COMPARISON OF BIAS AND PRECISION OF FIN RAYS, OTOLITHS, AND SCALES FOR AGEING BROOK TROUT (*SALVELINUS FONTINALIS*), BROWN TROUT (*SALMO TRUTTA*), AND RIO GRANDE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI VIRGINALIS*)

by

Phillip Mark Parsley

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
Master of Science in Biology

Middle Tennessee State University
May 2017

Thesis Committee:

Dr. Sarah E. Bergemann, Chair

Dr. Dennis M. Mullen

Dr. David R. Stewart

ACKNOWLEDGEMENTS

First of all, I would like to thank my advisor, Dr. Sarah E. Bergemann, for her advice throughout this project and the hours spent in revising thesis drafts. I would also like to thank Dr. David R. Stewart for his invaluable advice and assistance with every phase of this project. I would also like to thank Dr. Dennis M. Mullen for his support and encouragement throughout my career as a graduate student.

Thanks to Michael Gatlin (United States Forestry Service, Carson National Forest) for assistance by allowing a graduate student researcher from Middle Tennessee to travel to New Mexico and contribute in this project. It was greatly appreciated. I would also like to extend my thanks to Marlene Mueller who helped estimate the ages of fish. I would like to thank the MTSU Biology department for partial funding through scholarships (Padgett Kelly Research Scholarship, Thomas Hemmerly Graduate Research Support Fund) and grants in support for attending meetings.

Lastly, I want to thank my parents, Mark and Lisa Parsley, for their unconditional love and support throughout my entire graduate career at MTSU. Thank you for encouraging me to pursue my passion and for that I am grateful.

ABSTRACT

The precision, bias, and accuracy of age estimates from scales, pelvic fin rays, and otoliths were compared for Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), and Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*). Calcified structures were collected for each fish and age estimates were obtained by counting the number of annuli by two independent readers. The precision of age estimates was estimated by coefficient of variation (*CV*), and percent agreement (*PA*) between readers. Bias and accuracy were investigated through analysis of age-bias plots and Wilcoxon matched pairs rank tests. In all analyses, age estimates of Brook Trout, Brown Trout and the Rio Grande Cutthroat Trout were most precise using otoliths and fin rays. Typically, age estimates of otoliths and fin rays were more accurate than scales particularly for age classes > 4. Age estimates with scales typically underestimated the age of older fish whereas age estimates of fin rays and otoliths were closer in agreement. The differences of age estimates from fin rays, otoliths, and scales found in this study emphasize the importance of validating calcified structures for these species. Additionally, the accuracy and precision of age estimates from fin rays demonstrate that this method is a viable nonlethal alternative to the lethal sampling required for obtaining otoliths.

TABLE OF CONTENTS

Page

LIST OF TABLES v

LIST OF FIGURES vi

LIST OF APPENDICES vii

1. INTRODUCTION 1

2. MATERIALS AND METHODS 4

2.1. Study area 4

2.2. Sample collection and calcified structure removal 4

2.3. Calcified structure preparation and processing 5

2.4. Data analyses 6

3. RESULTS 9

4. DISCUSSION 11

REFERENCES 14

APPENDICES 18

LIST OF TABLES

Table 1. Age frequency table summarizing pairwise comparisons of age estimates from Brook Trout (*Salvelinus fontinalis*) sagittal otoliths (*n* = 105), pelvic fin rays (*n* = 106), and scales (*n* = 106) 19

Table 2. Age frequency table summarizing pairwise comparisons of age estimates from Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) sagittal otoliths (*n* = 59), pelvic fin rays (*n* = 59), and scales (*n* = 59) 20

Table 3. Age frequency table summarizing pairwise comparisons of age estimates from Brown Trout (*Salmo trutta*) sagittal otoliths (*n* = 99), pelvic fin rays (*n* = 101), and scales (*n* = 101) 21

Table 4. Precision of otoliths, fin rays, and scales determined from age estimates of Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), and Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis)* by two readers 22

LIST OF FIGURES

Figure 1. A representative scale used for ageing 23

Figure 2. A representative fin ray for ageing after sectioning 24

Figure 3. A representative sagittal otolith for ageing after sanding 25

Figure 4. Length frequency histograms of Brook Trout (*Salvelinus fontinalis*) (A), Brown Trout (*Salmo trutta*) (B), and Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) (C) collected from streams in Carson National Forest, New Mexico 26

Figure 5. Mean coefficient of variation (*CV*) estimates for otoliths, fin rays, and scales of each species [Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), and Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*)] 27

Figure 6. Pairwise age-bias plots of between reader age estimates for Brook Trout (*Salvelinus fontinalis*) sagittal otoliths (A), pelvic fin rays (B), and scales (C) 28

Figure 7. Pairwise age-bias plots of between reader age estimates for Brown Trout (*Salmo trutta*) sagittal otoliths (A), pelvic fin rays (B), and scales (C) 29

Figure 8. Pairwise age-bias plots of between reader age estimates for Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) sagittal otoliths (A), pelvic fin rays (B), and scales (C) 30

Figure 9. Age-bias plots comparing mean age estimates of calcified structures of Brook Trout (*Salvelinus fontinalis*) 31

Figure 10. Age-bias plots comparing mean age estimates of calcified structures of Brown Trout (*Salmo trutta*) 32

Figure 11. Age-bias plots comparing mean age estimates of calcified structures of Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) 33

LIST OF APPENDICES

APPENDIX A: TABLES AND FIGURES 19

APPENDIX B: AGE ESTIMATES OF BROOK TROUT (*SALVELINUS FONTINALIS*) 34

APPENDIX C: AGE ESTIMATES OF BROWN TROUT (*SALMO TRUTTA*) 36

APPENDIX D: AGE ESTIMATES OF RIO GRANDE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI VIRGINALIS*) 38

CHAPTER 1. INTRODUCTION

Age and growth studies are often used by fisheries biologists and managers to develop an understanding of population dynamics (DeVries and Frie 1996). Yet obtaining such information often requires lethal collection methods, expensive equipment to process the age samples, and experienced readers to accurately estimate age (DeVries and Frie 1996). Given these sets of challenges, it is a common temptation to acquire sample data using non-lethal methods or rely on outdated sample data from historical efforts to draw inference. Doing so provides data quicker, cheaper, and logistically easier. The inherent risk lies in providing age estimates that may be inaccurate and, in the worst case, inadvertently applied to misinform management plans (Campana 2001; Reeves 2003). Clearly, such outcomes counter the objectives of collecting accurate age data that management programs rely on (Catalano and Bence 2012).

Although many calcified structures have been used to estimate age, the efficacy of each structure is contingent on counting annual growth increments with accuracy, precision, and little bias (Campana et al. 1995; DeVries and Frie 1996). A major problem with studies that rely on nonlethal ageing techniques is that few of these structures can be used to accurately provide an unbiased estimate of the age of a fish (Maceina et al. 2007; Isely and Grabowski 2007). For example, ages obtained using scales often underestimate the age of older fish and slow growing fish as annuli become crowded and difficult to interpret (Hubert et al. 1987; Hining et al. 2000; Závorka et al. 2014). Fin rays can be obtained from fish by non-lethal sampling methods and are often used for ageing fish; however, studies have shown that fin ray samples are difficult to prepare and age estimates are inaccurate when annuli are hard to interpret (Beamish 1973; Hubert et al.

1987; Zymonas and McMahon 2009). Age estimates from otoliths are typically more precise and accurate than scales or fin rays (Kruse et al. 1997; Stolarski and Hartman 2008); however, the use of otoliths requires sacrificing the fish and this may not be feasible when the target species is endangered or threatened (Zymonas and McMahon 2009; Závorka et al. 2014). As a result, the accuracy and precision of obtaining age estimates from various calcified structures and the ease of preparation should be evaluated for each fish species.

In Colorado and New Mexico, both non-native and native salmonid species are managed to support highly popular recreational fisheries (Epifanio 2000; Penaluna et al. 2016). Of these, Brook Trout (*Salvelinus fontanalis*) and Brown Trout (*Salmo trutta*) are two widely distributed non-native trout species. The Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) is a native species and like many other inland cutthroat trout subspecies have experienced severe declines in their native range (Shemai et al. 2007). The Rio Grande Cutthroat Trout occupies about ten percent of its historic range (1 303 of the original 10 718 stream kilometers) and is currently limited in range to the Rio Grande basin of Colorado and New Mexico, including the upper Pecos drainage of New Mexico (Alves et al. 2008; Behnke 2010;). As a result of a decline in range, the Rio Grande Cutthroat Trout was considered a candidate for listing that would result in federal protection (USFWS 2014a). Yet, in 2014, federal listing of the Rio Grande Cutthroat Trout was not warranted (USFWS 2014a). However, given the range decline of Rio Grande Cutthroat Trout populations, it is important for fisheries biologists to collect accurate age data in order to inform sound management and conservation plans (USFWS 2014b). Collectively, the importance of these three trout species from both a conservation and management perspective requires that fisheries biologists understand respective population dynamics and assay trends in age population structure and mortality rates in any studies attempting to quantify mechanisms driving population trajectories.

The goal of the present study was to evaluate the efficacy of using calcified structures collected by non-lethal methods (i.e., scales and pelvic fin rays) as a comparison for ages obtained from a lethal collection method (otoliths) for two non-native (Brook Trout, Brown Trout) and one native trout (Rio Grande Cutthroat Trout), a species of concern in New Mexico. My objectives were to: (1) determine the precision of age estimates obtained from scales, pelvic fin rays, and sagittal otoliths; and (2) evaluate the relative accuracy of scale and fin rays by comparison with age estimates obtained by otoliths.

CHAPTER 2. MATERIALS AND METHODS

2.1. Study area

This study was conducted in Carson National Forest located among the Sangre de Cristo Mountains of the southern Rocky Mountain range in southern Colorado and northern New Mexico. The Carson National Forest encompasses a total area of 607 028 hectares that is managed by the United States Forestry Service as an area for recreation, livestock grazing, and recreational fishing. Elevation ranges from 1 828 meters to over 3 962 meters at its highest peak and (USDA FS Carson NF 2015). In the 643 kilometers of streams within Carson National Forest, there are four species of naturally reproducing trout populations (Brook Trout, Brown Trout, Rainbow Trout, and Rio Grande Cutthroat Trout); the Rio Grande Cutthroat trout are the only native trout species in the region (USDA FS Carson NF 2015).

