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## **Evaluation of Fin Rays and Otoliths for Estimating Age of Shoshone Sculpin**

Running footer: Shoshone Sculpin Ageing Structures

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## Abstract

Information on age and growth is central to understanding fish population dynamics and guiding conservation activities. However, using hard structures to age fishes from systems with stable water temperature has been largely ignored. We removed sagittal otoliths and pectoral fin rays from Shoshone Sculpin *Cottus greeniei*, a species endemic to spring systems in south-central Idaho, sampled from two spring systems during January–February, 2026. Otoliths and fin rays were mounted in epoxy and sectioned. Two readers independently aged the structures. Fish ( $n = 37$ ) varied in length from 46–153 mm (total length). Otoliths typically had discernable marks that were interpreted as annuli. Fin rays were deemed unsuitable for ageing and removed from the analysis. Assuming marks were annuli, Shoshone Sculpin varied from age 2 to age 8. Results of this research are consistent with other studies focused on using otoliths to age fishes in stable, spring habitats, and ages are concordant with research on other freshwater cottids. This study provides an important foundation for future research focused on validating annulus formation and characterizing the ecology of Shoshone Sculpin.

## Introduction

Understanding fish population dynamics is critically important for the management and conservation of fishes. Information on age structure and growth is particularly important since it provides insight into life history characteristics (e.g., age at sexual maturity, longevity) and informs population models that are central to evaluating population status and viability (Caswell 2001; Morris and Doak 2002). A variety of techniques have been used by fisheries scientists to obtain age data, but hard structures (i.e., otoliths, scales, fin rays) typically provide the most accurate and precise estimates (Quist et al. 2012; Whitley 2017).

Obtaining reliable information on age and growth is dependent on whether hard structures record interpretable information in the form of annular marks. The formation of annuli is caused by changes in growth and(or) physiology in response to annual variability in environmental conditions (Schramm 1989; Whitledge 2017). As such, stable environmental conditions (e.g., water temperature) may result in hard structures that do not regularly form annuli (Del Piccolo et al. 2024). The Shoshone Sculpin *Cottus greeniei* is endemic to spring systems along the Snake River canyon in south-central Idaho (Sigler and Zaroban 2018). Water in the springs originates from the Lost River basin where streams and rivers flow south and then disappear into the aquifer. The water flows south for about 160 km where it reemerges along the canyon walls of the Thousand Springs area (~65 km stretch of canyon) of the Hagerman Valley before entering the Snake River. The springs provide clear, well-oxygenated water with near uniform year-round temperatures typically around 13–16°C. Many of the springs have been developed for aquaculture production and irrigation for agriculture. The springs support a unique biotic community including three endemic freshwater snail species that are protected by the Endangered Species Act (ESA; U.S. Fish and Wildlife Service 1992). Populations of Shoshone Sculpin have been found in 25 locations, all of which are spring systems (Wallace et al. 1984; Campbell et al. 2023). In 1979, Shoshone Sculpin was petitioned for listing under the ESA but protection was deemed “non warranted” following various studies on the distribution and abundance of the species (Connolly 1983; Wallace et al. 1984; Kuda and Griffith 1993). Nevertheless, Shoshone Sculpin is rated as “vulnerable” globally (NatureServe, accessed June 2026) and is classified as a “species of greatest information need” by the Idaho Department of Fish and Game (IDFG 2024).

Previous research on Shoshone Sculpin has largely focused on describing its basic ecology (Connolly 1983), distribution (Wallace et al. 1984), and more recently, genetic diversity (Campbell et al. 2023). Efforts associated with estimating age of Shoshone Sculpin are limited to a single study and the conclusions are vague. Specifically, Connolly (1983) reported that otoliths (assumed to be sagittae) and pectoral fin rays were removed from 25 individual Shoshone Sculpin. Structures were sent to an external agency for analysis, but the author stated in the discussion of his thesis that the structures could not be aged due to the relatively stable conditions (i.e., water temperature) of spring habitats. No additional details were provided on specific issues with reading the structures. Instead, length-frequency distributions were used to assign ages to Shoshone Sculpin in his study. Based on those assignments, Shoshone Sculpin has been assumed to be a short-lived species (~3 years maximum age); no one has reevaluated the use of hard structures for estimating age.

