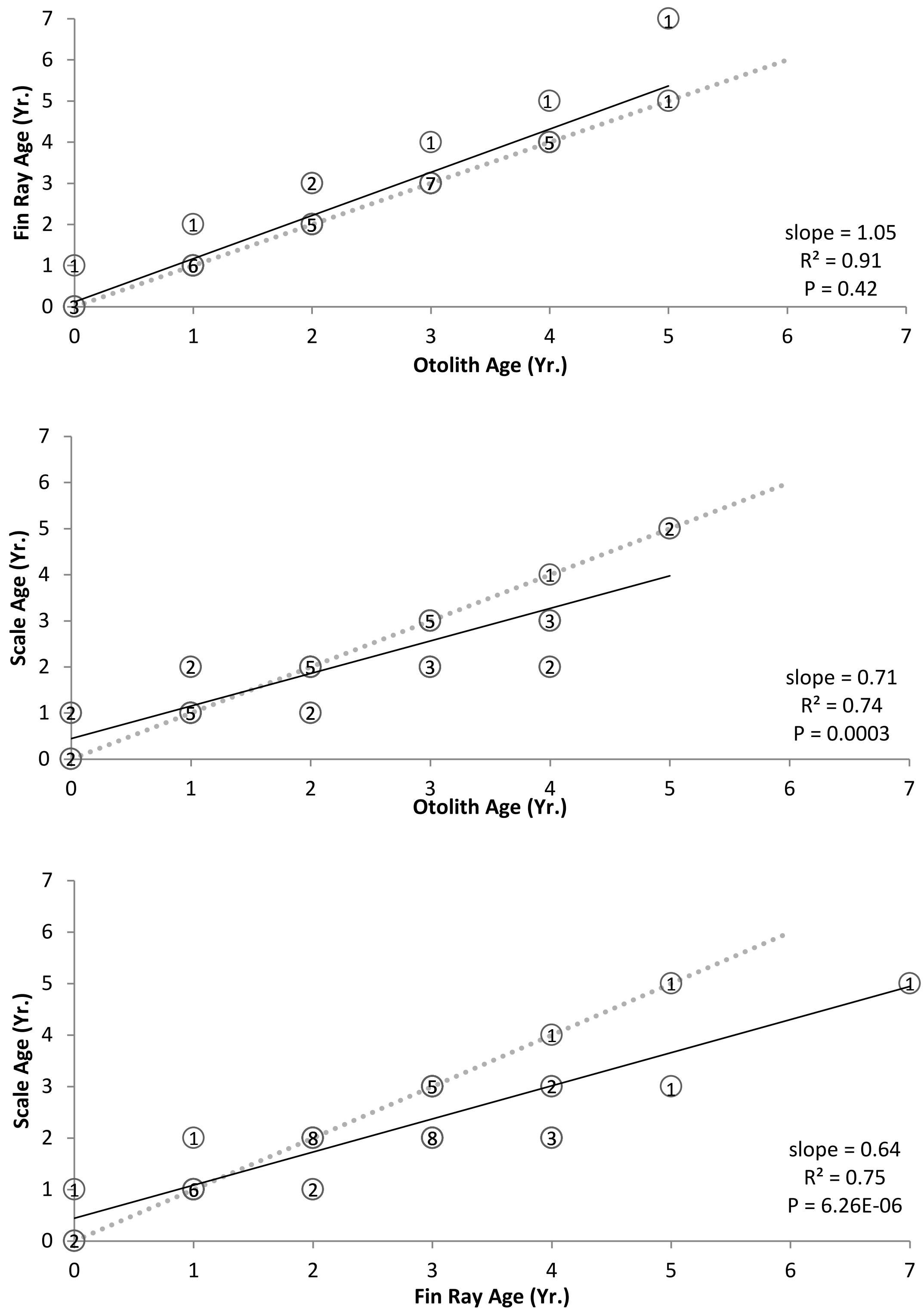

Figure 2:4: Pairwise regression comparisons of otoliths, fin rays and scales from Mores Creek. Solid line is the regression line for the age comparison. Dashed line is a hypothetical 1:1 relationship. P value represents a regression compared to a slope of one. Numbers in data points = n.

CHAPTER 3: A COMPARISON OF ASSIGNED AGE AND PRECISION OF SCALES, PECTORAL FIN RAYS, AND OTOLITHS OF BLUEGILL (*Lepomis macrochirus*)

Abstract

Bluegill are one the most important recreational fishes in North America, and are also sought by anglers in many Idaho waters. We sampled two lentic water bodies in southwestern Idaho, USA, to collect Bluegill of different age classes from young-of-the-year to the oldest age classes and compared age and precision estimates for sagittal otoliths, pectoral fin rays, and scales. Otoliths were found to be the most precise at both water bodies. Scale and fin ray age estimates differed in precision depending on water body. Scale age estimates were more precise at Atwood's Pond while fin ray age estimates were more precise at Bruneau Dunes Pond. Pairwise regression comparisons showed that scale age estimates significantly underestimate the age of fish when compared to that of otoliths, at both locations. There was not a significant difference between the assigned age of otoliths and fin rays at either location. We do not recommend the use of scales or fin rays as the primary aging structure for Bluegill. Although, we did not find assigned ages of otoliths and fin rays to differ, estimates of the latter demonstrated far less precision. The difference in precision concerns us. We suggest a study be undertaken to validate fin rays prior to them being used as a primary ageing structure for Bluegill in Idaho waters.

