SHOSHONE PUPFISH
Cyprinodon nevadensis shoshone (Miller)

Status: Critical Concern. The Shoshone pupfish faces extinction in the wild, given that just one confirmed population exists in a small, tenuous, artificial habitat.

Description: The Shoshone pupfish is a subspecies of the Amargosa pupfish (*Cyprinodon nevadensis).* All *C. nevadensis* subspecies are deep-bodied (especially in reproductive males), with total lengths that rarely exceed 50 mm. The head is blunt and slopes steeply to a small, terminal, oblique mouth. There is one row of tricuspid teeth on each jaw, with the central cusps being truncated or pointed. The morphology of the Shoshone subspecies is similar to the Saratoga Springs pupfish, *C. n. nevadensis*. However, it is characterized by larger scales and a somewhat narrower, more slender body. It also has fewer pelvic fin rays and scales than other subspecies of *C. nevadensis* (See Table 1). Reproductive males in breeding colors are bright blue with a black band at the posterior edge of the caudal fin. Reproductive females are drab olive-brown and develop 6-10 vertical bars along the sides which are often indistinct. An ocellus (eyespot) is typically present on the posterior base of the dorsal fin of females.

Taxonomic Relationships: The fossil record and past geologic events suggest that the western *Cyprinodon* species differentiated relatively recently, with most of this differentiation occurring during the early to mid-Pleistocene (Miller 1981). As the numerous large pluvial lakes in the Great Basin shrank after the Pleistocene, remnant populations of pupfish survived in isolation, leading to subspecific differentiation within *C. nevadensis*. Speciation of the Devils Hole pupfish (*C. diabolis*), which, together with *C. radiosus*, *C. nevadensis* and *C. salinus*, constitutes the *C. nevadensis* species complex, possibly occurred less than 20,000 years ago (Echelle and Echelle 1993). Further differentiation may have occurred in the last 10,000 years, following the final recession of pluvial waters.

C. nevadensis was first described from Saratoga Springs (now in Death Valley National Park) by Eigenmann and Eigenmann (1889). Following the initial description, the species was lumped with the desert pupfish (*C. macularius)* until Miller (1943) separated it again. Miller (1948) described six subspecies of *C. nevadensis*, four of which occur in California (The Saratoga Springs pupfish (*C. n. nevadensis)*, the Amargosa River pupfish (*C. n. amargosae)*, the Shoshone pupfish (*C. n. shoshone)*, the Tecopa pupfish (*C. n. calidae*) and two in Nevada (The Ash Meadows pupfish (*C. n. mionectes)* and the Warm Springs pupfish (*C. n. pectoralis*). *C. n. calidae* is now extinct (Moyle 2002).

At the time of their formal description, Shoshone pupfish were understood to exhibit a high degree of morphological variation between the headspring and downstream areas. Miller (1948) speculated on the origins of this variation and recommended but did not pursue studies to separate potential genetic and environmental causes. No pupfish have been observed below Old Highway 127 since before 2010, so the extant pupfish at Shoshone probably all descended from the captive breeding programs and reintroduction (S. Parmenter, CDFW, pers. comm. 2013) described in the Trends in Abundance section of this account.

Measure/ Count	*C. n. amargosae*			*C. n. nevadensis*			*C. n. shoshone*		
	male		female	male		female	male		female
		ALL			ALL			ALL	
Standard length (mm)		36			40			34	
*Body width	256		265	274		269	231		229
*Head length		305			312			307	
*Head depth	330		304	367		343	331		311
*Head width	240		259	257		256	233		231
*Snout length		101			97			89	
*Mouth width		117			115			114	
*Mandible length		198			95			93	
*Anal origin to caudle base	338		346	394		362	371		355
*Caudle peduncle length	264		237	277		253	263		251
*Anal fin base length	116		105	111		105	108		101
*Anal fin length	330		304	227		195	217		190
*Pelvic fin length	98		89	95		87	90		77
Anal fin ray count		10			10			10	
Dorsal fin ray count		10			10			10	
Pelvic fin ray count		6			6			4	
Pectoral fin ray count		16			16			16	
Caudal fin ray count		18			17			18	
Lateral line scales		26			26			26	
Predorsal scale count		19			18			18	
Dorsal fin to pelvic fin scale count		11			10			9	
Caudal peduncle circumference scale count		16			16			15	
Body circumference scale count		27			25			23	

*Expressed as percent of standard length x 1000.

Table 1. Comparative average morphometrics and meristics of three *Cyprinodon nevadensis* subspecies. Adapted from Miller (1948).