The lack of information on the population dynamics of Shoshone Sculpin is concerning given the conservation status and continued threats associated with alteration of spring habitats and the aquifer (e.g., aquaculture, municipal water supply, irrigation). This study was conducted as a step towards better understanding the limitations and potential use of hard structures to estimate age and growth of Shoshone Sculpin. Age and growth investigations require substantial effort, and removing otoliths requires sacrificing fish. Therefore, this study sought to evaluate whether sagittal otoliths and pectoral fin rays contained marks that may represent annuli. If so, this study would provide a critical foundation for future research focused on validating annulus formation and quantifying population dynamics of Shoshone Sculpin.

## Methods

Shoshone Sculpin were sampled from Riley Springs and Morgan Lake (also known as Banbury Springs Lake). Riley Springs Creek emerges approximately 4.0 km upstream of IDFG's Hagerman Hatchery where it serves as the primary water source for the hatchery. The stream formed by Riley Springs is relatively narrow (~7 m) with a low gradient and mixture of cobble, gravel, and sand substrates. Springs in the Banbury Springs complex originate from basalt cliffs and talus slopes approximately 50 m above the Snake River. Flow from the springs is captured by Morgan Lake, a small (3.8 ha) human-made lake formed by a levee on the bank of the Snake River. The system is relatively shallow (2-3 m) with fine substrate and abundant aquatic macrophytes. Both systems have relatively stable water temperatures (Figure 1). Water temperature in Riley Springs varies from about 14.5°C to 15.5°C throughout the year and averages 15.1°C (SD = 0.2; 2016–2025; D. Meyer, Idaho Power Company, unpublished information). Daily range (i.e., maximum – minimum) in water temperature averages 0.2°C (SD = 0.1). Water temperature at the inflow of Morgan Lake varied from 12.3°C to 14.9°C from 2016 to 2025 (mean  $\pm$  SD;  $14.1 \pm 0.5^\circ\text{C}$ ) and from 11.5°C to 16.2°C at the outflow ( $14.1 \pm 0.5^\circ\text{C}$ ). Daily variation in water temperature averages 0.6°C (SD = 0.2) at the inflow and 1.0°C (SD = 1.1) at the outflow.

Fish were sampled from Riley Springs using conical minnow traps and from Morgan Lake using a backpack electrofishing unit. Fish were sampled in January and February 2026, when personnel were available and to avoid potential confusion with annulus formation later in the year (e.g., spring when annuli often form). We sought to sample a variety of lengths while minimizing the number of sacrificed fish. As such, we attempted to include at least two fish per centimeter length group. Fish were euthanized and immediately frozen before being transported

to the University of Idaho for processing. Fish were measured to the nearest millimeter [total length (TL)]. Sagittal otoliths were removed from each fish, cleaned, and allowed to air dry (Long and Grabowski 2107). The leading left pectoral fin ray was removed at the junction with the body wall and allowed to air dry (Fischer and Koch 2017). Otoliths and pectoral fin rays were mounted in epoxy following standard methods (Koch and Quist 2007). Transverse sections (0.6–0.8 mm) of otoliths were sectioned by cutting on either side of the nucleus with a low-speed saw (Buehler Inc., Lake Bluff, Illinois). Cross sections (0.8–1.0 mm) of pectoral fin rays were cut close to the base of the fin ray. Sections were sanded and polished with sandpaper and then viewed under a microscope using transmitted light.

Two readers independently viewed each structure. Both readers had extensive experience reading otoliths; one of the readers had extensive experience ageing fin rays and the other was a novice with regard to fin rays. A presumed annulus was identified as an obvious discontinuity in the structure, typically as a change between optically opaque and transparent material (Quist et al. 2012; Fischer and Koch 2017; Long and Grabowski 2017). When age estimates differed, the readers attempted to identify a consensus age. Although we are unsure when annuli might be formed for Shoshone Sculpin, we adhered to standard convention (Quist et al. 2012) and added a year to the number of observed presumed annuli since fish were sampled after January 1.