Introduction

Fish are one of the most aged organisms with well over a million aged worldwide in 1999 (Campana and Thorrold 2001). Demographically, age and growth data are extremely important to fisheries managers for two reasons. First, fish do not have a maximum size at maturity, subsequently their growth is indeterminate. Fish also grow and mature at different rates in different habitats. The second reason is due to the economic and recreational exploitation of many fish stocks. The ability to age a population allows managers to determine production or harvest quotas and assess the effectiveness of management strategies.

Many structures have been used to study the age dynamics of fish populations: vertebrae (Brown and Gruber 1988), opercular bones (Baker and Timmons 1991), cleithra (Casselman 1990), scales (Gerking 1966, Schill et al. 2010), fin rays (Herbst and Marsden 2011), spines (Turner 1980), and otoliths (Hales and Belk 1992; Soupir et al. 1997; Campana and Thorrold 2001; Schill et al. 2010). Finding the structure that yields the best age estimate for the species, and stock, can be a challenge. By far, the two most commonly used of these structures are scales and otoliths (Maceina et al. 2007).

Scales have been shown to produce lower age estimates for most fish, especially in older individuals (Soupir et al. 1997; Campana 2001; Metcalf and Swearer 2005; Schill et al. 2010; Herbst and Marsden 2011). Nonetheless, they are still used on a regular basis as the primary ageing structure for many management agencies in North America in part because they do not require lethal sampling (Maceina et al. 2007). However, use of scales may be justified for Bluegill (*Lepomis macrochirus*), since a few older studies have validated their use (Regier 1962; Gerking 1966).

Otoliths are often shown to be accurate and precise (Casselman 1987), and have been validated for Bluegill in various geographic locations (e.g. Florida, Mantini et al. 1992; South Carolina, Hales and Belk 1992; Idaho, Chapter 1), but require lethal sampling.

This has led to recent interest in fin rays as a non-lethal ageing structure (Koch et al. 2008; Herbst and Marsden 2011). Some researchers have found that rays may be as precise and accurate as otoliths (Mills and Chalanchuk 2004). However, fin rays have a tendency to miss the first year class of some fish if not removed correctly (Metcalf and Swearer 2005). We are unaware of any prior studies evaluating Bluegill fin rays as an ageing structure.

Bluegill are one of the most popular recreational fishes in North America, and are found in 49 states and 6 Canadian providences (Quinn and Paukert 2009). They are also sought after in many Idaho waters. The recreational and economic importance of Bluegill makes it vitally important to correctly manage this species. Bluegill are prone to stunting in smaller sizes (under 150 mm) (Otis et al. 1998; Aday et al. 2002) and over exploitation of the larger fish (over 150 mm) in the same water body (Schneider and Lockwood 1997). This occurs when there is heavy fishing pressure on large Bluegill, while younger age classes have high recruitment, causing a bottleneck of resources available to the smaller fish (Schneider and Lockwood 1997).

Knowing the age distribution of a species experiencing stunting makes it critical to produce quality age estimates (Hall 1991; Hoxmeier et al. 2001). Because a majority of fisheries biologists prefer to sample fish non-lethally for ageing studies, despite the general superiority of otoliths, evaluation of prospective non-lethal ageing structures could promote more ageing work (Maceina et al. 2007). If fin rays or scales provide estimates similar to otoliths previously validated, this would provide a valuable tool in the age tool kit for managers.

Our objectives during this project were: 1) determine if otoliths, pectoral fin rays and scales produce similar age estimate for Bluegill, 2) compare the precision of otoliths, pectoral fin rays and otoliths of Bluegill, and 3) determine if there is a precise, non-lethal ageing structure for Bluegill.

Methods

We sampled two lentic waters, Atwood's Pond and Bruneau Dunes Pond, over a 17 month period. Atwood's Pond is a privately owned pond that is located next to the Payette River in Payette County, Idaho. The pond is a reclaimed gravel pit and is fed by hyporheic water from the Payette River. It has a maximum depth of 4.9 m and covers approximately 9.3 ha. The depth of Atwood's Pond fluctuates with the variation in Payette River flows. Bruneau Dunes Pond is located inside of Bruneau Dunes State Park, in Owyhee County, Idaho. Bruneau Dunes Pond is a manmade structure that is filled by pumping water from the Snake River through the winter months, generally November-March. The mean depth is 4.2 m and covers approximately 15.8 ha. The water level decreases slightly throughout the summer months and then increases throughout the winter months, time of pumping.

We used a Smith-root electrofishing boat for sampling purposes, but also towed a 1 x 2 m floating neuston net of 1mm bar mesh for sampling young-of-the-year fish. Collected fish were sacrificed, returned to the laboratory, and kept frozen until dissection. In the laboratory, each fish was thawed, measured to the nearest millimeter, weighed to the nearest tenth of a gram, and sex and maturity were determined, using the general method described in Downs et al. (1997). We removed scales, sagittal otoliths, and both pectoral fin rays. The fish used in this study were a sub-sample from a larger otolith validation study (Chapter 1). The fish were chosen, non-randomly, based on otolith age from throughout the 17 month sampling period, with a goal of seven fish (arbitrarily set) per age class from each sampling location. For some of the older age classes we were unable to meet our target sample size of seven fish (Table 3:1).