2.2. Sample collection and calcified structure removal

Brook Trout, Brown Trout, and Rio Grande Cutthroat Trout were sampled from multiple streams in Carson National Forest using backpack electrofishing and hook-and-line sampling methods (Armstrong 1971). Each fish was weighed (total weight; nearest g), and measured (total length (TL); nearest mm). Calcified structures (scales, pelvic fin rays, and sagittal otoliths) were removed and stored in a coin envelope labeled with an identifier. Scales were removed from an area above the lateral line and ventral to the anterior edge of the dorsal fin (Hubert et al. 1987; Zymonas and McMahon 2009). Pelvic fin rays were removed near the point of articulation at the fin base (Shirvell 1981; Erhardt and Scarnecchia 2013). Sagittal otoliths from each sample were collected by exposing the mouth of each fish revealing the ventral surface of the cranium before severing the cranium bone to expose the sagittal otoliths for removal using forceps (Schneidervin and Hubert 1986; Hubert et al. 1987). All ageing structures were stored in labeled coin envelopes and dried at room temperature.

2.3. Calcified structure preparation and processing

Scales were removed from the coin envelope by sectioning the portion of envelope with the scales and transferred to a 0.5 mL microcentrifuge tube filled with 500 µL of laboratory grade 5% aqueous pancreatin (Carolina Biological Supply) (Whaley 1991). Scales were immersed in the pancreatin solution for 48 hours to dissolve mucus and slime to clean the surface of the scale revealing clearer circuli (growth rings of scales) (Whaley 1991). After 48 hours, the scales were removed from the solution and examined under a dissecting scope. Three non-regenerated scales were chosen for ageing each sample. From these, one representative scale that yielded the highest age or contained the most definitive set of circuli was chosen for age estimation (Ericksen 1999). Each representative scale was mounted to a glass slide with superglue and covered using a glass coverslip before obtaining age estimates using a compound microscope at 50× magnification (Ericksen 1999) (Figure 1).

Pelvic fin rays were cleaned, trimmed, and fixed in an epoxy resin in 2.0 mL flat-top microcentrifuge tubes (Koch and Quist 2007). The cap of each microcentrifuge tube was detached, filled with non-drying modelling clay, and the tapered end of each tube was removed (Koch and Quist 2007). The distal edge of each fin ray was mounted in the modelling clay perpendicular to the cap and the detached hollow tube was placed over the structure and filled with a 1:1 solution of West System epoxy resin and hardener (Crumpton et al. 2012). The epoxy mixture was cured overnight (10-12 h), after which fin rays were dislodged from the tube using a hammer and a small wooden dowel. Cross-sections were cut perpendicular to the length of the fin rays using a scroll saw (Erhardt and Scarnecchia 2013). Sectioned fin rays were sanded with 220 grit sandpaper and finely polished by sanding with 1500 grit sandpaper. Fin rays were mounted on a glass microscope slide with superglue gel and examined at 50-200× using a compound microscope (Erhardt and Scarnecchia 2013) (Figure 2). The larger, ventral hemisegments of the fin rays were used for age estimation due to the presence of more defined annuli (Zymonas and McMahon 2009; Erhardt and Scarnecchia 2013).

Otoliths were cleaned by gently scraping with forceps and water to remove any dried tissue, placed sulcus side down on a clear glass slide, covered with superglue, sanded with 220-400 grit sandpaper to the sagittal midplane, and finely polished with 1500 grit sandpaper to expose the annuli. Age estimates were obtained using a dissecting scope at 40-50× magnification (Secor et al. 1996; Hining et al. 2000) (Figure 3).

2.4. Data analyses

All calcified structures were aged once by two independent readers with no prior knowledge of length, weight, or age estimates by the other reader. Precision for each structure was estimated using percent agreement (*PA*) and the coefficient of variation (*CV*). Percent agreement was estimated by the percentage of matching age estimates between Reader 1 and Reader 2. The coefficient of variation (*CV*) was estimated using the following equation provided by Campana et al. (1995):

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

where: X_{ij} = ith age determination of the jth fish

X_j = mean age of the jth fish

R = number of times each fish is aged

Differences in *CV* estimates were tested using a one-way analysis of variance (ANOVA) model. Variances between groups were checked prior to all analyses using a Bartlett test (Bartlett 1937) and transformed when necessary to meet the assumptions of homogeneity. If variances still remained heterogeneous after transformation, a nonparametric Kruskal-Wallis test was used. If significant differences were detected by the ANOVA or Kruskal-Wallis test, Tukey-Kramer (ANOVA) or Dunn's (Kruskal-Wallis) post-hoc tests were performed to test for differences in *CV* estimates.

To assess bias between readers, age-bias plots were constructed following the methods described by Campana et al. (1995). For each species, the age estimates of fin rays, otoliths, and scales of one reader were plotted against the corresponding age estimates by the second reader. Ninety-five percent confidence intervals were estimated for each age class and used to assess whether age estimates deviated from a 1:1 equivalence line (i.e. a linear function that results from 100% agreement of age estimates between readers) Campana et al. 1995). Bias is determined by significant departure from the equivalence line by the estimated 95% confidence intervals for each age class and aids in interpretation of systematic differences between reader age estimates (Campana et al. 1995).

Age-bias plots were also constructed to determine whether bias was evident in the mean age estimates comparisons of otoliths, fin rays, and scales (Campana et al. 1995). For each comparison, a least squares regression line was estimated and bias was determined if the slope (b_1) of the regression line was different from one and if the intercept (b_0) was different from zero (Campana et al. 1995). Additionally, Wilcoxon matched pairs rank tests were performed to test for differences in mean age estimates of different structures (Campana et al. 1995). An initial alpha (α) level of 0.05 was chosen for all statistical tests. Because multiple Wilcoxon tests were conducted, a Bonferroni adjustment was used to determine statistical significance for this set of results. The Bonferroni procedure accounts for type-I error by dividing the initial α (0.05) by k (the number of comparisons) and differences were not considered significant unless the P-value from the Wilcoxon tests were less than the Bonferroni-adjusted α level (Quist et al. 2007). All statistical tests were performed in R Statistical Package (R Core Team 2015).

CHAPTER 3. RESULTS

Ages were estimated from scales, fin rays, and otoliths for 106 Brook Trout (72-231 mm TL; Figure 4), 101 Brown Trout (123-307 mm TL; Figure 4), and 59 Rio Grande Cutthroat Trout (57-294 mm TL; Figure 4). The range of estimated ages was similar across species: 1-7 years for Brook Trout and Rio Grande Cutthroat Trout (Table 1 and 2), and 1-8 years for Brown Trout (Table 3).

Age estimates of otoliths and fin rays were the most precise calcified structures while scales provided substantially lower percent agreement and *CV* estimates for all species. For example, the age estimates of Brown Trout were in complete agreement between readers for 72% of otoliths ($CV = 5.10$) and 79% of fin rays ($CV = 3.62$) (Table 4). Brook Trout age estimates were in complete agreement for 77% of the otoliths ($CV = 6.11$) and 78% of fin rays ($CV = 5.69$) (Table 4). From Rio Grande Cutthroat Trout, age estimates were in complete agreement for 88% of fin rays ($CV = 2.02$) and 82% of otoliths ($PA = 82\%$, $CV = 4.04$) (Table 4). Scales provided the least precise age estimates for each species, indicated by the lowest percent agreement and highest *CV* estimates (Brook Trout: $CV = 10.23$, $PA = 62\%$; Brown Trout: $CV = 7.98$, $PA = 60\%$; Rio Grande Cutthroat Trout: $CV = 11.98$, $PA = 56\%$) (Table 4).

Coefficient of variation estimates did not differ for structures between species (One-way ANOVA, $F_{2,792} = 2.03$, $P \geq 0.05$), meaning *CV* estimates for scales, otoliths, and fin rays were similar across all species. However, *CV* estimates of calcified structures within species were different (Kruskal-Wallis, $H = 36.94$, $P \leq 0.05$) (Table 4, Figure 5). A Dunn's posthoc analysis revealed that mean *CV* estimates were similar for otoliths and fin rays ($P > 0.05$), but *CV* estimates of scales differed from otoliths and fin rays ($P \leq 0.05$) (Figure 5). Variability of age estimates between readers, as indicated by CV estimates, was highest for scales of each species, while fin rays and otoliths produced considerably less variation.

Age-bias plots of between reader age estimates for otoliths, fin rays and scales showed little evidence of bias based on minimal deviance of age estimates by the two readers (Figure 6, 7, and 8). Typically, where differences in reader age estimates occurred, Reader 1 assigned older ages by 1 year on average than Reader 2. This was particularly evident for scales but was not as apparent for fin rays and otoliths of any species. Regardless of structure, as age increased the variation between reader age estimates also increased.

Analysis of age-bias plots of structure comparisons did not indicate consistent differences in the mean age estimates for Brook Trout, Brown Trout, and Rio Grande Cutthroat Trout otoliths and fin rays (Figures 9, 10, and 11). Differences in ages were small (≤ 1 years of age) for each age class using these structures. Significant deviation was apparent in age-bias plots of scales paired with otoliths and fin rays (Figure 9, 10, and 11). Age estimates of scales were typically underestimated by 1 or 2 years for fish ≥ 3 years of age and large differences (> 3) occurred when assigning ages to fish > 5 years of age (Figures 9, 10, and 11). While analyses of age-bias plots revealed considerable bias between structures, results of the multiple comparison tests revealed a few instances where mean ages were different. Mean ages of otoliths differed from fin rays ($V = 249.5$, $P < 0.5$) and scales ($V = 590.0$, $P < 0.05$) for Rio Grande Cutthroat Trout. All other comparisons of mean age estimates between structures did not differ.

CHAPTER 4. DISCUSSION

In this study, age estimates were obtained from Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), and Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) by counting the annuli of scales, pelvic fin rays, and sagittal otoliths by two readers. This is the first study to investigate age estimates using calcified structures of Rio Grande Cutthroat Trout, a species of concern due to the decline of this species throughout its native range (Alves et al. 2008; Behnke 2010). Collectively, the results obtained here indicate sagittal otoliths and pelvic fin rays consistently provided age estimates that were more precise, more accurate, and less biased than age estimates obtained from scales for all species investigated.

