ARTICLE

# Thin-Sectioned Otoliths Reveal Sexual Dimorphism and a 10-Year Lifespan in the Endangered Pahrump Poolfish

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**Abstract**

   Basic life history information can be useful for informing the management of protected species such as the Pahrump Poolfish *Empetrichthys latos*, a goodeid (Cyprinodontiformes) that is endemic to the Mojave Desert in southern Nevada, USA, and the last surviving remnant of *Empetrichthys*. Critically endangered and extirpated from its native range, Pahrump Poolfish has been managed among constructed refuge habitats since 1971. To better understand sex-specific age demographics, we used thin-sectioned otoliths to age 121 Pahrump Poolfish from one population, collected in June 2017. These samples suggested a maximum age of 10 years for females and 7 years for males, with a substantial portion of all fish greater than 4 years old. This is more than twice the previously reported maximum age for this species. We report on the inadequacy of reading whole otoliths, opercula, and scales for age analysis of this species. Based on thin-sectioned otoliths, we also model growth in length as a function of age among individuals. We found significant sexual dimorphism across this fish's ontogeny, which has implications for selecting an appropriate sex ratio for founding populations.

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For poorly studied species, basic life history data such as sex-specific growth rates can be useful for directing management decisions. An estimate of sex-based differences in growth patterns may be especially important when selecting individuals to be used for establishing new populations. For instance, if growth patterns differ, larger fish will have an unequal sex ratio that in turn will reduce the effective population size (Wright 1969; Frankham 1995). Thus, fine-scale data on age and growth can be particularly useful for actively managed species.

Otolith-age validation studies that provide such fine-scale data on age and growth are relatively rare for small-bodied fishes (<10 cm), many of which are species of conservation concern (Spurgeon et al. 2015). This may be due to the presumption that small-bodied species are short-lived. Thus, most age estimates for small-bodied fishes have been based on size frequency distributions (Pearsons 1989; Duffy 1998; Herwig and Zimmer 2007; Tamura et al. 2019) or inspections of scale annuli (Jester and Suminski 1982; García-Berthou and Moreno-Amich 1992; Przybylski and García-Berthou 2004; Rogowski 2004; Deacon and Williams 2010; Patimar et al. 2010; Finch et al. 2013; Guezi et al. 2017), perhaps because these approaches are easily applied (Przybylski and García-Berthou 2004; Table 1).

A review of the literature revealed that scale-based estimates suggested maximum ages of 1 to 6 years for small-bodied fishes. Yet these estimates may misrepresent the age distribution of these species (Casselman 1990; Campana 2001; Scheerer and McDonald 2003). Thus, because thin-sectioned otoliths are the gold standard for estimating fish age (Campana 2001), they should also be applied to small-bodied fishes whenever possible, especially because extinction risk is greater for smaller-bodied fishes compared with large-bodied fishes (Reynolds et al. 2005; Olden et al. 2007). For example, Scheerer and McDonald (2003) reported age estimates for the endangered Oregon Chub Oregonichthys crameri of up to 9 years using thin-sectioned otoliths, far exceeding a previous estimate of 3 years based on length-frequency distributions (note, this species was delisted in 2015).

These observations inspired us to evaluate age and growth variation in the endangered Pahrump Poolfish Empetrichthys latos, the last remaining species within its genus. This species has been actively managed among refuge habitats since 1971, whereby translocations have regularly been used to establish/reestablish refuge populations (Goodchild 2015; Jimenez et al. 2017). The Pahrump Poolfish is considered sexually monomorphic, but in general, females reach larger sizes than males (Goodchild 2015). Aging work on this species is limited to scale analysis from historical samples of females that revealed a maximum longevity of 4 years (Deacon and Williams 2010).

