THE SOUTHWESTERN NATURALIST

EVALUATION OF OTOLITHS OF SALT CREEK PUPFISH (*CYPRINODON SALINUS*) FOR USE IN ANALYSES OF AGE AND GROWTH

Maria C. Dzul,* D. Bailey Gaines, Jesse R. Fischer, Michael C. Quist, and Stephen J. Dinsmore

Department of Natural Resource Ecology and Management, Iowa State University, 339 Science II, Ames, IA 50011 (MCD, JRF, SJD)
National Park Service, Death Valley National Park, 1321 South Highway 160, Pahrump, NV 89048 (DBG)
United States Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, Box 441141, Moscow, ID 83844 (MCQ)
**Correspondent: mariadzul@gmail.com*

The Southwestern Naturalist 57(4): 412–416 December 2012

EVALUATION OF OTOLITHS OF SALT CREEK PUPFISH (*CYPRINODON SALINUS*) FOR USE IN ANALYSES OF AGE AND GROWTH

Maria C. Dzul,* D. Bailey Gaines, Jesse R. Fischer, Michael C. Quist, and Stephen J. Dinsmore

Department of Natural Resource Ecology and Management, Iowa State University, 339 Science II, Ames, IA 50011 (MCD, JRF, SJD)
National Park Service, Death Valley National Park, 1321 South Highway 160, Pahrump, NV 89048 (DBG)
United States Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, Box 441141, Moscow, ID 83844 (MCQ)
**Correspondent: mariadzul@gmail.com*

Abstract—We collected Salt Creek pupfish (*Cyprinodon salinus salinus*) from Salt Creek, Death Valley, California, in November 2009 and May 2010. The purpose of our study was to determine whether otoliths displayed interpretable marks that might be used for estimating age and growth. Otoliths exhibited alternating bands of opaque and translucent material. Kendall rank correlation between number of bands on otoliths and length of fish were high for two readers ($\tau = 0.65$ and 0.79) and exact agreement between readers was 51%. Otoliths exhibited 0–5 bands, which provided evidence that longevity of Salt Creek pupfish likely is >1 year. Total length of fish collected in spring and autumn differed for fish with one and three bands on otoliths.

Resumen—Recogimos ciprinodóntidos de Salt Creek (*Cyprinodon salinus salinus*) en Salt Creek, Death Valley, California, en noviembre de 2009 y mayo de 2010. El propósito de nuestro estudio fue de determinar si otolitos exhibieron señales interpretables para estimar la edad y crecimiento. Los otolitos exhibieron franjas de materia alternativamente opaca o traslúcida. La correlación de rangos de Kendall entre la cantidad de franjas en los otolitos y el largo de los peces fue alta para dos leedores ($\tau = 0.65$ y 0.79) y el porcentaje de acuerdo exacto entre los dos leedores fue 51%. Los otolitos exhibieron 0–5 franjas, sugiriendo que la longevidad de los ciprinodóntidos de Salt Creek probablemente sea >1 año. El largo total de los peces recogidos en la primavera y en el otoño se diferenciaron entre peces con una y tres franjas en los otolitos.

Hard structures (e.g., otoliths, scales, spines, opercles) commonly are used to evaluate age and growth of fishes. Fishes that experience predictable metabolic changes on an annual basis (i.e., changes in growth due to season) often exhibit alternating opaque and translucent bands (annuli) on their otoliths. In these instances, otoliths can provide a historical record of patterns of somatic growth of individual fish as well as an estimate of age. Due to the importance of information on age and growth for understanding the ecology of a species, studies focused on applications of age and growth are numerous, including assessment of environmental influences on structure of populations (Burke et al., 1993; Korman and Campana, 2009; Goto and Wallace, 2010), evaluation of management practices (Olson et al., 1998; Quist et al., 2002; Fischer et al., 2010; Stewart, 2011), and determination of life-history characteristics (L'Abee-Lund et al., 1989; Jacobsen and Bennett, 2010, Loewen et al., 2010). Although data on age and growth are valuable, obtaining such information is dependent on whether hard structures record interpretable information. Formation of annuli often is influenced by changes in water temperature (Schramm, 1989; Neat et al., 2008), although seasonal changes in the wet–dry cycle (Warburton, 1978) and availability of food (Schramm, 1989) also can influence formation. Timing of formation of bands can help determine seasonal periods of stress (Scheerer and McDonald, 2003) due to increased energetic demands (e.g., spawning) or reduced metabolic activity (e.g., as a result of decreased water temperature). However, Johnson and Belk (2004) reported that otoliths of juvenile Utah chubs (*Gila atraria*) inhabiting a constant-temperature, desert-stream environment still exhibited seasonal patterns of growth, illustrating that mechanisms associated with formation of annuli may not be apparent.