Fin rays and otoliths consistently provided higher precision and accuracy of age estimates for Brook Trout, Brown Trout, and Rio Grande Cutthroat Trout. These results support previous studies that found fin rays and otoliths provided accurate and precise age estimates for Brown Trout and other salmonid species (Burnet 1969; Mills and Beamish 1981; Shirvell 1981; Sikstrom 1983; Chilton and Bilton 1986; Erhardt and Scarnecchia 2013). Based on previous research, a calcified structure with a *CV* estimate below 7.6 is considered acceptable for producing reliable age estimates (Campana 2001). All *CV* estimates of fin rays and otoliths in this study were lower than 7.6, and therefore suitable to use in ageing studies for these populations.

Age estimates based on scales led to underestimation of the age for Brook Trout, Brown Trout, and Rio Grande Cutthroat in higher age classes. These results are consistent with previous studies investigating scale age estimates of other salmonid species that showed the age estimates were typically inconsistent for cohorts >3 years of age (Silkstrom 1983; Downs et al. 1997; Kruse et al. 1997; Hining et al. 2002; Stolarski and Hartman 2008; Zymonas and McMahon 2009). In addition to scales being less accurate, higher *CV* estimates of scales for each species indicated that there is greater variability between readers as compared to otoliths or fin rays.

Although otoliths may offer precise and accurate age estimates relative to other structures, the lethal means required for sampling poses a problem for species of recreational value and species of conservation concern (Kruse et al. 1997, Zymonas and McMahon 2009). The Rio Grande Cutthroat Trout has been heavily impacted with a reduction of their native range due to anthropogenic land alterations, and competition and hybridization with nonnative salmonids (Quist and Hubert 2004; Behnke 2010). Additionally, the Rio Grande Cutthroat Trout are exploited for angling opportunities and are managed with the goal of providing a recreational fishery without contributing to further population declines (Rinne 1995). Similarly, managers typically avoid lethal sampling of Brook Trout and Brown Trout because of their popularity as a recreational species (Maceina et al. 2007). These management strategies highlight the need to provide accurate and unbiased age estimates without sacrificing fish.

The ability to collect accurate and unbiased age data is critical for effective management of fisheries resources (Isely and Grabowski 2007). Age data is often used to estimate parameters such as growth, mortality, and recruitment (Beamish and McFarlane 1983), and bias and inaccuracy in age estimation can lead to misguided management strategies (Buckmeier 2002). In short-lived species, such as trout, error in age estimates by one year can lead to unacceptable determinations of age class distributions and population parameters (Campana 2001). Further complicating matters is that fishery managers historically did not use accurate age data, and if any age data was collected it provided only minimal background information for the species and was accepted with an unknown degree of error (Buckmeier 2002). For species such as those in the present study, disregard for acquiring accurate age data could lead to serious errors in management approaches (Beamish and McFarlane 1983).

Where previous management policies were formulated from historical age data or a lack of age information entirely, this study provides supporting data that accurate and precise age estimates of Brook Trout, Brown Trout, and Rio Grande Cutthroat Trout can be obtained by non-lethal sampling of fin rays. This information affords fishery managers the ability to collect current and accurate age information for assessment of important population indices that lead to better informed management decisions without sacrificing fish. While the results of this study demonstrate the utility of fin rays as a non-lethal alternative to otoliths for ageing Brook Trout, Brown Trout, and Rio Grande Cutthroat, the differences in age estimates from the three structures in this study further emphasize the importance of validating structures to provide accurate age estimates.

REFERENCES

Alves, J.E., Patten, K.A., Brauch, D.E. and Jones, P.M., 2008. Rangewide status of Rio Grande cutthroat trout (*Oncorhynchus clarki virginalis*): 2008. Rio Grande Cutthroat Trout Conservation Team Report. Colorado Division of Wildlife, Fort Collins, Colorado.

Armstrong, R.H., 1971. Age, food, and migration of sea-run cutthroat trout, *Salmo clarki*, at Eva Lake, Southeastern Alaska. Transactions of the American Fisheries Society, 100:302-306.

Bartlett, M.S., 1937. Properties of sufficiency and statistical tests, Proceedings of the Royal Society of London A, 160:268-282.

Beamish, R.J., 1973. Determination of age and growth of populations of the white sucker (*Catostomus commersoni*) exhibiting a wide range in size at maturity. Journal of the Fisheries Research Board of Canada, 30:607–616.

Beamish, R. J., and McFarlane, G.A., 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society, 112:735-743.

Behnke, R., 2010. Trout and Salmon of North America. Simon and Schuster.

Buckmeier, D.L., 2002. Assessment of reader accuracy and recommendations to reduce subjectivity in age estimation. Fisheries, 27:10-14.

Burnet, A.M.R., 1969. An examination of the use of scales and fin rays for age determination of brown trout (*Salmo trutta* L.). New Zealand Journal of Marine and Freshwater Research, 3:147-151.

Campana, S.E., Ann, M.C., and McMillan, J.I., 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society, 124:131-138.

Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59:197-242.

Catalano, M.J., and Bence, J.R., 2012. The sensitivity to assumed ageing error of the stock assessment used to recommend lake whitefish yield for 2008 from management unit WFH01 of Lake Huron. QFC Technical Report 2011–01, Quantitative Fisheries Center, Department of Fisheries and Wildlife, Michigan State University, Michigan.

Chilton, D.E., and Bilton, H.T., 1986. New method for ageing Chinook Salmon (*Oncorhynchus tshawytscha*) using dorsal fin rays, and evidence of its validity. Canadian Journal of Fisheries and Aquatic Sciences, 43:1588-1594.

Crumpton, R.L., Henne, J.P., and Ware, K.M., 2012. Marking otoliths and fin spines of juvenile shortnose sturgeon with oxytetracycline and the effects of water temperature during treatment. North American Journal of Fisheries Management, 32:523-527.

DeVries, D.R., and Frie, R.V., 1996. Determination of age and growth. Pages 483-512 *in* Murphy, B.R. and Willis, D.W. (editors), Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Downs, C.C., White, R.G., and Shepard, B.B., 1997. Age at sexual maturity, sex ratio, fecundity, and longevity of isolated headwater populations of westslope cutthroat trout. North American Journal of Fisheries Management, 17:85-92.

Epifanio, J., 2000. The status of coldwater fishery management in the United States: an overview of state programs. Fisheries, 25:13-27.

Erhardt, J.M., and Scarnecchia, D.L., 2013. Precision and accuracy of age and growth estimates based on fin rays, scales, and mark-recapture information for migratory Bull Trout. Northwest Science, 87:307-316.

Ericksen, R.P., 1999. Scale aging manual for coastal cutthroat trout from Southeast Alaska (No. 99). Alaska Department of Fish and Game, Division of Sport Fish.

Hining, K.J., West, J.L., Kulp, M.A., and Neubauer, A.D., 2000. Validation of scales and otoliths for estimating age of rainbow trout from southern Appalachian streams. North American Journal of Fisheries Management, 20:978-985.

Hubert, W.A., Baxter, G.T., and Harrington, M., 1987. Comparison of age determinations based on scales, otoliths and fin rays for cutthroat trout from Yellowstone Lake. Northwest Science, 61:32-36.

Isely, J.J., and Grabowski, T.B., 2007. Age and growth. Pages 187-228 in Guy, C.S. and Brown, M.L. (editors), Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda, Maryland.

Koch, J.D., and Quist, M.C., 2007. A technique for preparing fin rays and spines for age and growth analysis. North American Journal of Fisheries Management, 27:782-784.

Kruse, C.G., Hubert, W.A. and Rahel, F.J., 1997. Using otoliths and scales to describe age and growth of Yellowstone cutthroat trout in a high-elevation stream system, Wyoming. Northwest Science, 71:30-38.

Maceina, M.J., Boxrucker, J., Buckmeier, D.L., Gangl, R.S., Lucchesi, D.O., Isermann, D.A., Jackson, J.R., and Martinez, P.J., 2007. Current status and review of freshwater fish aging procedures used by state and provincial fisheries agencies with recommendations for future directions. Fisheries, 32:329-340.

Penaluna, B.E., Abadía-Cardoso, A., Dunham, J.B., García-Dé León, F.J., Gresswell, R.E., Luna, A.R., Taylor, E.B., Shepard, B.B., Al-Chokhachy, R., Muhlfeld, C.C. and Bestgen, K.R., Rogers, K., Escalante, M.A., Keeley, E.R., Temple, G.M., Williams, J.E., Matthews, K.R., Pierce, R., Mayden, R.L., Kovach, R.P., Garza, J.C., and Fausch, K.D., 2016. Conservation of native Pacific trout diversity in western North America. Fisheries, 41:286-300.

Quist, M.C. and Hubert, W.A., 2004. Bioinvasive species and the preservation of cutthroat trout in the western United States: ecological, social, and economic issues. Environmental Science & Policy, 7:303-313.

Quist, M.C., Jackson, Z.J., Bower, M.R., and Hubert, W.A., 2007. Precision of hard structures used to estimate age of riverine catostomids and cyprinids in the upper Colorado River basin. North American Journal of Fisheries Management, 27:643-649.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reeves, S.A., 2003. A simulation study of the implications of age-reading errors for stock assessment and management advice. ICES Journal of Marine Science: Journal du Conseil, 60:314-328.

Rinne, J.N., 1995. Rio Grande cutthroat trout. Pages 24-27 *in* M.K. Young (editor), Conservation assessment for inland cutthroat trout. General Technical Report RM-256. USDA Forest Service, Fort Collins, Colorado, USA.

Schneidervin, R.W., and Hubert W.A., 1986. A rapid technique for otolith removal from salmonids and catostomids. North American Journal of Fisheries Management, 6:287-287.

Secor, D.H., Dean, J.M., and Laban, E.H., 1996. Manual for otolith removal and preparation for microstructural examination. Technical Publication No. 1991–01. Electric Power Research Institute and Belle W. Baruch Institute for Marine Biology and Coastal Research, Columbia, South Carolina.

Shemai, B., Sallenave, R., and Cowley, D.E., 2007. Competition between hatchery-raised Rio Grande cutthroat trout and wild brown trout. North American Journal of Fisheries Management, 27:315-325.

Sikstrom, C.B., 1983. Otolith, pectoral fin ray, and scale age determinations for Arctic grayling. The Progressive Fish-Culturist, 45:220–223.

Shirvell, C.S., 1981. Validity of fin- ray ageing for brown trout. Journal of Fish Biology, 18:377-383.