Given the limitations of using scales, additional information based on otolith annulus counts is needed to verify longevity and sex-specific growth rates for this species. Specifically, we evaluated sex-specific growth patterns to better understand the sexual size dimorphism that was reported previously by Goodchild (2015). Toward this objective, we compared the readability of thin-sectioned otoliths versus whole otoliths, opercula, and scales to determine whether a precise and credible aging structure can be used for this species. Further, we documented age demographics based on presumed annuli in thin-sectioned otoliths to determine whether they are consistent with pre-existing information for this species. We also studied sex-specific differences to determine whether there are any differences that could affect the way this species is managed. Using this information, we modeled growth characteristics of the population to inform its management.

## METHODS

*Background and sample collection.* — The Pahrump Poolfish was historically endemic to Manse Spring in Nye Country, Nevada, but anthropogenic disturbance led managers to transplant fish to additional sites prior to the extirpation of the native population by 1975 (Jimenez et al. 2017). Pahrump Poolfish have been actively managed since the early 1970s when they were translocated to three different refuge habitats. Of these initial attempts, only one refuge persisted, so all extant Pahrump Poolfish are derived from the 29 individuals that were transplanted in 1971 to Corn Creek on the Desert National Wildlife Refuge in Clark County, Nevada (Goodchild 2015; Jimenez et al. 2017). Since then, Pahrump Poolfish have been managed among a number of refuge habitats with periodic extirpations and repatriations (Jimenez et al. 2017). For instance, in 1972, a total of 16 Pahrump Poolfish was introduced to Shoshone Ponds in White Pine County, Nevada (now known as the Shoshone Ponds Natural Area of Critical Environmental Concern). However, by 1976, it was not clear whether this population had established, so another 50 fish were introduced. Thus, the current population was established with 50–66 fish between these two efforts. The Shoshone Ponds habitats include a large stock pond, a small stream, and a series of small ponds that have been managed as an ad hoc metapopulation (Goodchild 2015).

We examined 132 voucher specimens from the Shoshone Stock Pond population that were originally collected by using Gee's minnow traps in 2017. Specimens of adult Pahrump Poolfish from this population have served as a source for mesocosm experiments to evaluate their interactions with nonnative species (Goodchild 2015; Goodchild and Stockwell 2016; Paulson 2019; Paulson and Stockwell 2020). At an elevation of 1,768 m, Shoshone Stock Pond has average minimum water temperatures below 10°C from October through the end of April and temperatures

TABLE 1. Review of the aging studies on small fishes (<10 cm SL) that informed the present study. The symbols are defined as follows: Oto = otolith; ^=total length; L/F = length frequency; *=extremely low confidence in the age-scores assigned to *whole* otoliths after age 2.

| Reference | Species | Structure | Age range (years) | SL range (mm) | N |
|---|---|---|---|---|---|
| Jester and Suminski 1982 | White Sands Pupfish *Cyprinodon tularosa* | Scales | 1–5 | 1.6–5.6^ | 123 |
| García-Berthou and Moreno-Amich 1992 | Iberian cyprinodont (also known as Spanish Toothcarp) *Aphanius iberus* | Scales | 0–2 | 1.2–4.4 | ~500 |
| Zhang and Li 2002 | *Paracheilognathus imberbis* | Scales | 0–1 | 3.3–7.7^ | 204 |
| Beckman 2002 | Rainbow Darter *Etheostoma caeruleum* | Scales | 0–3 | up to 6.7^ | 113 |
| | | Oto - whole | 0–5 | up to 6.7^ | 108 |
| | | Oto - thin | 0–5 | up to 6.7^ | 112 |
| Koutrakis et al. 2003 | European Bitterling *Rhodeus amarus* | Scales | 0–5 | 1.2–8.3^ | 624 |
| Przybylski and García-Berthou 2004 | European Bitterling | Scales | 0–4 | 2.8–6.6 | 235 |
| | | Opercula | 0–4 | 2.8–6.6 | 235 |
| Deacon and Williams 2010 | Pahrump Poolfish *Empetrichthys latos* | Scales | 0–4 | 1.7–7.1 | 112 |
| Patimar et al. 2010 | European Bitterling | Scales | 1–5 | 2.7–8.4^ | 272 |
| Dzul et al. 2012 | Salt Creek Pupfish *Cyprinodon salinus* | Oto - whole | 0–5 | 1.6–5.5^ | 282 |
| Finch et al. 2013 | Eastern Sand Darter *Ammocrypta pellucida* | Scales | 0–3 | 1.9–6.4^ | 743 |
| Guezi et al. 2017 | Mediterranean Killifish *Aphanius fasciatus* | Scales | 1–6 | 1.7–6.0^ | 1,868 |
| Tamura et al. 2019 | *Acheilognathus tabira jordani* | L/F | 0–3 | 1.0–7.6 | 5,270 |
| This study | Pahrump Poolfish | Scales | 0 | 2.9–5.4 | 26 |
| | Pahrump Poolfish | Opercula | 0 | 2.9–5.4 | 26 |
| | Pahrump Poolfish | Oto - whole | 1–5* | 2.6–5.7 | 132 |
| | Pahrump Poolfish | Oto - thin | 1–10 | 2.6–5.7 | 121 |