Pupfishes (Cyprinodontidae) are known for their ability to tolerate wide ranges of temperature and salinity (Moyle and Cech, 1982). As such, certain species inhabit isolated desert environments that experience large annual fluctuations in water temperature, size of habitats, and salinity. While other studies have determined ages of pupfishes using scales (Jester and Suminski, 1982; Garcia-Berthou and Moreno-Amich, 1992), we are unaware of published studies that have used otoliths to determine age of pupfishes. Unlike scales, otoliths typically are not resorbed during periods of high stress (Marshall and Parker, 1982; DeVries and Frie, 1996). In addition, otoliths often provide more precise estimates of age relative to scales (e.g., Welch et al., 1993; Lowerre-Barbieri et al., 1994). One major disadvantage of using otoliths in analyses of growth is that it requires organisms to be sacrificed to extract otoliths. Consequently, use of otoliths for analyses of age and growth is impractical for imperiled (i.e., endangered, threatened) fishes.

Located on the floor of Death Valley, Inyo County, California, Salt Creek is a dynamic desert stream that is home to only one aquatic vertebrate, the endemic Salt Creek pupfish (*Cyprinodon salinus salinus*). Salt Creek is a temporally dynamic environment that experiences large annual fluctuations in wetted area, water chemistry, and water temperature. Water temperature of Salt Creek varies from near freezing (0°C) in winter to 40°C in summer, although there is less fluctuation in temperatures in deeper pools, which have an average temperature of 28°C (Moyle, 2002). Likewise, salinity can vary from 9.9 to 72.3 parts per thousand in different reaches of Salt Creek at different times of year, typically reaching highest values in shallow reaches in summer (Sada and Deacon, 1995). Pupfish inhabit $\leq$5 km of wetted length in Salt Creek during winter, but only 1.5 km of stream in summer due to a decrease in wetted length from high rates of evaporation (Moyle, 2002). Size of the population of Salt Creek pupfish also fluctuates annually by two orders of magnitude in response to environmental characteristics (Sada and Deacon, 1995). The population grows quickly during spring due to high reproductive output and declines in summer due to high mortality associated with evaporation of Salt Creek. Salt Creek pupfish exhibit two spawning peaks, one in spring and one in early autumn (Sada and Deacon, 1995). The Salt Creek pupfish does not receive federal protection but is listed as a Species of Special Concern by California Department of Fish and Game.

Because many pupfishes are subjected annually to various environmental and biological stressors, the influence of season on growth is poorly understood. For example, winter and summer could be seasons of reduced growth for Salt Creek pupfish. Salt Creek pupfish become inactive in winter, presumably due to low water temperatures (Sada and Deacon, 1995). In summer, Salt Creek pupfish experience high water temperatures and increased intraspecific competition for food. Furthermore, spring and autumn are periods of increased spawning activity and might result in decreased somatic growth of mature pupfish. As such, Salt Creek pupfish experience stressful conditions throughout the year, which might obscure patterns of growth on otoliths and preclude their use as a structure for evaluating age and growth. Thus, the question of whether otoliths contain interpretable marks is not trivial given that Salt Creek pupfish live in one of the harshest and most dynamic aquatic environments in North America. We examined otoliths from Salt Creek pupfish to determine whether otoliths showed alternating bands of opaque and translucent material that might correspond to annuli, there was a correlation between number of bands on otoliths and length of fish, and identification of marks was reproducible between two independent readers (i.e., verification). No validation of annuli was attempted; rather, our study represents an important step in determining whether an elaborate validation study might be warranted.