Life History: Shoshone pupfish exhibit many characteristics that adapt them to live in habitats with thermal and osmotic extremes (Miller 1981). The life-history characteristics of this subspecies, however, have not been studied in detail but are likely similar to the Saratoga Springs pupfish (*C. n. nevadensis*), for which the following characteristics are known. Optimal temperature for growth is 22˚C, with growth ceasing below 17˚C and above 32˚C. At optimal temperatures, growth is extremely rapid and fish reach sexual maturity within four to six weeks (Miller 1948). Such short generation time enables small populations to remain viable. Generation time varies among subspecies, with populations living in widely fluctuating environmental conditions exhibiting shorter generation times (Moyle 2002). Young adults (15-30 mm SL) of *C. nevadensis* usually constitute a majority of the biomass throughout the year (Naiman 1976). Highest densities and peak breeding season occur during summer, when water temperatures are higher and food is abundant (Kodric-Brown 1977). However, breeding may occur year-round in thermally stable habitat. Like the Saratoga Springs pupfish, the Shoshone pupfish presumably once bred year-round.

The Shoshone pupfish, like other spring-dwelling subspecies, exhibits reproductive behavior different from riverine forms (Kodric-Brown 1981). The males of spring-dwelling subspecies establish display territories. Both sexes are promiscuous and a single female may lay eggs with different males over time. The demersal eggs are sticky and, thus, adhere to substrates. Females may lay a few eggs each day (not necessarily on consecutive days) throughout the year. Territorial defense by males may confer some protection of eggs from predators, but otherwise parental investment is limited to gamete production (Kodric-Brown 1981).

Despite the Shoshone pupfish's ability to survive in a wide range of extreme conditions, their reproductive tolerance limits are likely narrow, 24-30° C, optimal being 28-29° C. The most sensitive phase of life history to thermal stress is oogenesis (Gerking 1981). Extreme temperatures affect egg production and egg viability (Shrode and Gerking 1977, Gerking 1981) and reproduction is greatly diminished at pH levels below 7 (Lee and Gerking 1980). Furthermore, reproductive performance does not improve despite generation-long acclimation to suboptimal temperatures (Gerking et al. 1979). Thus, any alterations to their habitat that would result in temperatures outside the range of their reproductive temperature optima would be potentially deleterious. Fertilized eggs, however, become resistant to environmental stresses within hours of being laid.

Shoshone pupfish, like other pupfishes, likely feed primarily on blue-green cyanobacteria and algae but will feed seasonally on small invertebrates, mostly chironomid larvae, ostracods, and copepods (Naiman 1975, 1976). Pupfishes forage continuously from sunrise to sunset and become inactive at night and have long convoluted guts characteristic of aquatic herbivores and teeth adapted for nipping (Moyle 2002).

Habitat Requirements: Historically, two holes in the upper portion of the Shoshone Springs province above the Old State Highway 127 provided velocity refuge for Shoshone pupfish from the channel's swift flows (Miller 1948). The larger, upper hole (known as Squaw Hole) was about 1 m in diameter and 0.75 m deep. The water was clear, with overhanging banks, and the pool bottom was muddy. Shoshone Spring water had lower salinity and boron content than other springs in the region but more calcium.

Miller (1948) noted the largest numbers of pupfish occurred on either side of Old Highway 127, where temperatures were cooler and more variable and channel slope was reduced. Since Miller's description in 1948, Shoshone Springs underwent severe alteration (Taylor et al. 1988, Castleberry et al. 1990). Notably, the present habitat for Shoshone pupfish also hosts one of 5 known populations of Sanchez's Springsnail (*Pyrgulopsis sanchezi*), *Tryonia variegata* (a regionally endemic springsnail), abundant non-native Red-rimmed Melania snails (*Melanoides tuberculata*); however, mosquitofish are absent (S. Parmenter, CDFW, pers. comm. 2013).

Distribution: The Shoshone pupfish was formerly found in Shoshone Spring and throughout its outlet creek in Inyo County (Miller 1948, Taylor et al. 1988). The spring source is at an elevation of 518 m, about 170 m above State Highway 127 on the east slope of a rocky lava hill. By approximately 50 years ago, most of the spring vents were enclosed and their water diverted to supply the town of Shoshone. In the 1980s, a small refuge pond was dug and supplied with water flowing from the last uncaptured spring vent. The refuge pond is approximately 5 m X 13 m, with banks vegetated by three-square bulrush (Scirpus americanus). In recent years, cattail infestation along the outflow ditch has been controlled and pupfish now utilize 135 meters of flowing channel, including a total of 5 pools between 5 and 50 m2. An additional isolated 20 m2 unconnected refuge pond is under construction, to be supplied by piped water from the enclosed spring vents. Plumbing has been installed to provide a backup water supply should flow fail from the single uncapped spring, which solely supplies the extant pupfish habitat. Shoshone pupfish are expected to colonize or be introduced into additional habitat, consisting of a former catfish aquaculture pond downstream of Old Highway 127, in the near future. Below Highway 127, the ouflow ditch provides perennial flow to a short segment of the Amargosa River. Mosquitofish occur in the floodplain, but not in the spring channel managed for pupfish (S. Parmenter, CDFW, pers. comm. 2013).

