TEMPERATURE AND SALINITY RELATIONSHIPS OF THE NEVADAN RELICT DACE

Steven Vigg[1]

ABSTRACT.— The relict dace, *Relictus solitarius*, represents the only genus and species of fish native to Ruby, Butte, Goshute, and Steptoe valleys in northeastern Nevada. In their natural habitats temperature ranges 0–25 C and salinity 175–1,158 mg/liter. The upper median thermal tolerance limit (96-hr TL50) of Butte Valley relict dace was 30.6 C when acclimated at 18–20 C. Relict dace tolerated total dissolved solids (TDS) of 11,043 mg/liter with no mortality during 96-hr exposures, but experienced 100 percent mortality at concentrations of 15,759 mg/liter with a mean resistance time of 23 hours. Tolerance of relict dace to 30 C was lowered as TDS was increased from 2,845 to 5,652 mg/liter. The Butte Valley fish were slightly more resistant to elevated salinities than the Goshute Valley sample, and conversely the Goshute Valley sample may be slightly more resistant to elevated temperature.

The relict dace, *Relictus solitarius*, is listed as "of special concern" by the Endangered Species Committee of the American Fisheries Society (Deacon et al. 1979). However, it was recently taken off the State of Nevada's protected species list.

Before the present study there were no quantitative data on the habitat requirements of relict dace. The purpose of this research was to determine a provisional upper lethal temperature limit (96-hr TL50), and to conduct range finding tests on the total dissolved solids (TDS) tolerance of this unique fish.

Relict dace apparently evolved during the past 1.5 to 2.0 million years in the contiguous drainage basins of pluvial lakes Franklin, Gale, Waring, and Steptoe just south of the conjoining parts of the Lahontan and Bonneville basins (Hubbs et al. 1974). As these Pleistocene lakes desiccated over the last 10,000 years, the relict dace was the only genus and species of fish to survive in the remnant springs of contemporary Ruby, Butte, Goshute, and Steptoe valleys, which comprise some 14,682 km^2 in northeastern Nevada. The species also occurs in Spring Valley, where it is believed to be an introduction (Fig. 1).

The taxon was first described by Hubbs and Miller (1972) from extensive research dating back to 1934. As well as being morphologically distinct from its closest relatives, *Gila* and *Rhinichthys*, this endemic cyprinid is also unique in terms of genetics (Lugaski 1980) and geographical distribution (Smith 1978).

Relict dace was once the most abundant of the four fish species native to the north central Great Basin (Hubbs et al. 1974). The species is losing populations and habitat (Hardy 1979), due to predation and competition by exotic fish species, modification of natural springs into stock ponds and irrigation channels, and groundwater mining.

METHODS

Temperature and salinity bioassays were conducted on relict dace from November 1980 to March 1981. Test fish were transported from their natural habitats in Ruby, Butte, and Goshute valleys (Fig. 1) to the Desert Research Institute (DRI) Bioresources Center laboratory in Reno. Upon arrival at DRI the fish were maintained in 425-liter holding tanks a minimum of four days for observation of handling stress. During this time the fish were acclimated at 18–20 C in dechlorinated Truckee River water prior to testing.

Relict dace from three different sources (Franklin Lake, Atwood Ranch, and Phalan Spring) were tested for 96 hours over a temperature range of 29.0 to 34.3 C. Two replicates of five fish each were tested in static 19-liter aquaria for each treatment. The test

[1]Bioresources Center, Desert Research Institute, P.O. Box 60220, Reno, Nevada 89506.