Stolarski, J.T., and Hartman, K.J., 2008. An evaluation of the precision of fin ray, otolith, and scale age determinations for brook trout. North American Journal of Fisheries Management, 28:1790-1795.

USDA FS Carson NF (U.S. Department of Agriculture - Forest Service, Carson National Forest). 2015. Assessment Report of Ecological, Social, and Economic Conditions, Trends, and Sustainability: Carson National Forest. Taos, New Mexico.

USFWS (U.S. Fish and Wildlife Service). 2014a. Endangered and threatened wildlife and plants; 12-month finding on a petition to List Rio Grande Cutthroat Trout as an endangered or threatened species. Federal Register 79:59140–59141.

USFWS (U.S. Fish and Wildlife Service). 2014b. Species status assessment report for the Rio Grande cutthroat trout. Albuquerque, New Mexico.

Whaley, R.A., 1991. An improved technique for cleaning fish scales. North American Journal of Fisheries Management, 11:234-236.

Závorka, L., Slavík, O., and Horký, P., 2014. Validation of scale-reading estimates of age and growth in a brown trout *Salmo trutta* population. Biologia, 69:691-695.

Zymonas, N.D., and McMahon, T.E., 2009. Comparison of pelvic fin rays, scales and otoliths for estimating age and growth of bull trout, *Salvelinus confluentus*. Fisheries Management and Ecology, 16:155-164.

APPENDICES

APPENDIX A

TABLES AND FIGURES

Table 1.
Age frequency table summarizing pairwise comparisons of age estimates from Brook Trout (*Salvelinus fontinalis*) sagittal otoliths (n = 105), pelvic fin rays (n = 106), and scales (n = 106). Data are the frequency of fish assigned each age by readers.

Age (years)									
	Reader 1								
	1	2	3	4	5	6	7	8	Total
Brook Trout Otolith Age Estimates									
Reader 2									
1	10								10
2		33	3	2					38
3		4	26	2	2				34
4			4	10	2				16
5		1		1	1	1	1		6
6					1				1
7							1		1
8									
Brook Trout Pelvic Fin Ray Age Estimates									
1	8								8
2	2	49	4						55
3		9	17	2					28
4			2	4	3				9
5					1	1			2
6						3			3
7							1		1
8									
Brook Trout Scale Age Estimates									
1	6								6
2	2	41	8	1					52
3		14	12	6		1			32
4			6	6	2	1			15
5				6	1				7
6									
7									
8									

Table 2.
Age frequency table summarizing pairwise comparisons of age estimates from Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) sagittal otoliths (n = 59), pelvic fin rays (n = 59), and scales (n = 59). Data are the frequency of fish assigned each age by readers.

Age (years)									
	Reader 1								
	1	2	3	4	5	6	7	8	Total
Rio Grande Cutthroat Trout Otolith Age Estimates									
Reader 2									
1	1								1
2		19	1						20
3		4	15						19
4			1	6					7
5				1	3				4
6				1		3	3		7
7							1		1
8									
Rio Grande Cutthroat Trout Pelvic Fin Ray Age Estimates									
1	1								1
2		28	1						29
3			12	1					13
4			1	5					6
5				1	3				4
6					1	2	2		5
7							1		1
8									
Rio Grande Cutthroat Trout Scale Age Estimates									
1	1	1							2
2		17	2	1					20
3		13	12	4	1				30
4			2	3	2				7
5									
6									
7									
8									

Table 3.
Age frequency table summarizing pairwise comparisons of age estimates from Brown Trout (*Salmo trutta*) sagittal otoliths (n = 99), pelvic fin rays (n = 101), and scales (n = 101). Data are the frequency of fish assigned each age by readers.

Age (years)									
	Reader 1								
	1	2	3	4	5	6	7	8	Total
Brown Trout Otolith Age Estimates									
Reader 2									
1									
2		16	2						18
3		2	15	3					20
4			6	21	4				31
5				4	12	4			20
6						7	3		10
7									
8									
Brown Trout Pelvic Fin Ray Age Estimates									
1									
2		18	1						19
3			18	2					20
4		1	1	21	2				25
5				3	16	7			26
6				1	1	5	1		8
7							2	1	3
8									
Brown Trout Scale Age Estimates									
1									
2		12	1						13
3		6	21	7	2				36
4			5	20	7	1			33
5				6	8	3			17
6					1				1
7						1			1
8									

Table 4.
Precision of otoliths, fin rays and scales determined from age estimates of Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), and Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis)* by two readers. Precision measures are average coefficient of variation ($CV = 100 \times SD$/mean), average percent error (*PE*), and percent agreement (%).

Structure	*n*	Average *CV*	Average *PE*	Percent agreement (%)
		Brook Trout		
Otoliths	105	6.11	4.32	77
Fin Rays	106	5.69	4.02	78
Scales	106	10.23	7.24	62
		Brown Trout		
Otoliths	99	5.10	3.61	72
Fin Rays	101	3.62	2.56	79
Scales	101	7.98	5.64	60
		Rio Grande Cutthroat Trout		
Otoliths	59	4.04	2.86	81
Fin Rays	59	2.02	1.43	88
Scales	59	11.98	8.47	56

Figure 1. A representative scale used for ageing.

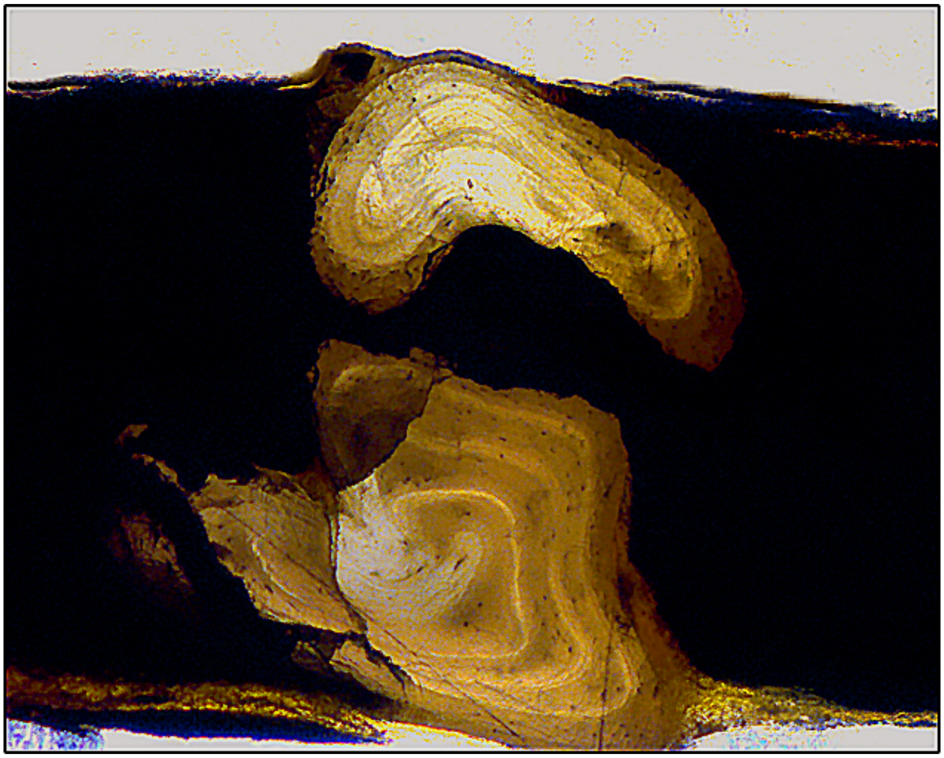

Figure 2. A representative fin ray used for ageing after sectioning.

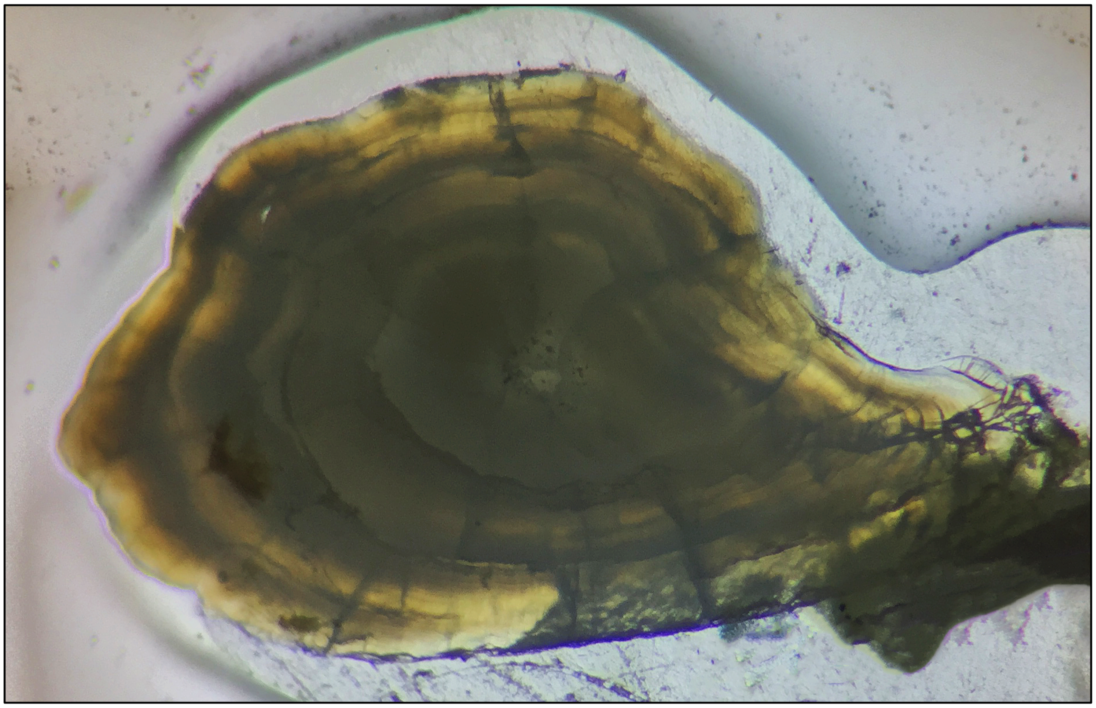

Figure 3. A representative sagittal otolith for ageing after sanding.