Scales were removed from the right side of the body midway between the dorsal fin and the lateral line (Quist et al. 2013). Scales were then stored in coin envelopes to dry. Pectoral fin rays were removed at the point where the pectoral fin articulates with the pectoral girdle (Koch et al. 2008) and stored in coin envelopes to dry. We also removed both sagittal otoliths with the use of the guillotine method (Secor et al. 1992). Otoliths were cleaned of soft tissue, dried and stored in 1.5 ml centrifuge tubes.

Scales were mounted between two glass microscope slides and digitally imaged at 12-40X depending on the size of the scale, using a Leica DC 500 camera mounted on a Leica DM 400B compound microscope. Scales were aged by two readers, independent of one another, and without knowledge of length, or assigned age of other structures.

The three leading edge fin rays were embedded in Buehler epothin, a clear epoxy, and sectioned with a Buehler low speed Isomet saw. Samples were sectioned serially (0.6 mm thickness 4 sections) starting at the proximal edge, and digitally imaged at 100x magnification. Fin rays were aged by two readers independently, without knowledge of length, or the age assigned to the other structures.

Whole otoliths were placed in a depression slide, sulcal groove down, submerged in water and digitally imaged, using the same equipment as above, at 12-40X magnification, depending on size of otolith. Otoliths were aged by two readers independently, without knowledge of length, or assigned age of other structures.

To determine if there was a difference in the precision of the age estimates between the three structures, we calculated exact percent agreement (PA0), within one year percent agreement (PA1) (Beamish and Fournier 1981), and between reader coefficient of variance (CV) (Chang 1982) for all three structures. We then compiled age bias plots to visually discern the variation between readers (Campana et al. 1995).

Pairwise comparisons using linear regression were also used to determine if assigned age differed significantly between structures. This was accomplished by building age plots and comparing the slope of the regression line to a slope of one using simple regression (Isermann et al. 2003). We used otoliths as our standard for comparison with the other structures; this due to otoliths being shown to be accurate and validated for Bluegill up to age two in our sample sites (Chapter 1) as well as for adults in other geographic locations (Schramm 1989; Mantini et al. 1992).

Results

We found that otoliths had the highest exact percent agreement, within one year agreement and the lowest between reader CV for both locations (Table 3:2). Scales were found to have a slightly higher exact percent agreement and within one year agreement as well as a lower between reader CV when compared to fin rays at Atwood's Pond (Table 3:2). The opposite was true at Bruneau Dunes where fin rays were found to have higher percent agreements and between reader CV than that of scales (Table 3:2). The age bias plots generally corroborated these results. Pectoral fin rays at Atwood's Pond and scales at Bruneau Dunes show considerable more variation than that of otoliths (Figure 3:1 and 3:2).

Pairwise comparison at Atwood's Pond showed that scales produced statistically different age estimates when compared to that of otoliths ($F_{(0.5)1, 41} = 10.56$, $p=0.002$) or fin rays ($F_{(0.5)1, 41} = 44.07$, $p=5.34\times10^{-8}$). Scales showed a trend of producing lower age estimates by age 4 when compared to fin rays, and by age 5 when compared to otoliths. Assigned age estimates did not differ significantly when comparing fin rays and otoliths ($F_{(0.5)1, 41} = 0.20$, $p = 0.66$) (Figure 3:3).

Pairwise comparisons for Bruneau Dunes showed that assigned ages for scales were statistically different than that of otoliths ($F_{(0.5)1, 50} = 5.85$, $p = 0.02$) and fin rays ($F_{(0.5)1, 50} =0.98$, $p =0.03$); with scales producing lower age estimates of fish 7 and older. Otoliths and fin rays did not differ on assigned ages ($F_{(0.5)1, 50} = 0.77$, $p = 0.39$) (Figure 3:4).

Discussion

Our results are in agreement with many other studies which found scales to produce less precise age estimates than that of otoliths for Bluegill (Hoxmeier et al. 2001; Edwards et al. 2005) as well as various other species (Sikstrom 1983; Muir et al. 2008; Zymonas and McMahon 2009; Schill et al. 2010). Further, we found scales to be more precise than fin rays at Atwood's Pond, while the opposite was true at Bruneau Dunes. The difference in precision of fin rays between our sample sites is not unexpected as fin rays have received mixed reviews in regard to both precision and accuracy. These range from being similar in precision to that of otoliths (Sikstrom 1983; Muir et al. 2008;

Zymonas and McMahon 2009) to be considered a poor choice or not forming an annulus each year (Besler 1999; Buckmeier et al. 2012). Additionally, we found fin rays to be considerably more difficult to read, particularly when attempting to discern true versus false annuli (Yosef and Casselman 1995). For example, a fish from Bruneau Dunes (fish 31) from June 2012, was aged at 4 and 5 using otoliths and scales respectively, and aged as 9 years old by both readers using fin rays. Further, some fin rays appeared to have a bi-annulus. During this study only one fish was not aged, this was due to the inability to discern the difference between true and false annuli on the fin rays.