consistently above 20°C from May through August (Goodchild 2015). During cooler months, Pahrump Poolfish presumably burrow into the substrate and enter a period of dormancy (Heckmann 2009). Thus, it is likely that seasonal growth patterns will be reflected in their otolith growth patterns, with opaque bands reflecting annual reductions in growth during the winter season.

*Body dissections.*— We removed the fish from the ethanol solution, placed them on a drying towel to remove excess liquid, and measured standard length (to $\pm 0.1$ cm) of each fish ($N = 132$). We dissected the fish to remove the gonadal tissue, which was inspected under a dissecting scope to determine sex.

From a subsample of 26 randomly selected fish, we removed scale samples and the opercula before extracting the otoliths. We removed scales laterally from the left side of each fish just dorsal to the lateral line and approximately midway between the pectoral and dorsal fins (Przybylski and García-Berthou 2004). We cleaned the opercula of excess tissue by gently rubbing it away with a paper towel. We immersed the scales and opercula in distilled water and viewed them under a dissecting microscope in both light- and dark-field modes for evidence of presumed annuli, following García-Berthou and Moreno-Amich (1992) and Przybylski and García-Berthou (2004). We photographed the scales and opercula on a dissecting microscope in dark-field mode at 10–50×.

*Otolith extraction and analysis.*— We removed at least one otolith from every fish in this study ($N = 132$) by following the general protocol for sagittal otolith extraction that is outlined by Bruch et al. (2009). We placed the sagittal otoliths immediately in microvials that were

prefilled with distilled water. We removed the residual cranial tissue (labyrinth organ) and other nonotolith material under a dissecting microscope. We photographed whole sagittal otoliths in water under a dissecting microscope at 50× using transmitted light in dark-field mode. We then air-dried the otoliths, weighed (to ±1 µg) them using a CAHN Electrobalance, and photographed them again at 75× on a compound microscope while they were immersed in mineral oil to increase visual clarity. These photographs helped determine the nuclear transect (to be thin-sectioned) and whether presumed annuli were apparent in the whole otoliths (Beamish 1979; Barber and McFarlane 1987).

We cleaned the otoliths of oil and mounted them in either ACE or Gorilla quick-setting epoxy and thin-sectioned them using twin diamond-embedded blades on a Buehler IsoMet 1000 low-speed saw to produce 200-µm sections (following Lackmann et al. 2019). We mounted these sections on a glass slide, immersed them in mineral oil, and photographed them at 75× or 188× on a compound microscope. From the 132 individuals, we extracted a total of 183 sagittal otoliths and produced 149 thin sections. Most of the additional ($N = 16$) sections were completed because a fraction of the initial sections was not readable, so another otolith was sectioned from that individual (if an otolith was still available). In one case, the additional otolith was sectioned to compare age estimates (see Results below).