Trends in Abundance: The Shoshone pupfish was once considered to be extinct (Selby 1977, CDFG 1980) but was rediscovered in 1986 (Taylor et al. 1988). Although the pupfish was found in "large numbers" through the outflow creek in the summer of 1986 (Taylor et al. 1988), its numbers had dwindled to perhaps less than 20 individuals by 1988 (J. Williams, unpubl. data). The decline may have been precipitated by the invasion of western mosquitofish (*Gambusia affinis*) into the outflow creek. Taylor et al. (1988) hypothesized that Shoshone pupfish survived in very low numbers until conditions became more favorable, when the population expanded. The pupfish may have passed through a genetic bottleneck during the period of severely reduced population size.

Because of the lack of suitable habitat and the abundance of mosquitofish, most Shoshone pupfish were removed from the wild and small stocks of approximately 12 fish each were kept at the University of Nevada, Las Vegas (UNLV), and the University of California, Davis (UCD). In the 1990s, captive-raised individuals from both UCD and UNLV were introduced into the refuge pond (Swift et al. 1993). By May, 2002 Shoshone pupfish still persisted in the pond, which was found to be 100% overgrown with cattail and only a handful of fish were detected. Since then, the property owner and CDFW have cooperated to improve and increase the available habitat. Sampling with Gee traps has produced captures of over 200 individuals, but no formal population estimates have been made (S. Parmenter, CDFW, pers. comm. 2013). Fish were common in July, 2010 when three of the authors visited the site.

Nature and Degree of Threats: Shoshone pupfish survive today due to human intervention in an intensively managed refuge habitat. They are particularly threatened by their extremely limited distribution and potential for additional genetic impacts, should their population size again shrink due to reduced flows, habitat alteration, invasion of alien species or other factors.

Agriculture and urbanization. As discussed in the Saratoga Springs pupfish account, pumping of ancient aquifers to supply water for farming, industrial-scale solar development, and, increasingly, the City of Las Vegas, has the potential to eventually reduce or eliminate flows from Shoshone Spring, if it is connected to the pumped aquifers. For potential threats to the groundwater source of Shoshone Spring, see the Amargosa River pupfish account in this report.

Rural residential. The existence of Shoshone pupfish is entirely dependent on the maintenance of artificial habitats. The spring is privately owned and its water is used as a water supply for the town of Shoshone, as well as for the pupfish refuge. Despite the fact that the current owners of the site are dedicated to the preservation of this unique fish, the fact that Shoshone pupfish are now largely confined to a small, artificial, habitat means that they are extremely vulnerable to random acts of vandalism, to the introduction of other fishes and pathogens into their habitat, to the degradation of the spring habitat by colonization of cattail, which can completely crowd out open water habitat, and to the potential for changes in land ownership and decreased commitment to their preservation.

Alien species. Introduced mosquitofish undoubtedly preyed on pupfish eggs and young and were the immediate factor threatening the pupfish with extinction before they were rescued. While no mosquitofish were observed in the refuge spring, they may prevent the re-establishment of pupfish in the outflow ditch, even if water quality issues were corrected (e.g. chlorinated water from the swimming pool).

	Rating	Explanation
Major dams	n/a	
Agriculture	Medium	Regional ground water pumping for agriculture, even at great distances, has potential to affect flow in Shoshone Spring
Grazing	n/a	
Rural residential	Critical	Diversion of Shoshone Springs for use as a water supply for the town of Shoshone nearly caused extinction
Urbanization	Low	Regional ground water pumping to support Las Vegas and to service industrial-scale solar farms has potential to affect flow in Shoshone Spring
Instream mining	n/a	
Mining	Low	Occurs in area but no known impact
Transportation	Low	Spring outlet affected by road
Logging	n/a	
Fire	n/a	
Estuary alteration	n/a	
Recreation	Medium	Public access to the only known habitat increases risk of pollution, as well as introduction of alien species and pathogens
Harvest	n/a	
Hatcheries	n/a	
Alien species	High	In the past, interactions with mosquitofish was major factor in declines, but the greatest threat is now degradation of open water habitat by cattails

Table 2. Major anthropogenic factors limiting, or potentially limiting, viability of populations of Shoshone pupfish. Factors were rated on a five-level ordinal scale where a factor rated “critical” could push a species to extinction in 3 generations or 10 years, whichever is less; a factor rated “high” could push the species to extinction in 10 generations or 50 years whichever is less; a factor rated “medium” is unlikely to drive a species to extinction by itself but contributes to increased extinction risk; a factor rated “low” may reduce populations but extinction is unlikely as a result. A factor rated “n/a” has no known negative impact. Certainty of these judgments is high. See methods section for descriptions of the factors and explanation of the rating protocol.