Scales produced a statistically lower age estimate when compared to both otoliths and fin rays in the current study. This lower age estimate appears to become more prevalent as the assigned age reaches and exceeds seven years. Our findings are in agreement with other studies of various species which have shown scales produce lower age estimates of older fish when compared to otoliths (Metcalf and Swearer 2005; Schill et al. 2010; Herbst and Marsden 2011).

We are confident in our age assignments using otoliths; but not so using fin rays or scales. Spiegel et al. (2010) suggested applying a confidence rating to each ageing structure as it is aged. We did not do this, but it would have proven helpful by allowing us to subjectively determine if one structure provided us with more, or less, confidence in our age assignments. One reason for the increased confidence in ageing using otoliths over fin rays and scales may be that both readers had considerably more ageing experience using otoliths than fin rays or scales. This may have led to a bias towards better otolith precision (Figures 3:1 and 3:2). However, we believe the bias to be small as many other studies comparing these structures, on a variety of species, have also found otoliths to be more precise than fin rays and scales (e.g. Largemouth Bass (*Micropterus salmoides*), Maraldo and MacCrimmon 1978; Arctic Grayling (*Thymallus arcticus*), Sikstrom 1983; Catostomids and Cyprinids, Quist et al. 2007; and Dolly Varden (*Salvelinus malma*), Stolarski and Sutton 2013).

Though, we did not validate Bluegill fin rays or scales during this investigation, several prior authors have. Despite our poor results with scales in the present study, Regier (1962) and Gerking (1966) validated scales for this species. Otoliths of fish up to age two have been validated in our sample sites (Chapter 1) as well as other geographic locations (Schramm1989; Mantini et al. 1992). However, we are unaware of any studies that validated fin rays for Bluegill.

Interestingly we observed that the zonation patterns of Bluegill otoliths and fin rays appeared to be reversed. Otoliths had a wide translucent zone which is the growth zone (Chapter 1), while fin rays had a wide opaque zone, presumably the growth zone. Such a shift in the zoning pattern was not observed in the cold-water Redband Trout (Chapter 2). This difference is intriguing and may explain some of the confusion in age estimates. Considerably more research should be undertaken. These results should be confirmed with a chemical mark and recapture study. One possible explanation for this reversal may be that otoliths reside in the endolymphatic fluid, which is heavily controlled by the endocrine system (Kalish 1989; Campana 1999; Alvarez et al. 2008), while the fin rays are supported by the circulatory system. This difference in controlling systems may play a role in the reversal of these zones on the two structures.

Conclusions

Our results showed that the assigned age of fin rays and otoliths did not differ significantly from one another. However, we do not suggest the use of fin rays or scales for the ageing of Bluegill. Though otoliths are lethal, they appear far more precise.

We agree with Spiegel et al. (2010) that assigning a confidence rating to each structure would lead to a better understanding of ageing structures. If future researchers desire to use fin rays to age Bluegill, a study should be undertaken to validate fin rays as forming one annuls per year on Bluegill, as all structures to be used for ageing purposes should be validated (Beamish and McFarlane 1983). We suggest such an effort be completed prior to fin rays being used as a primary ageing structure for Bluegill.

The three structures compared during the course of this investigation have been compared for many different species in various geographic locations (Quist et al 2007; Stolarski and Hartman 2008; Herbst and Marsden 2011; Buckmeier et al. 2012). We found that otoliths are the most precise, and scales produce lower relative age estimates, especially for older fish, which is in agreement with other studies (Soupir et al 1997; Campana 2001). Based on the overall results of this study, we recommend that otoliths be used for ageing Bluegill in South Idaho waters.

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Table 3:1: Sample size per age group for Atwood's Pond and Bruneau Dunes, dashes represent no sample for that age class. Age is based on otolith ages.

Age	Bruneau Dunes	Atwood's Pond
0	7	7
1	7	7
2	7	7
3	7	7
4	7	7
5	3	3
6	7	-
7	2	3
8	1	-
9	3	-
10	1	-

Table 3:2: The exact percent agreement (PA-0), within one year Percent agreement (PA-1), and the between reader coefficient of variance (CV) for Bluegill from Atwood's Pond and Bruneau Dunes Pond. Assigned age is based on the pairwise regression analysis, and different letters represent statical differences in the between structure comparison of assigned age.