## Results

Thirty-seven Shoshone Sculpin varying in length from 46–153 mm were included in the analysis (Figure 2). We used various techniques to enhance readability of fin rays, including sanding sections exceptionally thin, adding immersion oil, and using different light sources and angles. However, fin rays appeared as homogenous tissue and did not contain interpretable marks

(Figure 3). As such, fin rays were excluded from consideration as a viable ageing structure. Otoliths from four individuals (49 mm and 51 mm individuals from Riley Springs; 56 mm and 77 mm individuals from Morgan Lake) were not readable. Specifically, the otolith sections were exceptionally opaque and no structure in the otolith could be discerned even though the sections were further reduced in thickness, finely polished, and enhanced with immersion oil. Otolith sections from the remaining fish contained marks that were presumed annuli (Figure 3). The otoliths were often fairly opaque, particularly near the nucleus, but marks were observed after manipulating the angle and intensity of transmitted light and by adding immersion oil. Readers agreed on all age estimates except for two fish (i.e., 94.5% exact agreement). In both instances, age estimates differed by one year and a consensus age was identified. Estimated ages varied from age 2 to age 8, but the majority (57.6%) of fish in the sample were age 4 and younger (Figure 4). The number of presumed annuli typically increased with length, particularly for fish in Morgan Lake.

## Discussion

Sagittal otoliths from Shoshone Sculpin had discernable marks that were presumed to be annuli. The otoliths were fairly opaque compared to many other taxa (e.g., percids, centrarchids), but were consistent with our observation of other sculpin otoliths (i.e., Bear Lake Sculpin *Cottus extensus*, Shorthead Sculpin *C. confusus*) examined in our laboratory. The only other study that has attempted to age Shoshone Sculpin with hard structures was Connolly (1983). However, the author failed to provide any details other than stating that Shoshone Sculpin could not be aged with otoliths and fin rays, presumably due to the stable conditions of spring habitats. The formation of annuli is typically a response to changes in growth and(or) physiology caused by

seasonal variation in environmental conditions (Schramm 1989; Whitledge 2017). Historically, variability in some aquatic systems was considered so low that investigations of age and growth in these systems were thought to be pointless. An excellent example is provided by tropical systems where the idea of constant environmental conditions was so pervasive that most scientists assumed tropical fishes would not exhibit any discernable or useful marks on their hard structures (Pannella 1979; Longhurst and Pauly 1987; Fowler 2009). As such, the usefulness of hard structures for ageing tropical fishes was ignored until the late 20<sup>th</sup> century (Fowler 2009). Since that time, scientists have discovered that tropical systems experience seasonal variation in environmental conditions (sometimes subtle) that is recorded as annuli on hard structures, particularly otoliths (Choat and Robertson 2002; Fowler 2009; Moltschaniwskyj and Cappel 2009). In other systems, annuli have been shown to form in response to seasonal changes in the wet-dry cycle (Warburton 1978) and food availability (Schramm 1989).

Spring systems are among the world's most stable aquatic systems, particularly with regard to water temperature (Botosaneanu 1998). Nevertheless, environmental conditions in springs are often temporally variable in response to variability in discharge, allochthonous inputs (e.g., terrestrial invertebrates), and solar irradiance (Ilmonen and Paasivirta 2005; Cantonati et al. 2012). Few studies have been published on ageing fish from spring systems, but those that have been conducted have successfully aged fish. For instance, Dzul et al. (2012) evaluated the use of sagittal otoliths for ageing Salt Creek Pupfish *Cyprinodon salinus salinus* in Salt Creek, California. Although they collected fish from a diversity of habitats, fish from spring habitats were included in the study. They found that otoliths, including those from fish in springs, contained clear marks that were interpreted as annuli. Johnson and Belk (2004) evaluated annulus formation in lapilli otoliths from Utah Chub *Gila atraria* in Big Spring, Utah. Big

Spring is a spring system in the North American Great Basin with nearly constant water temperatures ( $\sim 17^{\circ}\text{C}$ ). The authors observed clear marks on the otoliths and then used marginal increment analysis to confirm that the marks were annuli. Although temperatures were nearly constant, seasonal changes in resource availability (e.g., prey resources) were suggested to result in annulus formation. Although thermal conditions in both Riley Springs and Morgan Lake are not constant, they are quite stable (i.e., relative to most temperate systems) with mean temperatures varying over the year by about  $1^{\circ}\text{C}$  in Riley Springs and  $2\text{--}3^{\circ}\text{C}$  in Morgan Lake. As such, small changes in water temperature may be sufficient to elicit the formation of marks (presumed annuli) on Shoshone Sculpin otoliths. The formation of presumed annuli may also be a function of system productivity similar to what was observed by Johnson and Belk (2004). Regardless, efforts focused on seasonal variation in system productivity would be useful in better understanding the mechanisms associated with presumed annulus formation.