Multiple readers viewed all of the whole otolith ($N = 183$) images to assess their potential utility for determining age. The otolith thin sections ($N = 149$) were then analyzed by the primary reader and digitally marked for presumed annuli if they were deemed readable. Approximately 1 year after these initial age estimates, the primary reader independently scored the otolith thin sections a second time. The initial age scores were ultimately used, as both readings were consistent (see Results). Because all of the Pahrump Poolfish were collected on a single date in June and this species spawns during the growing season (Goodchild 2015), we standardized the age scores by using the otolith edge as a presumed annulus on all specimens. Growing season is defined as the period during which the Pahrump Poolfish in the Shoshone Stock Pond experience rapid gonadal growth and associated egg production during late spring and summer. If a thin section was not readable, then we thin-sectioned another otolith from that specimen (if one was available). We compared the thin-sectioned otolith images with their corresponding whole otolith images.

*Statistical analysis.*— We used the von Bertalanffy growth function (von Bertalanffy 1938) to model growth in size such that $SL = L_\infty \times \left[1 - e^{-k \times (\text{age} - t_0)}\right]$, where age is in years, parameter $L_\infty$ is asymptotic length, parameter $k$ is the exponential rate of approach to asymptotic size (Schnute and Fournier 1980), and parameter $t_0$ is age at length 0. We developed eight models for SL based on combinations of parameters $L_\infty$ and $k$ that varied by sex, and $t_0$ was fixed (at 0) or estimated. We used linear regression to model sagittal otolith mass as a function of age, sex, and an interaction between the two variables. We used information-theoretic methods (Burnham and Anderson 2002) to determine the highest ranked models based on the relative Akaike's information criterion corrected for small sample sizes ($\Delta AIC_c$) when comparing multiple models in a suite. We used JMP 14 Pro Statistical Discovery for statistical analysis and graphical output.

**Results**

The scales did not show evidence of annuli. Although opercula are often considered better than scales for age analyses (Przybylski and García-Berthou 2004), they also did not show any evidence of annuli, with no opaque bands apparent despite substantial size variation among the specimens that we inspected (2.9–5.4 cm; Table 1). The corresponding thin-sectioned otoliths contained 1 to 6 annuli. Thus, we deemed the scales and opercula inadequate aging structures for these specimens.

We produced age-scorable sections for ~92% (121 of 132) of the sampled fish (see Figure 1 for examples). The average precision between the primary and secondary readings was a coefficient of variation of 7.5%. The 11 fish for which a thin-sectioned otolith could not be scored consisted of nine females and two males. These nonaged females ranged from 3.4 to 5.7 SL, with a median of 4.2 cm SL. The nonaged males were both 3.2 cm SL.

Presumed annuli were only partially visible or indiscernible in the whole otoliths, but they were clearly apparent in the thin sections (Table 1; Figures 1 and 2). Often the first presumed annulus was visible in the whole otolith image that was taken on the compound microscope (Figure 2B inset arrow), but the outer annuli in older fish remained concealed unless they were thin-sectioned (Figure 2C; Table 1). After this finding, and because whole otoliths are widely known to produce underestimates of fish age (Hoyer et al. 1985; Hales and Belk 1992; Campana 2001), we did not pursue age-reading whole otolith images. Thus, we concluded that otolith thin sections were the only reliable method for determining presumed annulus counts for this species.

Evidence from the presumed annuli in the otolith thin sections showed that females ranged from 1 to 10 years and males 1–7 years of age. Both sexes had a modal age of 2 years and a median age of 3 years. The majority of individuals (85%) were 1–4 years old, belonging to the 2013–2016 year-classes (Figure 3).