Structure	PA-0	PA-1	CV	Assigned Age
Atwood's Pond				
Otoliths	98%	100%	0.5	A
Fin Rays	65%	91%	11.4	A
Scales	67%	95%	8.3	B
Bruneau Dunes				
Otoliths	88%	98%	1.8	A
Fin Rays	60%	92%	6.7	A, B
Scales	58%	92%	13.1	B

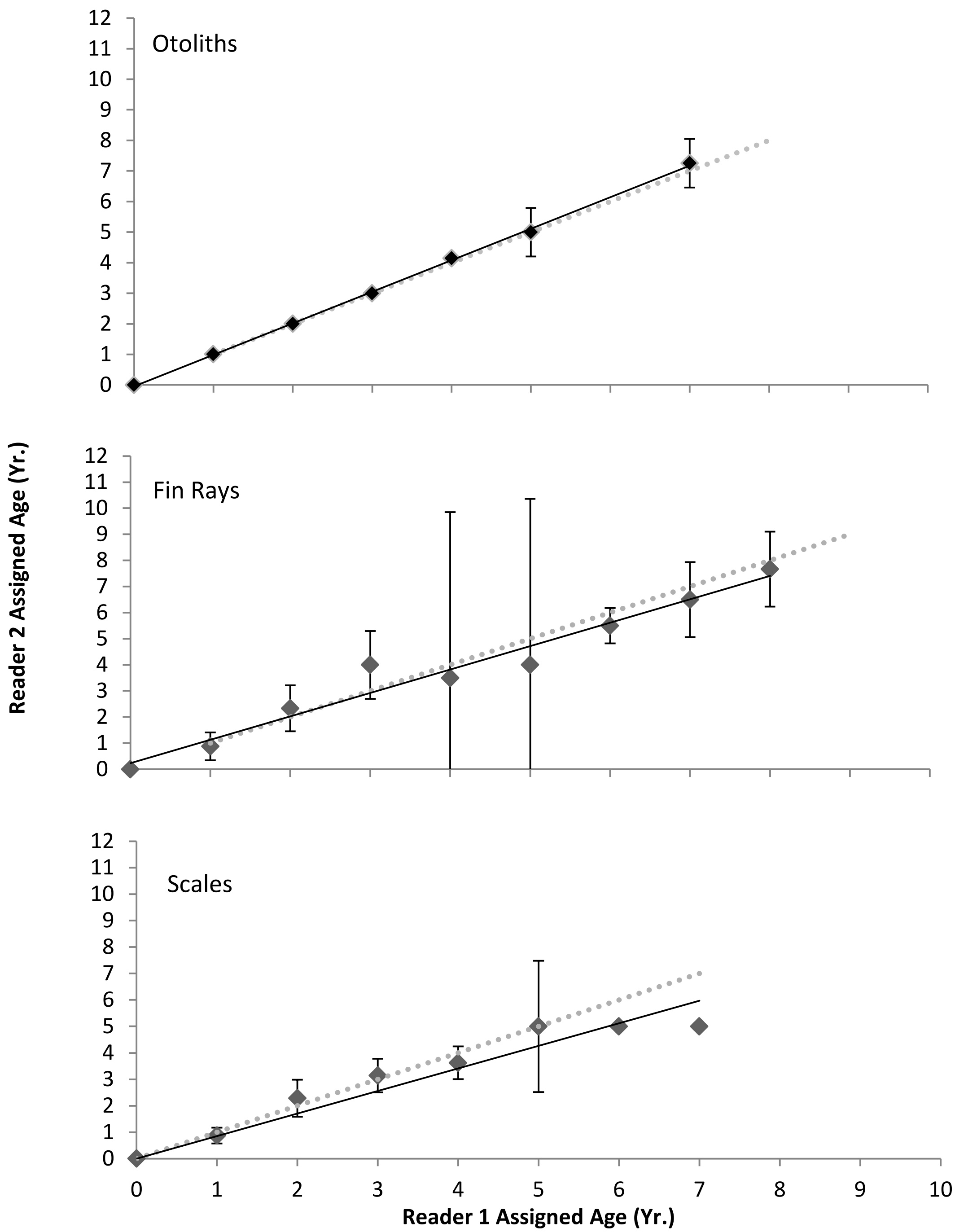

Figure 3:1: Age (years) comparisons for Atwood's Pond Bluegill. These plots compare the ageing precision between two readers for each structure. Diamonds equals means ± (95% CI) between brackets. Dashed line: hypothetical perfect agreement, solid line: actual agreement between readers.