Materials and Methods—We collected Salt Creek pupfish from Salt Creek in November 2009 and May 2010. To ensure that all categories of size were represented in the sample, 10 individuals/5-mm-length group for fish 15–20 and 35–55 mm in total length, and 20 individuals/5-mm-length group for fish 21–35 mm were sampled in November 2009. To further increase size and resolution of samples, we collected 10 individuals/2-mm-length group in May 2010. Fish were preserved in 95% ethanol and transported to the laboratory for processing of otoliths.

Total length of each fish was measured using digital calipers. Otoliths were removed dorsally from fish using forceps following Morales-Nin (1992). When possible, both sagittal otoliths were removed. Otoliths were mounted whole on a glass microscope slide using thermoplastic cement. A small drop of immersion oil was placed on the otoliths, and otoliths were then placed on the stage of a microscope and examined at 56× magnification. A growth band was identified as an adjacent pair of opaque and translucent markings that formed complete, uninterrupted bands around the otolith. Otoliths were read independently by two readers. If readers disagreed on age of an otolith, the otolith was later re-read by both observers together. If observers could not agree on number of bands on an otolith, it was removed from analyses.

All statistical analyses were conducted in R version 2.8.1 (R Development Core Team, Vienna, Austria). Kendall's rank correlation coefficient (τ) was used to evaluate the relationship between number of growth bands on otoliths and length of fish. Percentage of exact agreement between readers was calculated by dividing number of otoliths with exact agreement by total number of otoliths read. After both readers agreed on number of bands on otoliths, specimens were grouped by date of collection and number of annuli exhibited on otoliths. Hereafter, these groupings are referred to as cohorts. Mean lengths were calculated for each cohort from each date of collection. Mean lengths of cohorts from both dates of collection were compared using a two-sample t-test for unequal variances and unequal samples (Zar, 2010).

Results—A total of 282 Salt Creek pupfish was collected during both sampling periods. The collection in November 2009 included 108 fish, while 174 fish were collected in May 2010. Otoliths exhibited interpretable alternating bands of opaque and translucent material, although otoliths varied in quality (Fig. 1). Estimates of the number of bands by both readers were correlated positively with total length. Percentage of exact agreement between readers was 51%. Of the remaining 49% of otoliths where readers disagreed, 86% of estimates were within one band. Maximum difference between estimates was three bands. Reader 2 tended to overestimate number of bands relative to reader 1. Kendall's rank correlation between number of bands on