Fin rays are often a preferred ageing structure for species with poor conservation status because they do not require sacrificing fish and because age estimates from rays are often concordant with otolith ages (Griffin et al. 2017; Wilson et al. 2025). Fin rays have also been shown to underestimate age compared to otoliths or simply not provide interpretable marks (e.g., Klein et al. 2017; Quist et al. 2023; Del Piccolo et al. 2024). In the present study, sectioned pectoral fin rays were quickly removed from consideration as an ageing structure for Shoshone Sculpin. The authors have extensive experience working with fin rays. Despite our best efforts to produce interpretable sections from fin rays, the sections were quite homogenous and simply lacked repeatable structure that could be interpreted as annuli. In contrast, sagittal otoliths from Shoshone Sculpin contained marks. Of the few studies that have investigated age of sculpin, most have either used length-frequency distributions or sagittal otoliths (Luksenburg and

Pedersen 2002; Meyer et al. 2008; Brown et al. 2025). Connolly (1983) used length-frequency distributions to assign ages to Shoshone Sculpin which resulted in the narrative that Shoshone Sculpin are short-lived ( $\leq 3$  years). Using length-frequency distributions to establish age is tenuous for all but the youngest ages (Quist et al. 2012). Other studies have used sagittal otoliths and have reported that most freshwater sculpin have lifespans exceeding 3 years. Meyer et al. (2008) described age structure of Wood River Sculpin *C. leiopomus*, a species endemic to the Wood River, Idaho, using sagittal otoliths. Fish varied from less than 15 mm to 112 mm TL; although the majority (76%) of fish in their study were age 3 and younger, they observed fish up to age 8. Mottled Sculpin *C. bairdii* in the Lower Blue River, Colorado, varied from about 40 mm to 119 mm TL and from age 0 to age 9 (Brown et al. 2025). Similar to other sculpin populations, Shoshone Sculpin may not be as short-lived as previously reported by Connolly (1983) which has important implications for management and conservation of the species.

This study provides an important first step in better understanding techniques for ageing Shoshone Sculpin and other species in spring habitats. We found that sagittal otoliths are likely a suitable structure for ageing Shoshone Sculpin. However, additional research (e.g., Buckmeier et al. 2017) on the validity and timing of annulus formation is needed. If annulus formation is described and validated, a diversity of important questions can be answered regarding the population ecology and conservation of Shoshone Sculpin and its habitat.