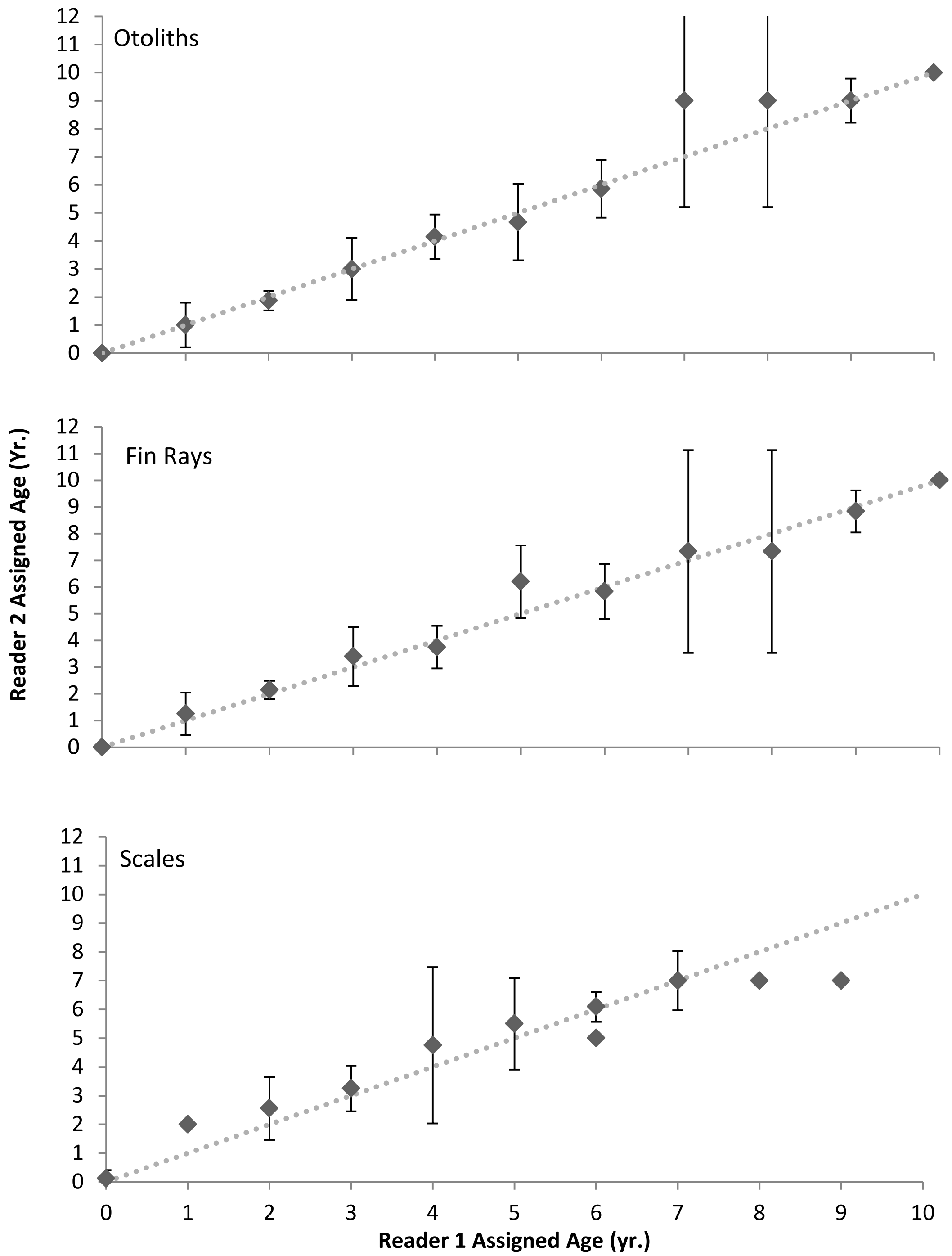

Figure 3:2: Age (years) comparisons for Bruneau Dunes Pond Bluegill. These plots compare the ageing precision between two readers for each structure. Diamonds equals means ± (95% CI) between brackets. Dashed line: hypothetical perfect agreement, solid line: actual agreement between readers.