Effects of Climate Change: As one might expect of oasis species, climate change poses a direct threat to the continued existence of desert pupfish species, including Shoshone pupfish. Although all pupfishes of the American southwest are remarkably well adapted to the wide range of salinity and temperature found in their arid range, they are also remarkably vulnerable to change. Isolated desert springs and rivers fed by subsurface flow systems are precarious ecosystems, vulnerable to geologic and anthropogenic disruption. Fed by rain and snow melt at high elevation in the desert mountain ranges (Riggs and Deacon 2004), desert aquifers in the Death Valley region will likely receive less recharge as the regions warms. This will be compounded by the growing human demand for water in southern Nevada, which will invariably increase as the climate becomes hotter and drier. Moyle et al. (2013) considered Shoshone pupfish as "highly vulnerable" to extinction as the result of climate change, but other factors are more likely to drive it to extinction first.

Status Determination Score = 1.1 – Critical Concern (see Methods section, Table 2). The only purported population of Shoshone pupfish exists in a small, artificial, habitat and is one of the most endangered fishes in California (Table 2). It is possible that this population is comprised of hybrids, in which case the Shoshone pupfish may already be extinct. Jelks et al. (2008) list it as Endangered, while NatureServe considers it to be "Imperiled" with a high risk of extinction due to very restricted range.

It remains unclear if the fish currently in Shoshone Spring are distinct from Amargosa River pupfish (*C. n. amargosae*). If genetic analysis indicates that the two populations are the same, then the Shoshone pupfish is either extinct or was never genetically distinct to begin with. If the results, instead, confirm a distinct genetic lineage, then the Shoshone pupfish will also bear the dubious distinction of being one of the most threatened fish in California.

Metric	Score	Justification
Area occupied	1	Confined to one heavily modified springbrook
Estimated adult abundance	1	Population extremely small
Intervention dependence	1	Additional refuge populations must be established
Tolerance	2	Although remarkably adapted to high temperatures and salinities, outside their refuge they encounter conditions at the very edge of their reproductive tolerances
Genetic risk	1	Single, small, population is vulnerable to genetic bottlenecking and/or drift
Climate change	1	Threatened by increases in temperature and reductions of flow resultant from limited recharge of the aquifer, compounded by increasing human water demand
Anthropogenic threats	1	See Table 2
Average	1.1	8/7
Certainty (1-4)	4	Well studied

Table 3. Metrics for determining the status of Shoshone pupfish, where 1 is a major negative factor contributing to status, 5 is a factor with no or positive effects on status, and 2-4 are intermediate values. See methods section for further explanation.

Management Recommendations: Two artificial pools were created in the headsprings area of Shoshone Spring during 1988 and stocked with Shoshone pupfish from refuge populations at UCD and UNLV. The pools were subsequently enlarged into a single pool that serves as the principal refuge for the species. The headsprings area should be managed as a preserve and the refuge pool monitored frequently (at least monthly) to establish baseline water conditions and check for presence of alien fishes. In the past, cattails (*Typha* spp.) have threatened to completely take over the refuge pool, which would eliminate open water habitat (S. Parmenter, CDFW, pers. comm. 2009). Cattail control entails laborious hand removal. In an effort to prevent cattail reestablishment, S. Parmenter (CDFW) has cultivated naturally occurring three-square bulrush (*Schoenoplectus americanus*). Once established, the bulrush competes with and prevents cattail reinvasion and has the added benefit of not colonizing deeper water, thereby stabilizing open water habitat. The initial phase of this “gardening” is labor intensive, but is critical to the maintenance of preferred Shoshone pupfish habitat and, therefore, must continue. Funds should be permanently allocated for this endeavor.

The concrete ditch between the Old State Highway and State Highway 127 has experienced at least two fish kills due to chlorinated outflow from the swimming pool (D. Castleberry and B. Bolster, pers. comms.). Chlorinated discharges, therefore, must be avoided. Conditions in the outflow creek between State Highway 127 and the Amargosa River should be monitored to determine if it is suitable for pupfish reintroduction.

A groundwater study is also needed to identify the source aquifer to guard against overexploitation.

Figure 1. Distribution of Shoshone pupfish, *Cyprinodon nevadensis shoshone* (Miller), in California.