We measured otolith mass for 114 of the 121 age-scored otolith sections. The remaining seven individuals had just one otolith extracted per fish, and we had sectioned them before we had documented the otolith masses. There was a significant interaction between sex and age

FIGURE 1. Thin-sectioned sagittal otoliths from Pahrump Poolfish of presumed ages 1–4 and 7–9 years. Note the well-defined opaque bands and how the otoliths grow laterally (appearing here as depth) as they age. The white dots mark each presumed annulus. Scale bar = 1 mm.
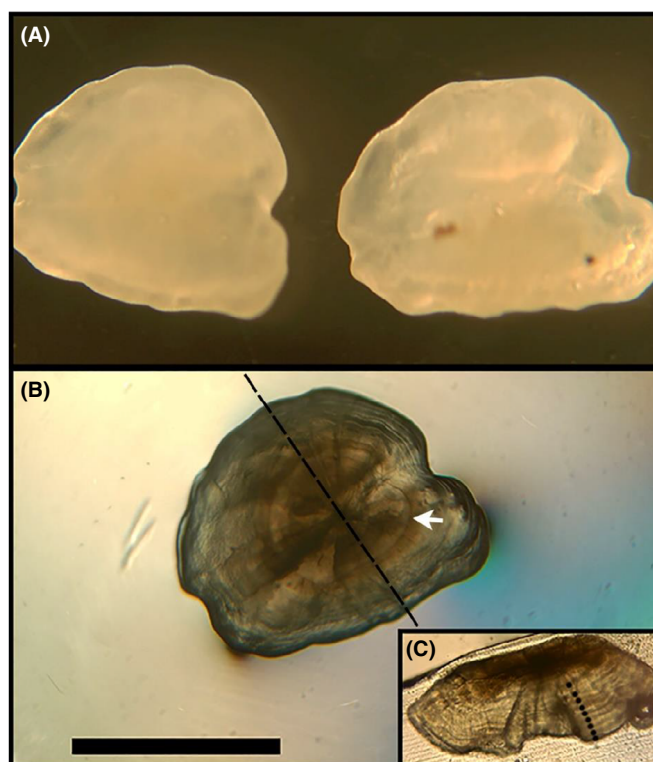


FIGURE 2. Whole versus thin-sectioned otoliths from the presumed 9-year-old female Pahrump Poolfish in this study: (A) sagittal otoliths that were photographed on a dissecting microscope, (B) the leftmost otolith in (A) photographed under transmitted light and oil on a compound microscope (the dashed line denotes the thin section transect, and the arrow denotes the first annulus), and (C) the same sagittal otolith in (B) after being sectioned, shown also under a compound microscope via transmitted light and oil. The other otolith thin section from this specimen is shown in Figure 1, the bottommost otolith section (9 years). Note the well-defined annuli on both thin sections from this specimen (Figures 1 and 2C) and annuli that cannot be discerned from whole otoliths alone (A, B). Scale bar = 1 mm applies to all panels.

(Table 2), with females showing higher growth rates (Figure 4; $R^2 = 0.72$, $P < .001$). The other models, without the interaction term, were less explanatory (Table 2; see the Methods section for the model selection technique).

We detected a significant sexual dimorphism in somatic growth and size. Of 35 males, 28 (80%) were less than 3.5 cm SL, whereas 86 of 97 (~89%) females were greater than 3.5 cm (Table 3). These sex-specific differences in size are significant throughout the fish's development, as evidenced in the von Bertalanffy growth model (Table 4). Females reach $L_\infty$ at a standard length of 5.6 cm, whereas males reach $L_\infty$ at 3.6 cm (Figure 5). Not only are males smaller; they also reach their asymptotic length faster ($k = 0.464$ for males versus $k = 0.262$ for females).