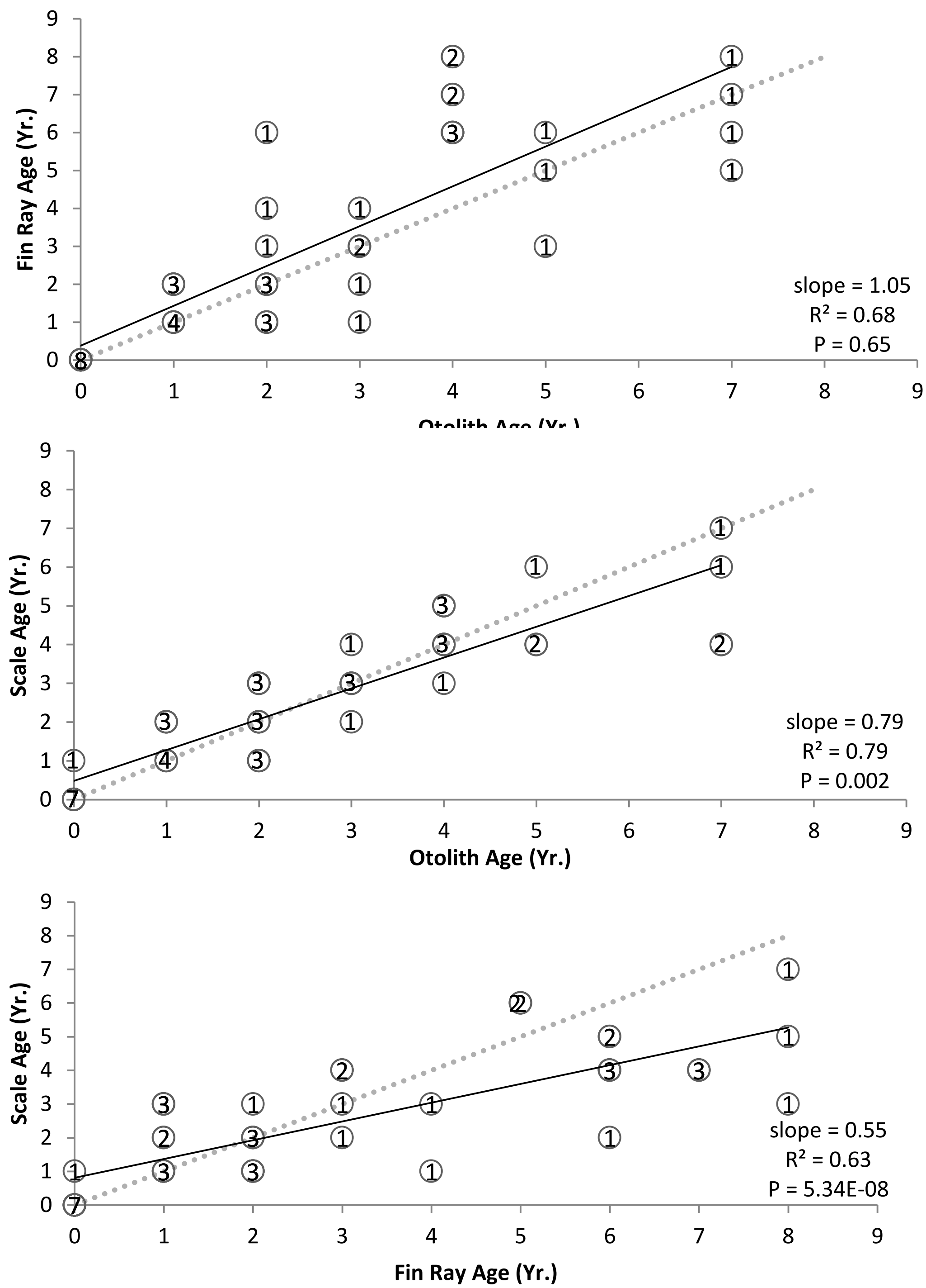

Figure 3:3: Pairwise regression comparisons of otoliths, fin rays and scales from Atwood's Pond. The numbers in the data points are n. Solid line is the regression line for the age comparison. Dashed line is a hypothetical 1:1 relationship. P value represents a regression compared to a slope of one. Numbers in data points = n.

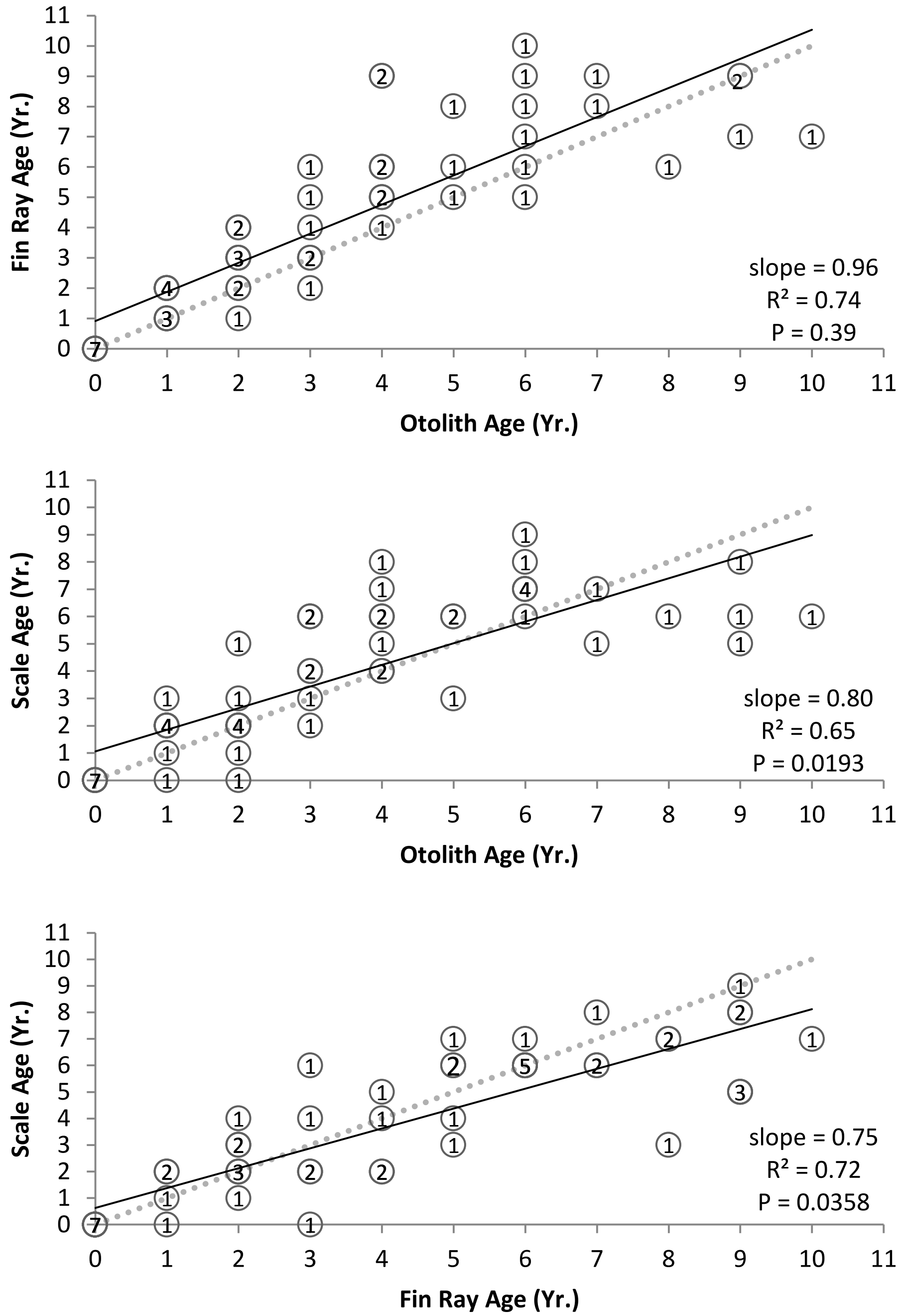

Figure 3:4: Pairwise regression comparisons of otoliths, fin rays and scales from Bruneau Dunes Pond. The numbers in the data points are n. Solid line is the regression line for the age comparison. Dashed line is a hypothetical 1:1 relationship. P value represents a regression compared to a slope of one. Numbers in data points = n.