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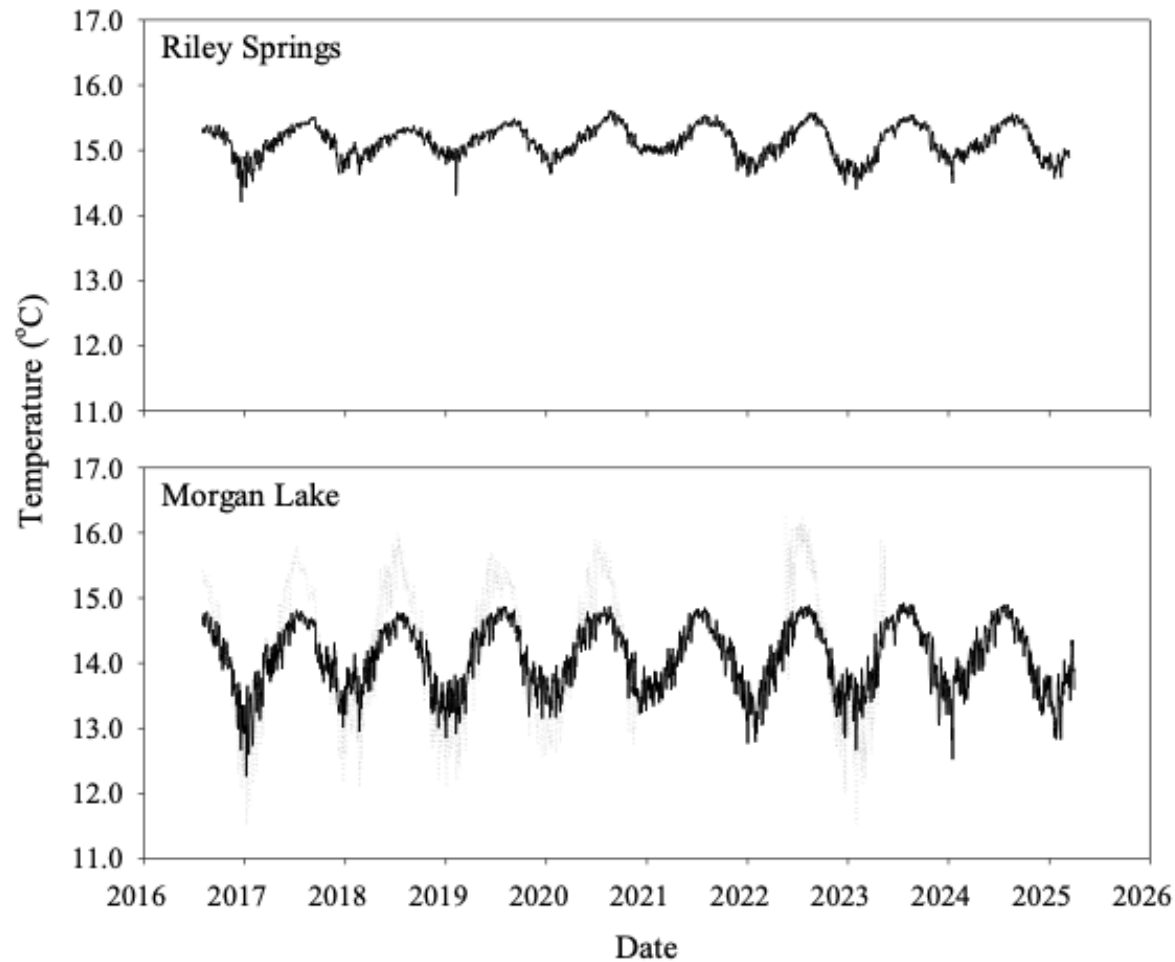
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