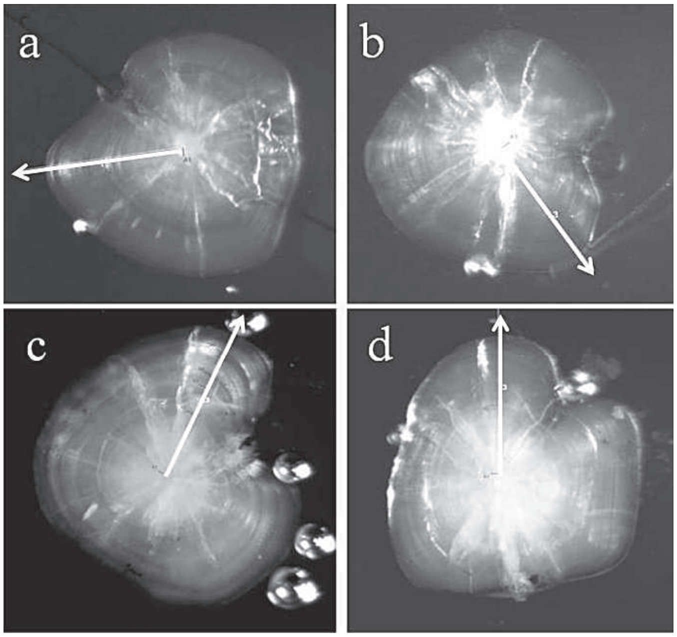

FIG. 1—Otoliths of Salt Creek pupfish (*Cyprinodon s. salinus*) in Salt Creek, Death Valley, Inyo County, California, varied in quality and sometimes in assessments of number of bands by two readers: (a) estimated as two bands by both readers; (b) estimated as one band by reader 1 and zero bands by reader 2; (c) estimated as four bands by both readers; (d) estimated as three bands by reader 1 and two bands by reader 2. Expression of banding patterns varied among otoliths, with some showing more subtle bands. Bands near the center of large otoliths tended to be opaque and difficult to see. Cracking in large otoliths was common and also obscured banding patterns.

otoliths and lengths of fish increased after both readers had reached consensus. Percentage agreement increased to 99.6% after both readers determined age of otoliths together. Only one otolith did not display bands. The cohort-0 otolith was from a 15.8-mm individual collected in November 2009. Differences in mean lengths were observed between sampling periods. Specifically, mean length of fish from odd-numbered cohorts (i.e., one, three, and five bands) collected in November 2009 were larger than respective cohorts collected in May 2010 (Table 1). However, mean lengths of all other cohorts (i.e., two and four bands) were similar between collection periods. Measurement of widths of bands was attempted; however, transitions between adjacent bands often were gradual and not abrupt. Consequently, determination of exact measurement points was highly subjective. Notably, boundaries between bands were more discernible in small otoliths (i.e., from fish aged 0–2) than for larger otoliths, thereby, suggesting that studies of growth might be feasible for fishes with 0–2 bands in otoliths.

DISCUSSION—High concordance between estimates of age and total length of the fish by both readers suggests that there is strong evidence of predictable seasonal

TABLE 1—Mean total length (size of sample in parentheses) of different cohorts of Salt Creek pupfish (*Cyprinodon s. salinus*) collected from Salt Creek, Death Valley, Inyo County, California, in November 2009 and May 2010. *P*-values were generated from a *t*-test for unequal samples.

Cohort	November 2009	May 2010	*P*-value
0	15.8 (1)	—	—
1	23.4 (40)	25.2 (29)	<0.01
2	30.8 (25)	30.7 (38)	0.55
3	38.3 (19)	40.8 (65)	0.02
4	48.8 (15)	49.0 (33)	0.43
5	50.9 (5)	53.9 (3)	0.06

effects on somatic growth of Salt Creek pupfish that is recorded on their otoliths. Detection of five bands on otoliths is surprising, as longevity of cyprinodontids is believed to be ca. 1 year (Kodric-Brown, 1977; Soltz and Naiman, 1978). Because validation of bands was not attainable in our study, more research is necessary to determine the length of time associated with formation of bands in otoliths. Accordingly, we cannot conclude that longevity of Salt Creek pupfish exceeds 1 year because we do not understand seasonal patterns of growth. In particular, multiple bands might form on otoliths during a year due to short-term fluctuations in environmental conditions. Similar to results of our study, Jester and Suminski (1982) estimated that scales of the White Sands pupfish *Cyprinodon tularosa* displayed 1–5 bands. Additionally, mean lengths at age of Salt Creek pupfish were smaller compared to those of *C. tularosa* as estimated from scales by Jester and Suminski (1982). In contrast to Jester and Suminski (1982) and our study, Garcia-Berthou and Moreno-Amich (1992) estimated that *Aphanius iberus,* a cyprinodontid endemic to southern Spain, displayed ≤2 bands on scales.

Although we detected differences between collections of fish in May and November with one, three, and five bands, it is unclear whether these values have an interpretable biological meaning or if they are the result of some form of sampling error. The difference between lengths of fish from cohort 1 might be attributed to a greater range of smaller fish in the collection in November compared to May. Specifically, the collection in November included three fish with total length <20 mm, whereas the collection in May did not include any fish <20 mm. Because otoliths can serve as records of local environmental conditions, there is another possible biological explanation for the observed pattern of unequal lengths in fish with odd numbers of bands. Specifically, Salt Creek pupfish might annually experience two slow-growth periods (i.e., winter and summer) and two fast-growth periods (i.e., spring and autumn). Under this circumstance, otoliths from pupfish spawned in autumn would display a different number of bands compared to otoliths from fish spawned in spring.