## DISCUSSION

The evidence from the thin-sectioned otoliths suggests that Pahrump Poolfish in the Shoshone Stock Pond population can live up to 10 years, or 2.5 times longer than has

been previously suggested for this species. Further, one third of the sampled fish in this study was 4 years old or older. Previous age estimates for Pahrump Poolfish were determined via scale analysis on female fish from the native habitat at Manse Spring (Deacon and Williams 2010). Similarly, other studies have shown that coarse-scale aging approaches often underestimate actual ages (Scoppettone 1988; Johnson et al. 1995; Scheerer and McDonald 2003).

Our findings are striking, as earlier work showed that fish from the Shoshone Stock Pond population were much smaller than Pahrump Poolfish from another refuge population that is located approximately 333 km to the south-southwest (Goodchild 2015). This earlier work suggested that the smaller sizes of Pahrump Poolfish in Shoshone Stock Pond were probably due to limited growth rates that are related to the colder temperature and reduced
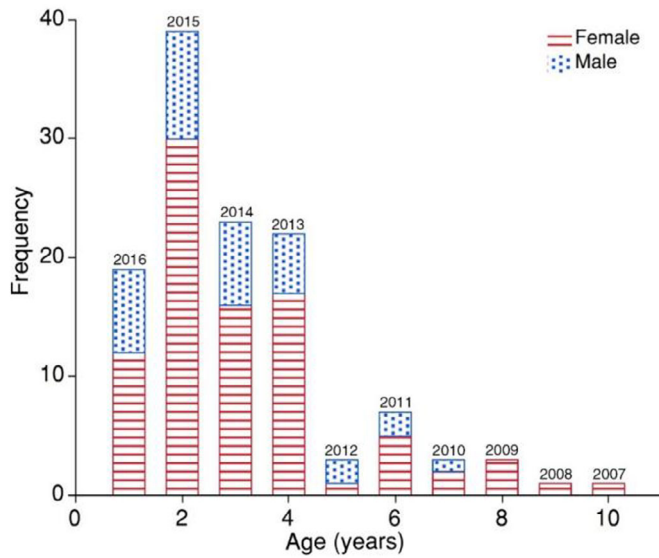
FIGURE 3. Distribution of aged Pahrump Poolfish ($N = 121$) by sex and labeled by year-class (2007–2016). Females: $N = 88$, males: $N = 33$.



FIGURE 4. Sagittal otolith mass ($N = 114$) versus age for Pahrump Poolfish modeled by the highest-ranked model ($F_{4, 109} = 94.7$, df = 4, $P < 0.001$, $R^2 = 0.72$).

TABLE 2. Model selection statistics for the otolith mass versus age model suite ($N = 114$). $AIC_c$ = Akaike's information criterion corrected for small sample sizes.

| Model | SSE | k | $AIC_c$ | $\Delta AIC_c$ | F ratio | P | $R^2$ |
|---|---|---|---|---|---|---|---|
| Linear + sex + sex × age | 3.2 | 4 | −395.8 | 0.0 | 93.8 | <0.001 | 0.72 |
| Linear + sex | 3.4 | 3 | −390.5 | 5.3 | 129.4 | <0.001 | 0.70 |
| Linear | 4.5 | 2 | −362.7 | 33.2 | 174.8 | <0.001 | 0.61 |

TABLE 3. Sex ratios within SL size categories for Pahrump Poolfish ($N = 132$) in the present study.

| SL range (cm) | ♀(N) | ♂(N) | Total (N) | %♀ |
|---|---|---|---|---|
| 2.500–2.999 | 3 | 7 | 10 | 30.0 |
| 3.000–3.499 | 8 | 21 | 29 | 27.6 |
| 3.500–3.999 | 34 | 5 | 39 | 87.2 |
| 4.000–4.499 | 27 | 2 | 29 | 93.1 |
| 4.500–4.999 | 14 | 0 | 14 | 100.0 |
| 5.000–5.499 | 8 | 0 | 8 | 100.0 |
| 5.500–5.999 | 3 | 0 | 3 | 100.0 |

length of the growing season at Shoshone Stock Pond (Goodchild 2015). Thus, our estimates for longevity should be interpreted specifically for this habitat until Pahrump Poolfish from other populations are studied.