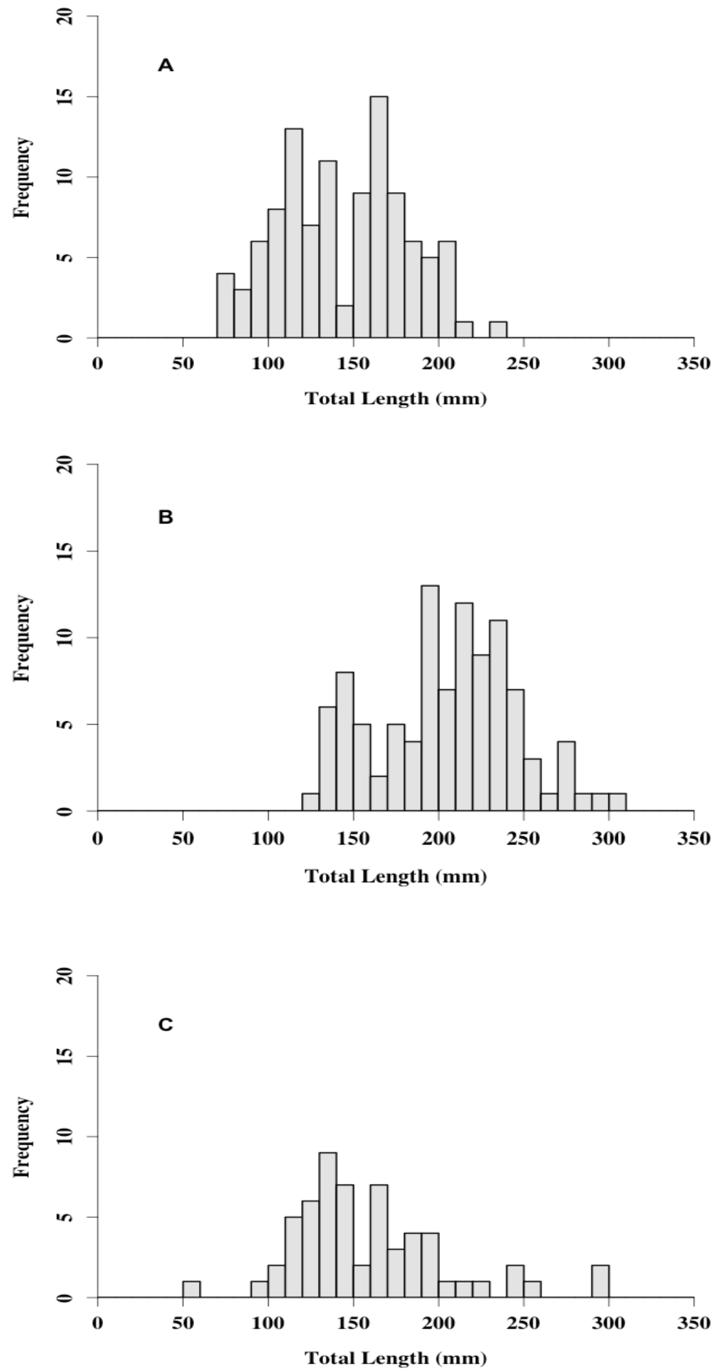

Figure 4. Length frequency histograms of Brook Trout (*Salvelinus fontinalis*) (A), Brown Trout (*Salmo trutta*) (B), and Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) (C) collected from streams in Carson National Forest, New Mexico.

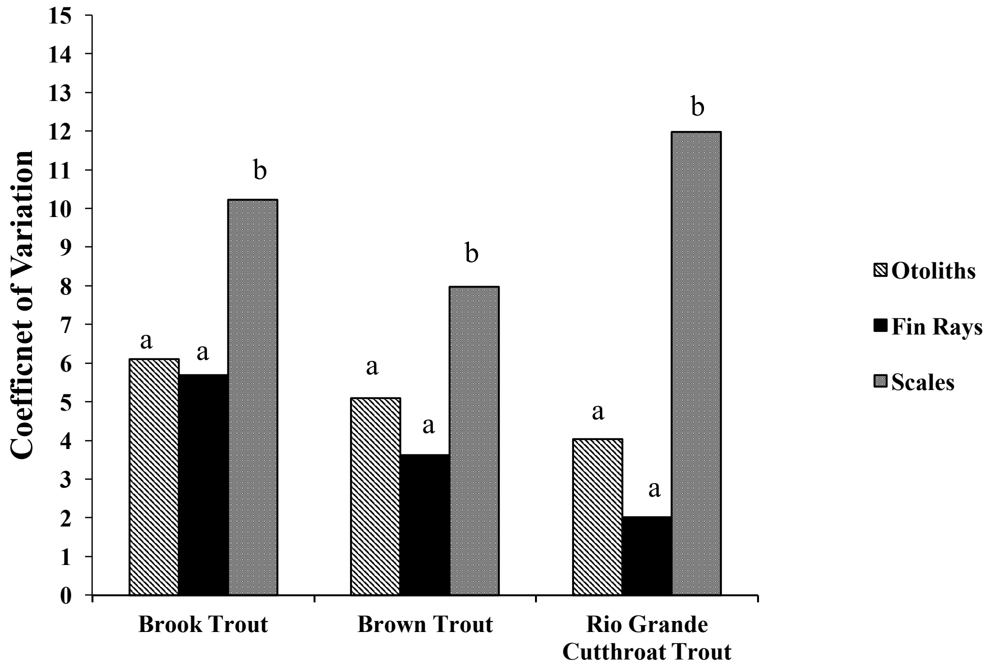

Figure 5. Mean coefficient of variation (*CV*) estimates for otoliths, fin rays, and scales of each species [Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), and Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*)]. Different letters above columns representing *CV* estimates indicate differences in mean *CV* estimates between structures for each species.

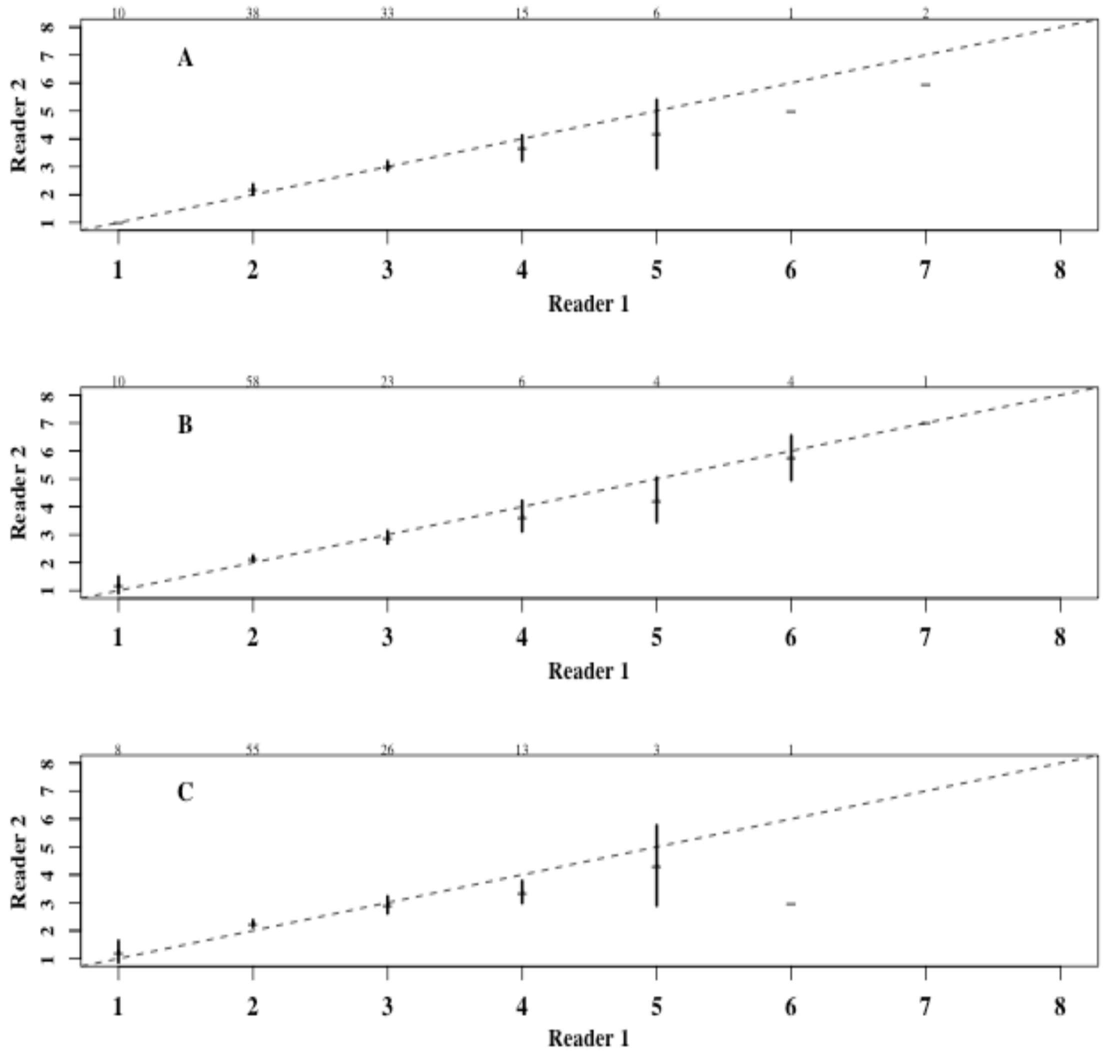

Figure 6. Pairwise age-bias plots of between reader age estimates for Brook Trout (*Salvelinus fontinalis*) sagittal otoliths (A), pelvic fin rays (B), and scales (C). The dashed line indicates 1:1 pairwise agreement between reader age estimates. Solid bars representing estimated 95% confidence intervals are shown for every age where $n \geq 3$.