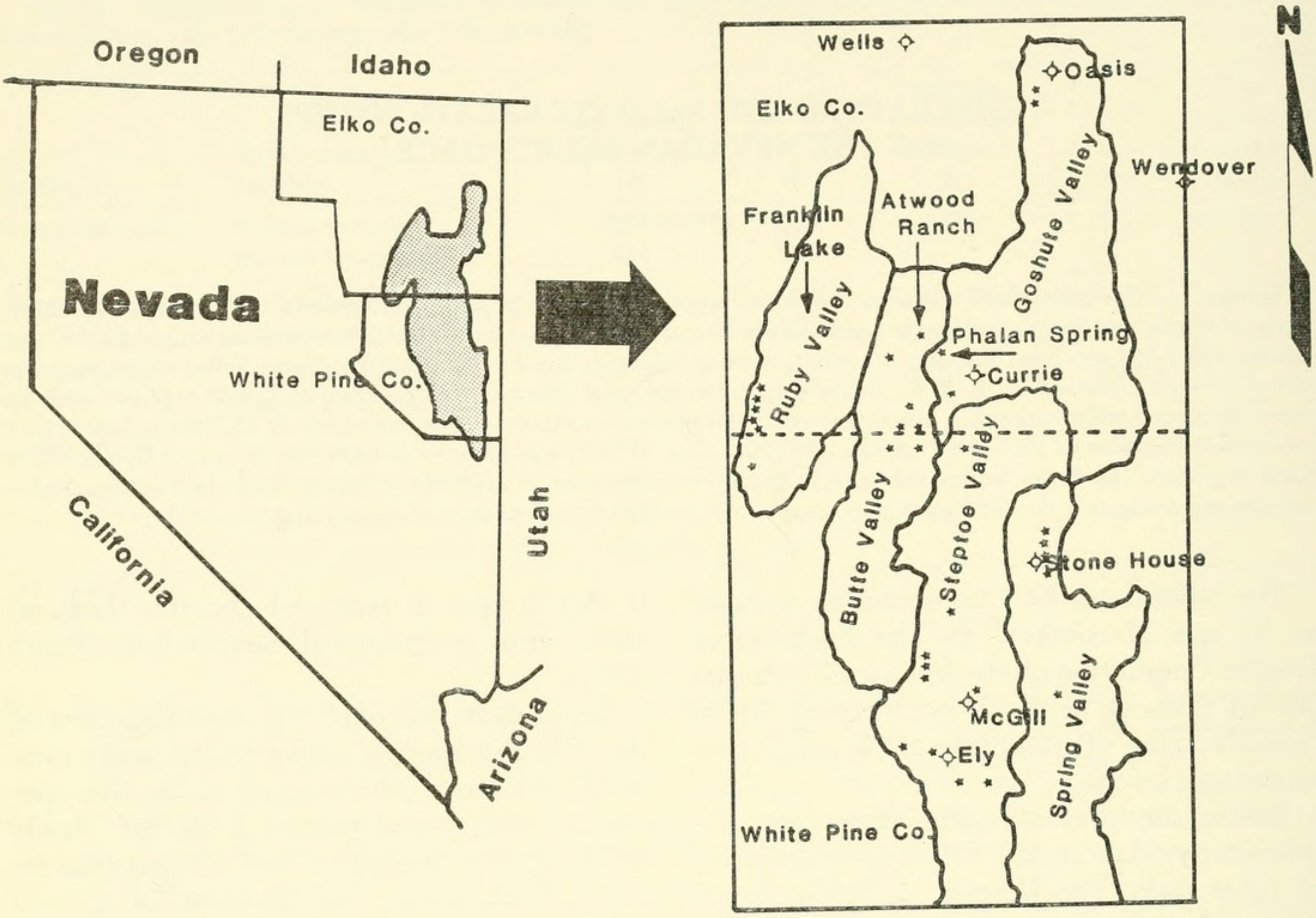

Fig. 1. Areal distribution of relict dace in five valleys in northeastern Nevada. The shaded area indicates known distribution of relict dace. Arrows mark the location of habitats where fish were collected. Asterisks (*) identify relict dace habitats documented by Hubbs et al. (1974).

aquaria were placed in a thermostatically controlled water bath to maintain a constant temperature (± 0.1 C). Five control fish were placed in dechlorinated Truckee River water in a 19-liter aquarium at room temperature (approximately 20 C) for each treatment; no control fish died.

Table 1. Salinity related measurements of test media for TDS-alkalinity bioassays with relict dace, *Relictus solitarius*.