Moreover, if growth in spring differed from growth in autumn, only fish with odd numbers of bands on otoliths would have unequal lengths when spring and autumn collections were compared; a result supported by our data. In addition to growth-season dynamics, other factors such as microhabitat, sex, and long spawning periods likely influence patterns of growth and, thereby, contributed to variability in growth among individual pupfish. Our study was designed to determine if otoliths were viable structures to determine age and growth of Salt Creek pupfish and not to rigorously test the observed pattern of growth and potential influences. Thus, future studies attempting to determine age of Salt Creek pupfish should collect specimens from the same sampling sites, sample equal numbers of males and females from each length group, target small fishes <15 mm total length, and attempt to quantify spawning.

In conclusion, age and growth of otoliths of *Cyprinodon* remains unexplored, yet we provide preliminary evidence that studies of age and growth might be fruitful. Life-history characteristics of many cyprinodontids are not well understood because tagging individuals with unique marks is difficult due to their small size. As such, studies of age and growth could provide valuable information about relationships between pupfishes and their environments. For example, studies of age and growth could help quantify differences in survival and growth of Salt Creek pupfish spawned in spring compared to autumn. However, a validation study of pupfishes must occur before otoliths are used to make inference about age, growth, or both.

We thank K. Wilson (National Park Service), M. Bower (United States Forest Service), S. Kyriazis (National Park Service), and S. Parmenter (California Department of Fish and Game) for assistance with logistics and for ecological insight. We thank S. Wilson and M. Colvin for helpful comments on an early version of the manuscript. This project was supported, in part, by Iowa State University, the Death Valley Natural History Association, and the Idaho Cooperative Fish and Wildlife Research Unit. The research was conducted under Iowa State University Institutional Animal Care and Use Committee protocol 1-10-6851-I. The use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the United States Government.

Literature Cited

Burke, J. S., D. S. Peters, and P. J. Hanson. 1993. Morphological indices and otolith microstructure of Atlantic croaker, *Micropogonias undulatus*, as indicators of habitat quality along an estuarine pollution gradient. Environmental Biology of Fishes 36:25–33.

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

Fischer, J. R., M. C. Quist, S. L. Wigen, A. J. Schaefer, T. W. Stewart, and T. M. Isenhart. 2010. Assemblage and population-level responses of stream fish to riparian buffers at multiple spatial scales. Transactions of the American Fisheries Society 139:185–200.

Garcia-Berthou, E., and R. Moreno-Amich. 1992. Age and growth of an Iberian cyprinodont, *Aphanius iberus* (Cuv. & Val.), in its most northerly population. Journal of Fish Biology 40:929–937.

Goto, D., and W. G. Wallace. 2010. Bioenergetic responses of a benthic forage fish (*Fundulus heteroclitus*) to habitat degradation and altered prey community in polluted salt marshes. Canadian Journal of Fisheries and Aquatic Sciences 67:1566–1584.

Jacobsen, I. P., and M. B. Bennett. 2010. Age and growth of *Neotrygon picta, Neotrygon annotata* and *Neotrygon kuhlii* from North-east Australia, with notes on their reproductive biology. Journal of Fish Biology 77:2405–2422.

Jester, D. B., and R. R. Suminski. 1982. Age and growth, fecundity, abundance, and biomass production of the White Sands pupfish, *Cyprinodon tularosa*, in a desert pond. Southwestern Naturalist 27:43–54.

Johnson, J. B., and M. C. Belk. 2004. Temperate Utah chub form valid otolith annuli in the absence of fluctuating temperature. Journal of Fish Biology 65:293–298.

Kodric-Brown, A. 1977. Reproductive success and the evolution of breeding territories in pupfish (*Cyprinodon*). Evolution 31:750–766.