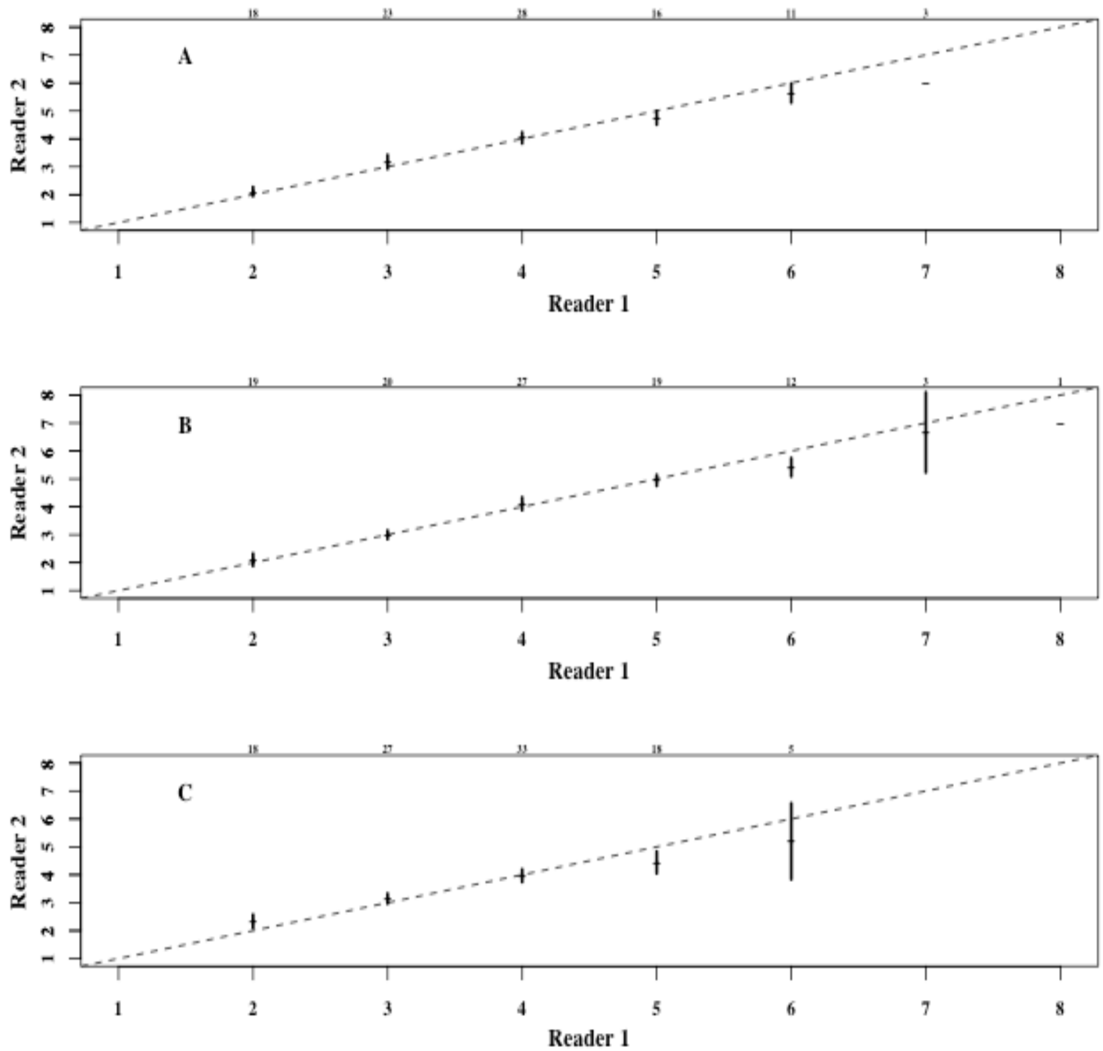

Figure 7. Pairwise age-bias plots of between reader age estimates for Brown Trout (*Salmo trutta*) sagittal otoliths (A), pelvic fin rays (B), and scales (C). The dashed line indicates 1:1 pairwise agreement between reader age estimates. Solid bars representing estimated 95% confidence intervals are shown for every age where $n \geq 3$.

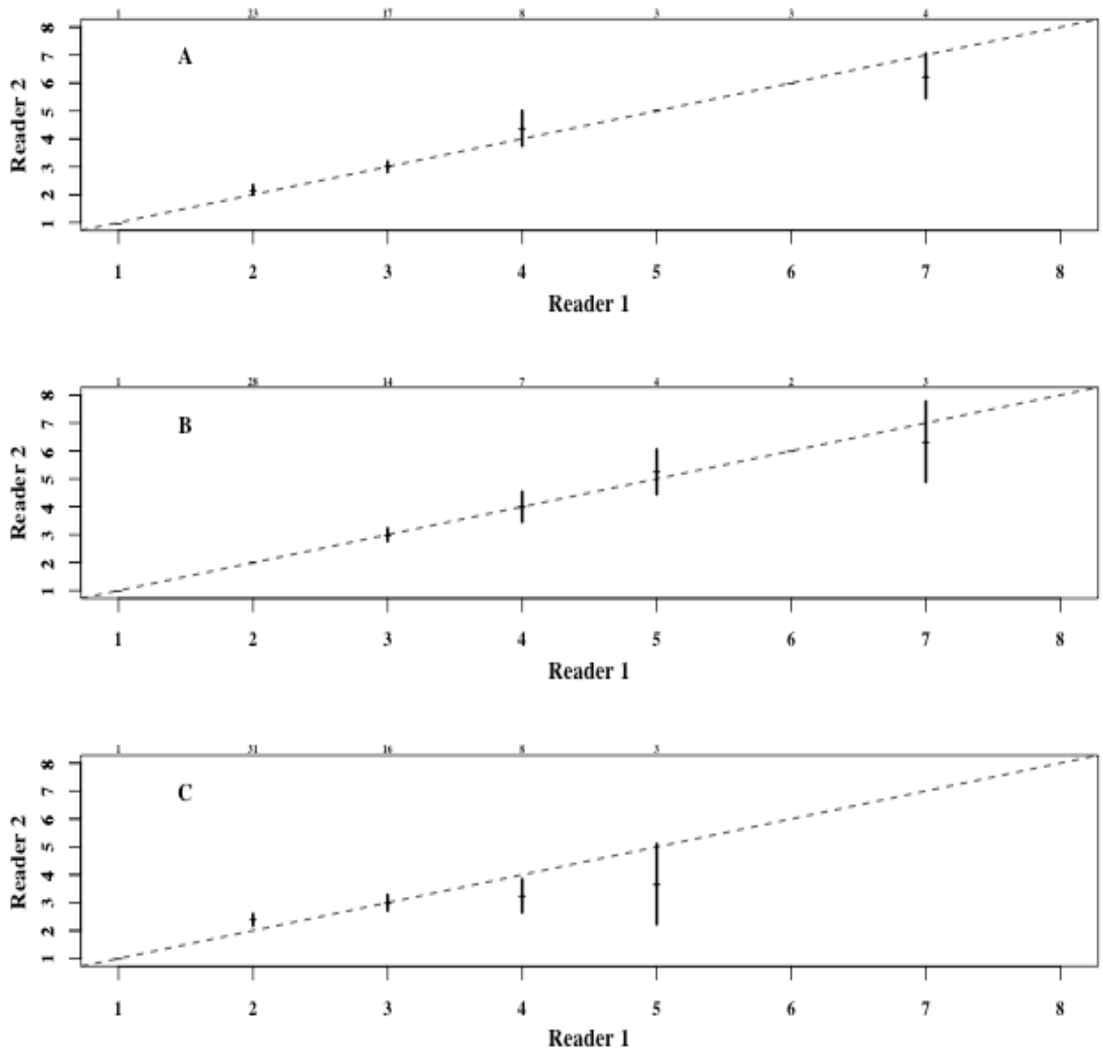

Figure 8. Pairwise age-bias plots of between reader age estimates for Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*) sagittal otoliths (A), pelvic fin rays (B), and scales (C). The dashed line indicates 1:1 pairwise agreement between reader age estimates. Solid bars representing estimated 95% confidence intervals are shown for every age where $n \geq 3$.

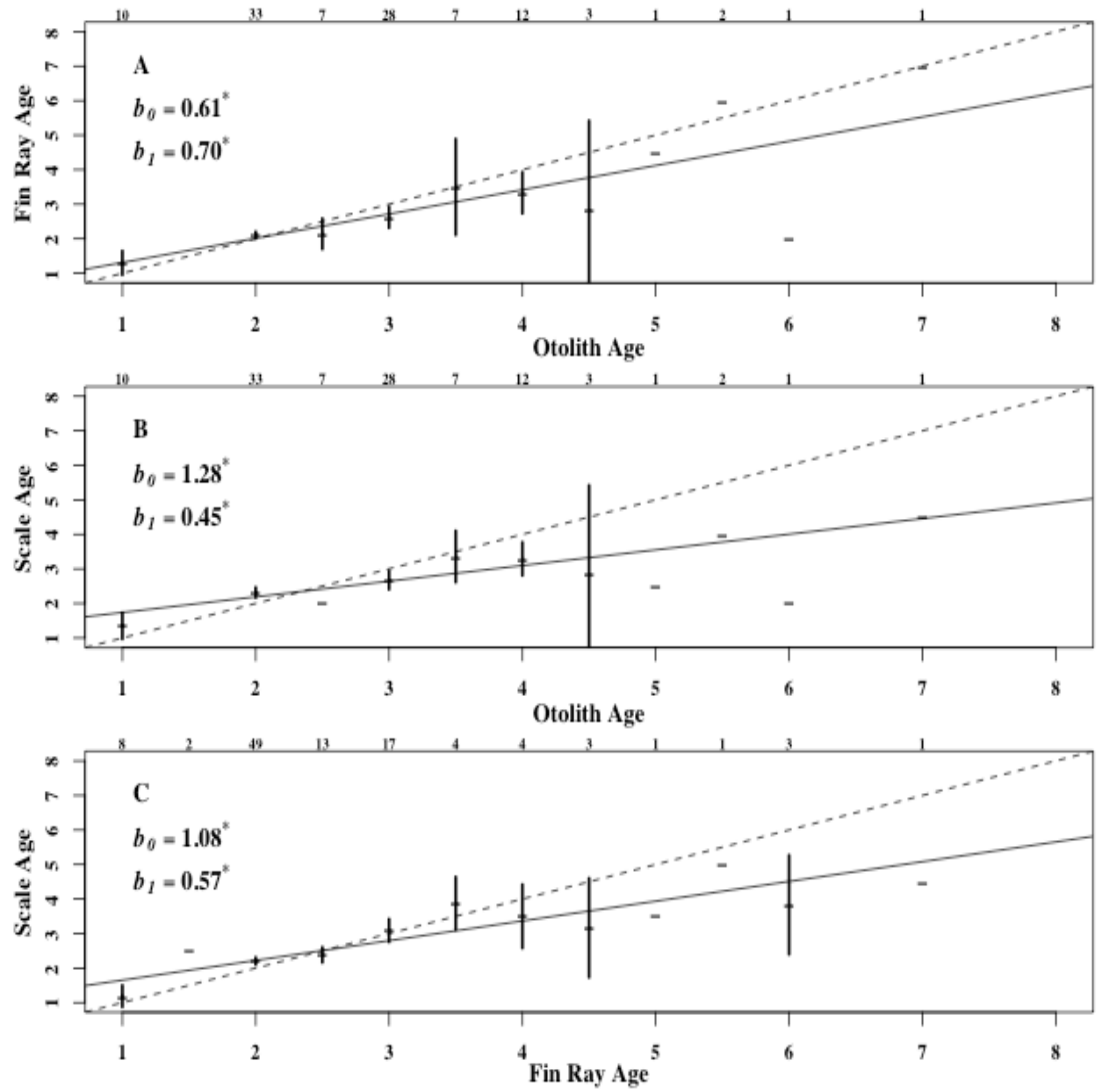

Figure 9. Age-bias plots comparing mean age estimates of calcified structures of Brook Trout (*Salvelinus fontinalis*). The dashed line indicates 1:1 agreement between ages estimated by calcified structures. The solid line represents the estimated least squares regression line. Ninety-five percent confidence intervals represented by solid vertical bars are shown for every age where $n \geq 3$. Estimates of the Y-intercept (b_0) that differ from zero and slope estimates (b_1) that differ from one ($P < 0.05$) are noted with an asterisk (*).