We found no evidence of scale and operculum annuli, even though opercula are known to be better than scales at exhibiting presumed annuli (Przybylski and García-Berthou 2004). That we found no evidence of annuli in these structures is not surprising considering that these tissues are prone to cellular resorption (Casselman 1990; Campana 2001). Deacon and Williams (2010) and S. C. Goodchild (personal observation) previously found evidence of scale annuli in other samples, but our results suggest that scale annuli are inconsistently present. Otoliths are unique in that they are metabolically inert stones, with growth being permanently deposited (Campana 2001). Indeed, nonotolith structures are often considered to be less reliable and less accurate because they consistently
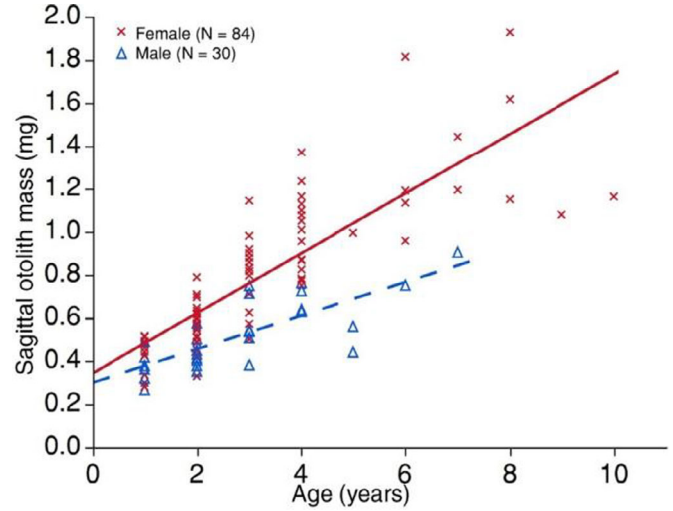
result in systematic aging errors that underestimate true age (Campana 2001; Sylvester and Berry 2006).

Although false annuli (checks) may occur in otoliths during the first few years of early rapid growth (Li et al. 2008; Buckmeier and Smith 2020), checks are less likely to occur once fish are mature because annuli become more distinct and follow a consistent growth pattern (Campana et al. 2008; Lackmann et al. 2019). However, environmental variation or physiological stress can affect the spacing of annuli. For example, high reproductive effort affects the spacing of annuli in some species (Irgens et al. 2019). The formation of false annuli associated with reproductive effort is unlikely for the Shoshone Pahrump Poolfish population because poolfish lay their eggs singly across the growing season. In fact, Goodchild (2015) reported that reproductive effort in number of eggs was constant, with reproductive allocation varying from 6% to 8% of body mass (gonadosomatic index) across the breeding season (Goodchild 2015).

We also report on a distinct sexual dimorphism in Pahrump Poolfish from this location, with females

TABLE 4. Model selection statistics for all von Bertalanffy growth functions in the SL versus age model suite ($N = 121$). The global model has sex-specific $L_\infty$ and $k$ and $t_0$ unfixed; NSS = not sex-specific.