CONCLUSIONS

In order to correctly age a fish the biologist must understand the zonation of the ageing structure they are using. Given our results we found that when working with montane Redband Trout otoliths the translucent zone is formed during the winter and should be counted as the annulus. Conversely, Bluegill otoliths in our study sites formed the opaque zone during fall-spring and should therefore be counted as the annulus. The zones of the two fishes are indeed reversed. These findings help address some of the confusion surrounding the interpretation of otoltih zonation. The reason there is differences in interpretation of otoltih zonation stems from the fact that the zones are forming at opposite times of the year, when Redband Trout and Bluegill are compared.

We found otoliths to be the most precise for both species in all water bodies. Our finding showed that assigned age of pectoral fin rays were similar to that of otoliths. However, we found fin rays to be less precise for both species in all locations. Though we found fin ray ages to be less precise than that of otoliths, the difference was small. Given this date we feel pectoral fin rays may be an acceptable non-lethal ageing structure for montane Redband trout. However, this is suggested with caution and we highly suggest a study be undertaken to validate fin rays as forming one annulus per year prior to them be used as a primary ageing structure.

We do not suggest the use of pectoral fin rays as an ageing structure for Bluegill due to the high variability we found. Further, we do not suggest the use of scales as an acceptable ageing structure for either species. Scale age estimates were statistically lower than those of otoliths and were also considerably more variable for both species in all our sample sites. Based on our results we feel otoliths are the superior ageing structure when compared to fin rays or scales and highly suggest their use for Bluegill in South Idaho waters.

FUTURE RESEARCH

The mechanisms controlling otoliths zone reversals we showed in the current study should be investigated further. Also the intriguing observation that the zones of fin rays and otoliths in Bluegill appear to be reversed, but Redband Trout do not show this phenomenon need to be corroborated and studied further. Specifically, a study using chemical analysis should be undertaken to determine the difference between the timing and composition of the different zones on species that show a reversal of the otolith zones, e.g. Redband Trout and Bluegill. This chemical mark should include otoliths and fin rays. In addition we suggest future research investigate the mechanisms behind this variation. Specifically, what hormones are controlling the endolymphatic fluid, and is there a difference in what is being expressed during different times of the year between species with otolith zone reversals. We encourage others to follow up with work on various other species in other geographic locations to verify our current findings. Beyond the zonation confusion; we strongly urge that a study be undertaken to validate pectoral fin rays as forming one annulus per year before they are used as the primary ageing structure. This should be undertaken for both Redband Trout and Bluegill.

APPENDIX

Percent of Otoliths That Had an Opaque Edge Starting in June 2011 Through October 2012

Table A1: **Percent of otoliths from Bluegill with an opaque edge starting in September 2011 through October 2012. Dashes represent no sample for that species during that month of sampling. Sample size is generally less than five per month for each species, and all age classes are combined for these calculations.**

	Atwood's Pond				Crane Falls Lake				Bruneau Dunes
	Black Crappie	Largemouth Bass	Pumpkinseed	Warmouth	Black Crappie	Largemouth Bass	Pumpkinseed	Yellow Perch	Largemouth Bass
June	0	-	-	-	-	-	-	-	-
July	0	-	-	-	-	-	-	-	-
August	42	31.25	25	60	-	-	-	-	50
September	100	87.5	100		0	50	100	100	55
October	100	100	-	50	-	50	100	0	90
November	100	100	-	100	100	100	100	100	100
December	-	-	-	-	-	-	-	-	-
January	-	-	-	-	-	-	-	100	100
February	100	100	-	100	100	100	100	100	100
March	100	100	-	100	100	50	-	100	100
April	100	100	-	100	-	50	100	100	57
May	-	40	-	100	-	50	67	0	75
June	0	17	33	0	-	33	50	0	20
July	0	0	0	-	0	0	0	0	0
August	0	0		0	33	0	0	0	17
September	100	43	100	100	100	20	40	0	70
October	100	100		100	0	83	0	50	86

Table A2: Percent of otoliths from Redband Trout with an opaque edge starting in September 2011 through October 2012. Dashes represent no sample for that species during that month of sampling. Sample size is generally less than five per month for each species, and all age classes are combined for these calculations.

	Mores Creek	
	Brook Trout	Sculpin
June	-	-
July	-	-
August	-	-
September	100	14.28571429
October	0	0
November	0	0
December	-	0
January	-	0
February	-	0
March	0	0
April	-	0
May	-	100
June	-	100
July	100	100
August	50	100
September	100	40
October	0	20