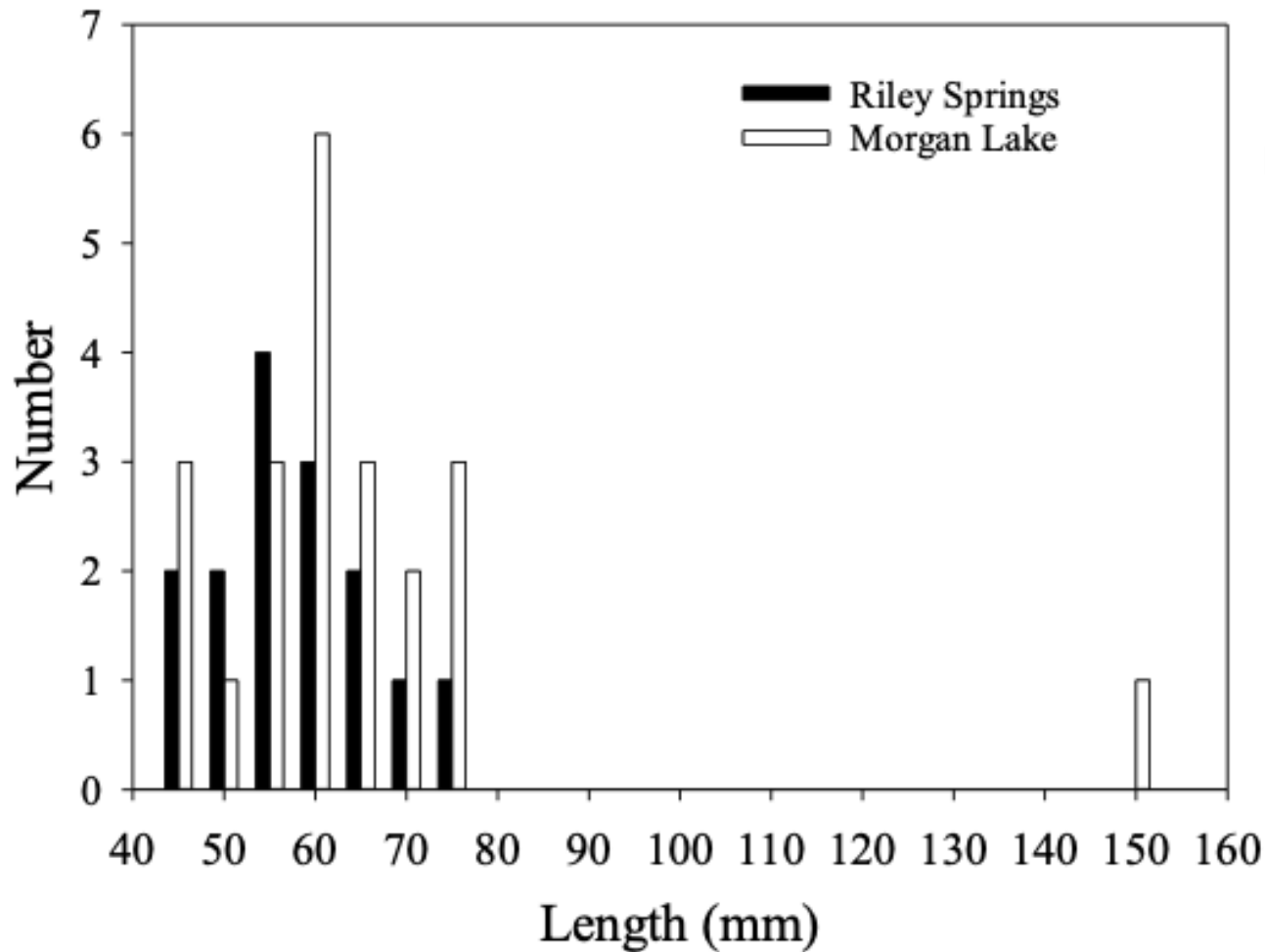
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336