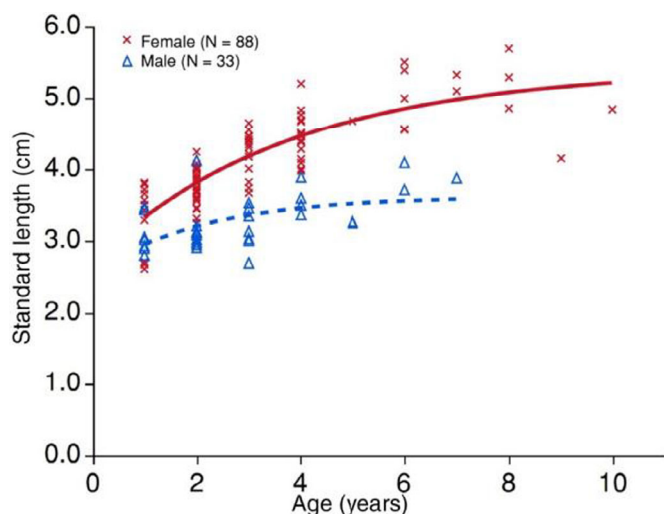| Model | SSE | $k$ | $AIC_c$ | $\Delta AIC_c$ | $F$ ratio | $P$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| Global | 12.8 | 5 | −259.4 | 0.0 | 91.0 | <0.001 | 0.76 |
| $k$ NSS | 13.7 | 4 | −253.3 | 6.1 | 111.7 | <0.001 | 0.74 |
| $L_\infty$ NSS | 14.7 | 4 | −244.7 | 14.6 | 101.4 | <0.001 | 0.72 |
| Full, $t_0 = 0$ | 19.4 | 4 | −211.1 | 48.3 | 67.3 | <0.001 | 0.63 |
| $k$ NSS, $t_0 = 0$ | 22.4 | 3 | −196.0 | 63.4 | 80.4 | <0.001 | 0.58 |
| $L_\infty$, $k$ NSS | 28.8 | 3 | −165.2 | 94.2 | 49.1 | <0.001 | 0.45 |
| $L_\infty$ NSS, $t_0 = 0$ | 32.1 | 3 | −152.2 | 107.1 | 38.1 | <0.001 | 0.39 |
| $L_\infty$, $k$ NSS, $t_0 = 0$ | 37.6 | 2 | −135.3 | 124.0 | 48.3 | <0.001 | 0.29 |



FIGURE 5. Growth in length. Standard length versus age for all aged Pahrump Poolfish in this study ($N = 121$) and the highest ranked ($F_{5,\,115}$ = 91.8, df = 5, $P < 0.001$, $R^2 = 0.76$) von Bertalanffy growth model (solid line = females, dashed line = males), with different parameters for asymptotic length ($L_\infty$) and growth rate ($k$) for females ($L_\infty = 5.4$, 95% CI [5.02, 6.21], $k = 0.262$ [0.147, 0.397]) compared with males ($L_\infty = 3.6$ [2.9, 4.1], $k = 0.464$ [0.309, 0.805]), with age-at-0 length parameter $t_0 = -2.6$ [−4.6, −1.5].

significantly larger and older than males. The maximum longevity for females and males was 10 years and 7 years, respectively. Further, females accounted for 7 of 8 individuals that were greater than 6 years old. Goodchild (2015) also noted sexual dimorphism for both the Shoshone Stock Pond population and another refuge population at Lake Harriet at Spring Mountain Ranch State Park, Nevada. Our work shows that sexual size dimorphism is evident for this species generally throughout its ontogeny. Indeed, model selection for both otolith growth and SL growth models indicate significant sexual dimorphism.

In the case of Pahrump Poolfish, these findings have implications for founding new populations of this species in novel habitats. For example, 96% of all of the aged fish that were at least 4.0 cm were females. Thus, future transplants should only include individuals below this size threshold to avoid a highly unequal sex ratio. This is especially important for establishing new refuge populations, as a balanced sex ratio is predicted to increase the effective genetic population size (Wright 1969; Frankham 1995).

Because age estimates from thin-sectioned otoliths require sacrificing the individuals, the authors recommend the prudent use (i.e., investigation of otoliths) of any Pahrump Poolfish that succumb to natural mortality or can otherwise be investigated for otolith analysis. The authors recommend age validation on thin-sectioned otoliths of this species (e.g., via edge analysis or oxytetracycline mark–recapture) to conclusively determine whether the opaque banding structure that is documented is indeed annual. Until such work is done, the authors advise using otolith age-demographic data to inform policy.

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