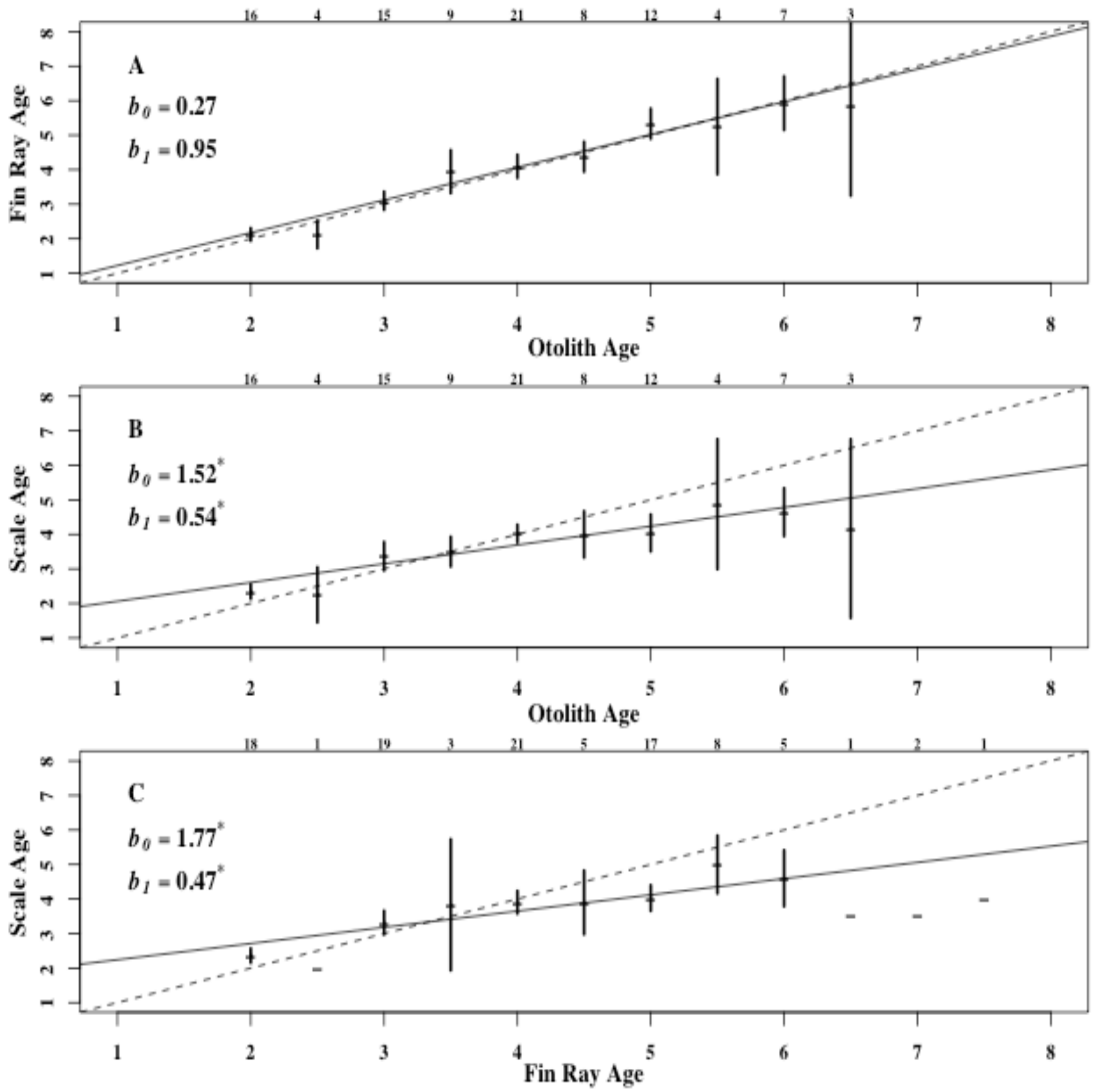

Figure 10. Age-bias plots comparing mean age estimates of calcified structures of Brown Trout (*Salmo trutta*). The dashed line indicates 1:1 agreement between ages estimated by calcified structures. The solid line represents the estimated least squares regression line. Ninety-five percent confidence intervals represented by solid vertical bars are shown for every age where $n \geq 3$. Estimates of the Y-intercept (b_0) that differ from zero and slope estimates (b_1) that differ from one ($P < 0.05$) are noted with an asterisk (*).

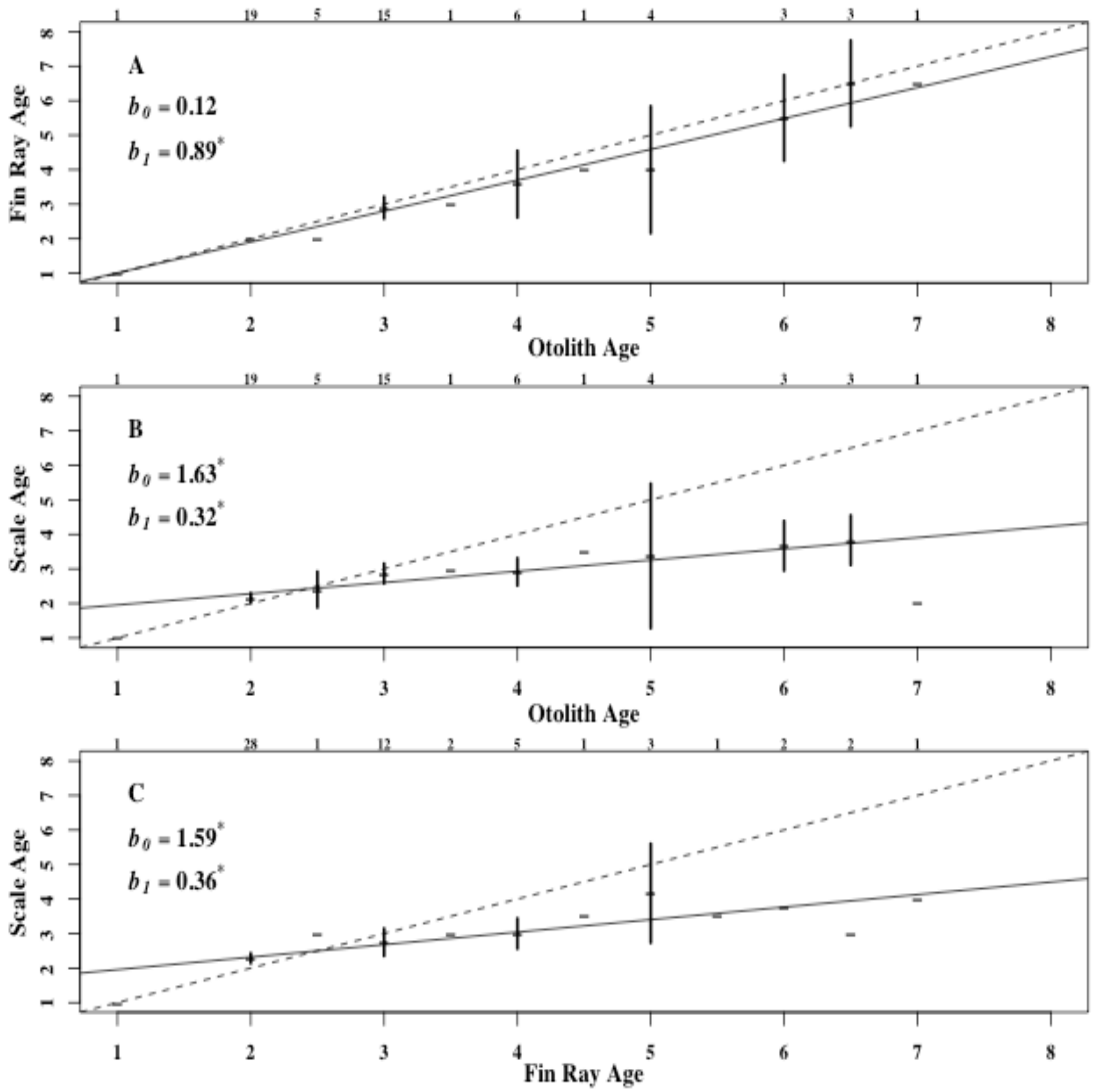

Figure 11. Age-bias plots comparing mean age estimates of calcified structures of Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*). The dashed line indicates 1:1 agreement between ages estimates. The solid line represents the estimated least squares regression line. Ninety-five percent confidence intervals represented by solid vertical bars are shown for every age where $n \geq 3$. Estimates of the *Y*-intercept (b_0) that differ from zero and slope estimates (b_1) that differ from one ($P < 0.05$) are noted with an asterisk (*).