337 Figure 1. Mean water temperature (°C) for Riley Springs and Morgan Lake in south-central Idaho, 2016–2025. Temperature data for  
338 Morgan Lake include temperatures at the inflow (solid line) and outflow (dotted line).



339

340 Figure 2. Number of Shoshone Sculpin (by 5-mm length group) collected to evaluate ageing structures. Fish were sampled from Riley  
 341 Spring and Morgan Lake in south-central Idaho, January–February 2026.

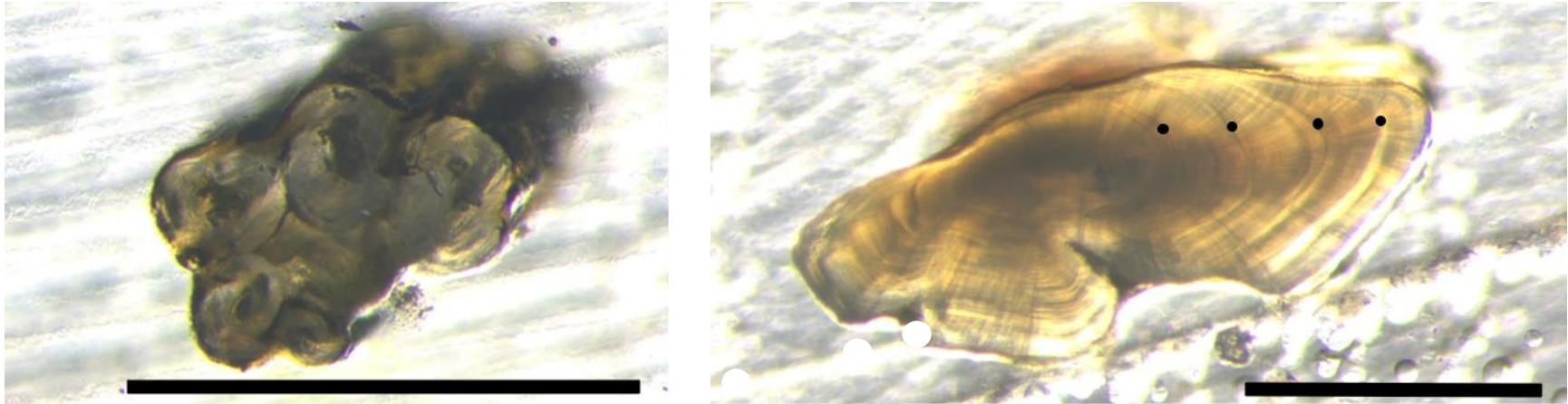
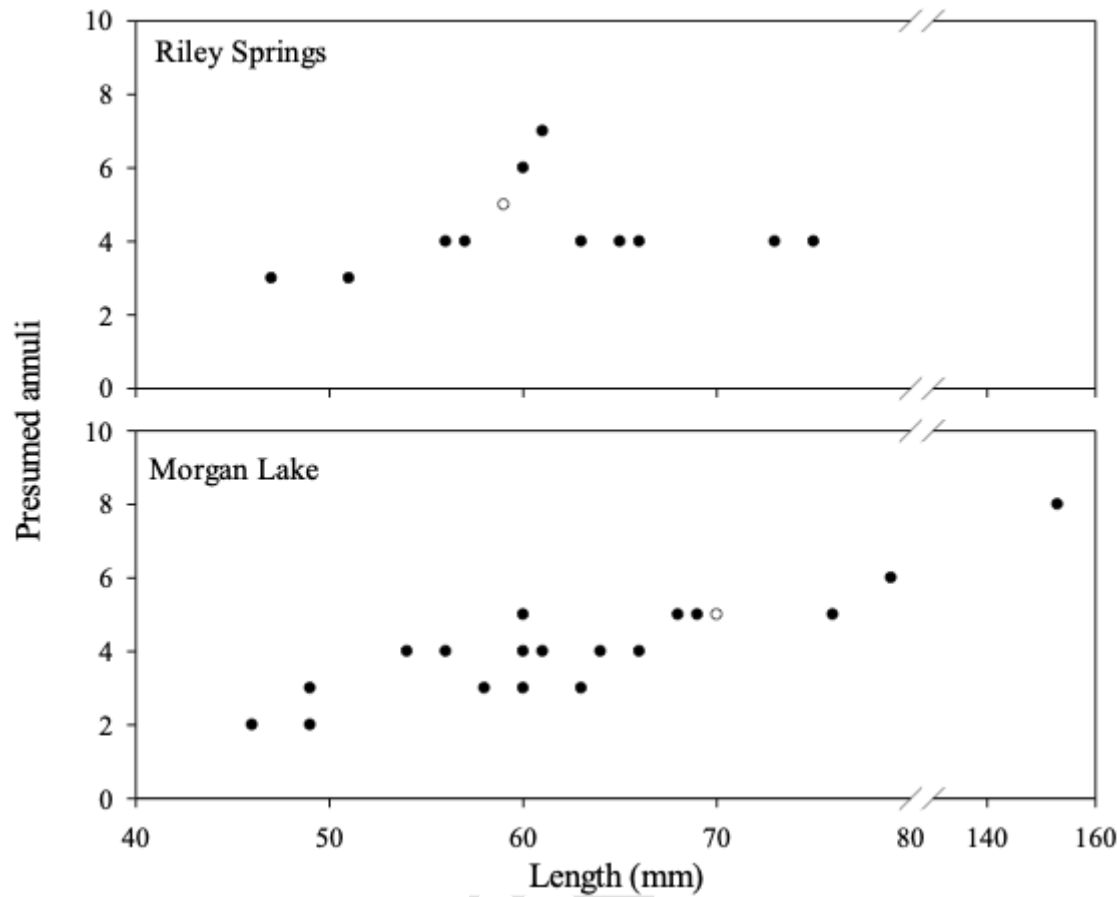


Figure 3. Sectioned pectoral fin rays (left panel) and sectioned sagittal otolith (right panel) from a 59 mm Shoshone Sculpin sampled from Riley Springs, Idaho, in February 2026. Solid symbols represent presumed annuli and the scale bar is 0.5 mm. We also assumed an annulus on the edge for the purpose of assigning an estimated age (i.e., this individual was considered age 5).



347  
 348 Figure 4. Number of presumed annuli from Shoshone Sculpin by length (note break in x-axis). Fish were sampled from Riley Springs  
 349 and Morgan Lake in south-central Idaho, January–February 2026. Solid symbols represent a single individual. Open symbols represent  
 350 two individual fish.