Korman, J., and S. E. Campana. 2009. Effects of hydropeaking on nearshore habitat use and growth of age-0 rainbow trout in a large regulated river. Transactions of the American Fisheries Society 138:76–87.

L'Abee-Lund, J. H., B. Jonsson, A. J. Jensen, L. M. Saettem, T. G. Heggberget, B. O. Johnsen, and T. F. Naesje. 1989. Latitudinal variation in life-history characteristics of sea-migrant brown trout *Salmo trutta*. Journal of Animal Ecology 58:525–542.

Loewen, T. N., D. Gillis, and R. F. Tallman. 2010. Maturation, growth, and fecundity of Arctic charr, *Salvelinus alpines* (L.), life-history variants co-existing in lake systems of southern Baffin Island, Nunavut, Canada. Hydrobiologia 650:193–202.

Lowerre-Barbieri, S. K., M. D. Chittenden, Jr., and C. M. Jones. 1994. A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. Fishery Bulletin 92:555–568.

Marshall, S. L., and S. S. Parker. 1982. Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths. Canadian Journal of Fisheries and Aquatic Sciences 39:542–547.

Morales-Nin, B. 1992. Determination of growth in bony fishes from otolith microstructure. Food and Agriculture Organization Fisheries Technical Paper 322:1–51.

Moyle, P. B. 2002. Inland fishes of California. University of California Press, Berkley.

Moyle, P. B., and J. J. Cech, Jr. 1982. Fishes: an introduction to ichthyology. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Neat, F. C., P. J. Wright, and R. J. Fryer. 2008. Temperature effects on otolith pattern formation in Atlantic cod *Gadus morhua*. Journal of Fish Biology 73:2527–2541.

Olson, M. H., S. R. Carpenter, P. Cunningham, S. Gafny, B. R. Herwig, N. P. Nibbelink, T. Pellet, C. Storlie, A. S. Trebitz, and K. A. Wilson. 1998. Managing macrophytes to improve fish growth: a multi-lake experiment. Fisheries 23(2):6–12.

Quist, M. C., C. S. Guy, M. A. Pegg, P. J. Braaten, C. L. Pierce, and V. H. Travnichek. 2002. Potential influence of harvest on shovelnose sturgeon populations in the Missouri River system. North American Journal of Fisheries Management 22:537–549.

Sada, D. W., and J. E. Deacon. 1995. Spatial and temporal variability of pupfish (genus *Cyprinodon*) habitat and populations at Salt Creek and Cottonball Marsh, Death Valley National Park, California. National Park Service Report 8000-2-9003:1–76.

Scheerer, P. D., and P. J. McDonald. 2003. Age, growth, and timing of spawning of an endangered minnow, the Oregon chub (*Oregonichthys crameri*), in the Willamette Basin, Oregon. Northwestern Naturalist 84:68–79.

Schramm, H. L., Jr. 1989. Formation of annuli in otoliths of bluegills. Transactions of the American Fisheries Society 118:546–555.

Soltz, D. L., and R. J. Naiman. 1978. The natural history of native fishes in the Death Valley system. Natural History Museum of Los Angeles County, Science Series 30:1–76.

Stewart, J. 2011. Evidence of age-class truncation in some exploited marine fish populations in New South Wales, Australia. Fisheries Research 108:209–213.

WarBurton, K. 1978. Age and growth determination in a marine catfish using an otolith check technique. Journal of Fish Biology 13:429–434.

Welch, T. J., M. J. Van Den Avyle, R. K. Betsill, and E. M. Driebe. 1993. Precision and relative accuracy of striped bass age estimates from otoliths, scales, and anal fin rays and spines. North American Journal of Fisheries Management 13:616–620.

Zar, J. H. 2010. Biostatistical analysis. Fifth edition. Prentice Hall, Inc., Upper Saddle River, New Jersey.

Submitted 10 July 2011. Accepted 24 May 2012.
Associate Editor was Christopher M. Taylor.