APPENDIX B

AGE ESTIMATES OF BROOK TROUT (*SALVELINUS FONTINALIS*)

BROOK TROUT ID	READER 1 OTOLITHS	READER 2 OTOLITHS	READER 1 FIN RAYS	READER 2 FIN RAYS	READER 1 SCALES	READER 2 SCALES
1	2	2	2	2	2	2
2	2	2	2	2	2	2
3	3	3	2	2	2	2
4	3	4	6	6	4	3
5	3	3	3	3	4	3
6	4	4	4	4	4	4
7	4	4	3	3	4	4
8	3	3	6	5	5	5
9	2	3	3	3	2	2
10	4	4	4	4	3	3
11	1	1	2	2	1	2
12	3	3	2	3	3	2
13	3	4	3	3	4	4
14	2	2	2	2	2	3
15	2	2	2	3	2	3
16	2	2	2	2	3	3
17	2	2	2	2	3	4
18	3	2	2	3	2	2
19	2	2	2	2	2	2
20	2	2	2	3	3	3
21	5	3	4	3	4	3
22	4	4	5	4	4	3
23	3	3	2	2	2	3
24	2	2	2	2	3	3
25	3	3	2	3	2	2
26	4	5	2	2	2	3
27	4	4	4	3	5	4
28	2	2	3	3	2	3
29	3	3	3	2	2	3
30	3	3	3	3	3	4
31	5	4	3	2	2	2
32	3	3	3	4	3	4
33	3	3	2	2	2	3
34	7	5	2	2	2	2
35	3	3	3	3	2	2
36	4	2	2	2	2	2
37	2	3	2	2	2	2
38	2	2	2	2	2	2
39	2	2	2	3	2	3
40	1	1	2	2	2	3
41	1	1	2	2	2	2
42	3	3	2	2	2	2
43	3	3	2	2	3	2
44	3	3	2	2	2	2
45	4	4	2	2	2	2
46	2	2	2	2	2	2
47	2	3	2	2	2	2
48	3	3	3	2	3	2
49	4	4	2	2	3	3
50	3	2	2	2	2	2
51	4	2	2	2	2	2
52	4	3	2	2	3	2
53	3	2	2	2	2	2
54	3	4	2	2	2	2
55	2	3	1	2	2	2
56	3	3	2	2	2	2
57	2	2	1	2	3	3
58	2	2	2	3	2	3
59	3	3	1	1	2	2
60	5	3	2	2	2	2

BROOK TROUT ID	READER 1 OTOLITHS	READER 2 OTOLITHS	READER 1 FIN RAYS	READER 2 FIN RAYS	READER 1 SCALES	READER 2 SCALES
61	3	3	3	3	3	2
62	3	3	3	3	3	3
63	5	5	5	4	3	2
64	7	7	7	7	6	3
65	4	4	3	3	3	4
66	3	3	3	3	3	2
67	3	3	3	2	3	3
68	3	4	3	3	4	4
69	2	2	2	2	2	3
70	2	2	2	2	2	2
71	3	3	3	3	3	4
72	3	3	3	3	3	3
73	3	3	3	3	3	3
74	2	2	2	2	2	3
75	6	5	6	6	3	4
76	4	4	5	4	4	3
77	5	4	4	4	4	4
78	4	4	4	4	4	2
79	5	6	6	6	5	4
80	4	3	3	4	4	4
81	2	5	5	5	4	3
82	2	2	2	3	2	2
83	2	2	2	2	2	2
84	2	2	2	2	2	3
85	2	2	2	2	2	2
86	3	3	3	3	3	3
87	2	2	2	3	2	2
88	2	2	2	2	2	2
89	2	2	2	2	2	2
90	2	2	2	2	2	2
91	2	2	2	2	2	2
92	3	3	3	3	3	3
93	2	2	2	2	2	2
94	2	2	2	2	2	2
95	1	1	1	1	1	2
96	2	2	2	2	3	2
97	2	2	2	2	2	3
98	NA	NA	2	2	2	2
99	2	2	2	2	2	2
100	2	2	2	2	2	2
101	1	1	1	1	1	1
102	1	1	1	1	1	1
103	1	1	1	1	1	1
104	1	1	1	1	1	1
105	1	1	1	1	1	1
106	1	1	1	1	1	1

APPENDIX C

AGE ESTIMATES OF BROWN TROUT (*SALMO TRUTTA*)

BROWN TROUT ID	READER 1 OTOLITHS	READER 2 OTOLITHS	READER 1 FIN RAYS	READER 2 FIN RAYS	READER 1 SCALES	READER 2 SCALES
1	3	4	4	4	4	3
2	3	3	3	3	4	4
3	4	5	4	4	4	4
4	3	3	2	4	2	2
5	6	6	6	5	5	4
6	2	2	2	2	2	2
7	3	3	4	4	3	2
8	2	2	2	2	2	2
9	3	4	5	5	5	4
10	3	3	3	3	4	4
11	3	3	3	3	3	3
12	4	4	4	4	4	4
13	6	5	6	5	6	7
14	4	4	6	6	4	4
15	4	4	5	5	4	5
16	3	3	3	3	3	3
17	6	6	6	6	5	4
18	4	4	4	4	4	5
19	3	4	5	4	4	4
20	5	5	5	5	3	3
21	4	4	4	5	4	4
22	3	2	2	2	2	2
23	2	2	2	2	2	3
24	5	4	4	4	4	3
25	2	3	2	2	3	3
26	2	3	2	2	2	2
27	2	2	2	2	2	3
28	6	5	6	6	4	4
29	4	4	3	3	3	3
30	5	4	5	5	5	5
31	6	6	5	5	6	5
32	3	3	3	3	4	5
33	4	4	4	3	4	4
34	2	2	2	2	2	2
35	4	4	4	4	5	5
36	4	4	4	4	5	4
37	5	5	5	5	4	4
38	5	5	7	7	4	4
39	3	3	3	3	3	4
40	4	4	4	4	4	5
41	5	5	6	6	6	5
42	5	5	6	5	3	3
43	4	4	4	3	3	3
44	5	5	5	5	5	3
45	5	5	6	6	5	5
46	3	3	3	3	3	4
47	3	3	3	4	4	5
48	4	4	4	4	4	3
49	5	5	5	4	5	5
50	3	4	4	4	3	3
51	6	6	7	6	4	3
52	4	3	3	3	3	3
53	4	4	3	3	4	4
54	3	2	3	2	2	2
55	5	5	5	5	3	3
56	3	4	3	3	3	3
57	5	5	5	5	4	3
58	2	2	2	2	2	3
59	4	4	5	5	4	4
60	4	3	3	3	4	4

BROWN TROUT ID	READER 1 OTOLITHS	READER 2 OTOLITHS	READER 1 FIN RAYS	READER 2 FIN RAYS	READER 1 SCALES	READER 2 SCALES
61	2	2	2	2	2	2
62	3	3	2	2	2	3
63	2	2	2	2	2	2
64	2	2	2	2	3	3
65	2	2	2	2	3	3
66	3	3	3	3	3	3
67	2	2	2	2	2	2
68	2	2	3	3	2	2
69	4	4	4	6	3	4
70	2	2	2	2	2	3
71	NA	NA	4	5	3	3
72	4	4	4	5	4	3
73	7	6	5	5	5	4
74	4	4	3	3	4	4
75	6	5	6	5	5	5
76	6	6	6	5	5	5
77	4	5	5	5	3	3
78	4	4	4	4	4	3
79	2	2	2	2	3	3
80	6	5	4	4	4	4
81	7	6	7	7	3	3
82	3	4	4	4	3	3
83	NA	NA	5	6	6	5
84	6	6	6	5	5	6
85	5	4	5	5	5	4
86	4	4	4	4	5	5
87	4	3	5	5	3	4
88	3	3	4	4	3	4
89	4	4	4	4	4	5
90	4	5	4	4	5	5
91	5	4	4	4	4	4
92	6	6	8	7	5	3
93	5	5	5	5	5	4
94	7	6	6	5	6	4
95	4	5	4	4	3	3
96	5	5	5	5	4	4
97	4	4	4	4	4	4
98	3	3	3	3	4	4
99	2	2	2	2	2	2
100	3	3	3	3	3	3
101	2	2	3	3	2	3

APPENDIX D

AGE ESTIMATES OF RIO GRANDE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI VIRGINALIS*)

RGCT ID	READER 1 OTOLITHS	READER 2 OTOLITHS	READER 1 FIN RAYS	READER 2 FIN RAYS	READER 1 SCALES	READER 2 SCALES
1	5	5	5	5	5	4
2	2	2	2	2	2	2
3	3	3	2	2	3	3
4	3	3	3	4	3	3
5	3	3	3	3	3	3
6	3	3	3	3	4	4
7	3	3	3	3	3	3
8	2	2	2	2	2	3
9	2	2	2	2	2	3
10	4	4	4	4	3	3
11	7	6	7	7	4	4
12	2	2	2	2	2	3
13	3	3	2	2	3	3
14	2	2	2	2	2	2
15	2	2	2	2	2	2
16	2	2	2	2	2	3
17	2	2	2	2	2	2
18	2	2	2	2	2	2
19	2	2	2	2	2	2
20	2	2	2	2	2	2
21	3	4	3	3	3	3
22	3	3	3	3	2	3
23	2	3	2	2	2	2
24	2	2	2	2	2	2
25	2	3	2	2	2	3
26	6	6	5	6	4	3
27	4	4	4	4	4	2
28	5	5	3	3	2	3
29	5	5	5	5	5	4
30	4	4	4	5	4	3
31	3	3	4	3	3	3
32	4	5	4	4	3	4
33	3	3	3	3	4	3
34	2	2	2	2	2	3
35	2	2	2	2	2	2
36	2	3	2	2	2	2
37	4	4	3	3	2	3
38	2	3	2	2	2	3
39	3	3	3	2	3	3
40	2	2	2	2	2	3
41	3	2	2	2	3	3
42	3	3	3	3	2	2
43	3	3	4	4	3	2
44	4	6	3	3	2	2
45	4	4	2	2	2	3
46	2	2	2	2	3	2
47	7	7	7	6	2	2
48	6	6	6	6	4	4
49	7	6	6	6	3	4
50	3	3	2	2	2	3
51	6	6	5	5	4	3
52	7	6	7	6	5	3
53	3	3	3	3	3	3
54	3	3	3	3	2	2
55	4	4	4	4	3	3
56	2	2	2	2	2	2
57	1	1	1	1	1	1
58	2	2	2	2	2	1
59	2	2	2	2	2	2