Test media	TDS* (mg/*l*)	Total alkalinity (mg/*l*)	Specific conductance (μmhos/cm at 25 C)	pH
Truckee River	157	78	187	7.5
½ Pyramid Lake ½ Distilled	2,845	639	4,730	9.2
1X Pyramid Lake	5,652	1,262	8,560	9.2
2X Pyramid Lake	11,043	2,580	16,900	9.2
3X Pyramid Lake	15,759	3,670	22,900	9.2
4X Pyramid Lake	20,475	4,620	28,200	9.1

*Summation of all constituents.

Test fish were not fed 24 hours prior to the bioassay nor during the tests. Dissolved oxygen (DO) and pH were measured at least once and usually three times daily. The aquaria were continuously aerated, and mean DO values always exceeded 6 mg/liter. Temperature was measured with a calibrated digital thermometer at least three times daily in each aquarium.

Range finding tests were conducted to determine the TDS-alkalinity tolerance of relict dace. Concentrated Pyramid Lake water (2X, 3X, 4X) was used to achieve elevated salinity levels (Table 1). Fish were derived from two sources, Atwood Ranch Spring and Phalan Spring, to test for differences by population. Procedures similar to those of the thermal bioassays were used: replicates of five fish/test in 19-liter continuously aerated aquaria with selected environmental parameters monitored. Temperature was held constant (21.4–22.3 C) via the thermal water bath. Data were interpreted in terms of resistance time as well as mortality.

Another series of tests was performed to evaluate salinity-temperature interactions, utilizing only the Atwood Ranch Spring population. Pyramid Lake water (1X), and ½ Pyramid - ½ distilled water were utilized as the test media for normal (≃21–22 C) and elevated (≃30 C) temperatures. For all salinity tests, Truckee River water (low TDS) was used for controls. Electrical conductivity and alkalinity determinations for each treatment were determined by the DRI water chemistry lab, using standard analytical procedures (APHA et al. 1975). TDS was determined by summation of all measured constituents.

The total and mean weight of each group of five test fish were determined (in water) to the nearest 0.1 gram prior to testing. Dead fish were immediately removed and preserved in 10 percent formalin; time of each death was recorded. The criteria for death were no opercular movements and no response to touch. Statistical analyses were conducted utilizing programs from SPSS (Nie et al. 1975).

RESULTS

Temperature

Range finding tests were conducted on relict dace from Franklin Lake in the Ruby Valley. No mortality occurred at 29.0 C during 96 hours, and 100 percent mortality occurred at 34.3 C within 27 hours (10 fish/test). More definitive results were derived from the Butte Valley (Atwood Ranch) population with four replicate tests over a range of 30–31 C (Table 2). The 96-hr median tolerance limit (TL50) for relict dace collected at the Atwood Ranch Spring was 30.6 C.

Concurrent tests on relict dace from Atwood Ranch and Phalan Spring at 30.7 C indicated that the latter population may be slightly more resistant to high temperatures. The Phalan Spring population experienced only 40 percent mortality at 30.7 C, but the Atwood Ranch Spring fish suffered 80 percent mortality. The higher temperature tolerance of the Phalan Spring population may be due to the long-term physiological acclimatization at 19.5–23 C compared to 16.5–21 C at the Atwood Ranch Spring (Table 3).

TDS-Alkalinity

Relict dace tolerated total alkalinity (HCO_3^- + $CO_3^=$ as $CaCO_3$) and corresponding TDS of 2,580 mg/liter and 11,043 mg/liter, respectively, for 96 hours without experiencing any mortality (Table 4). TDS levels of 15,759 and 20,475 mg/liter resulted in 100 percent mortality of both populations of relict dace tested. Analysis of variance illustrated a significant difference in resistance times by concentrations ($P<0.001$), fish population ($P<0.05$), and interaction between

TABLE 2. Temperature tolerance tests (96-hr) of Butte Valley relict dace (*Relictus solitarius*) conducted from November 1980 to March 1981 in dechlorinated Truckee River water at mean acclimation temperatures of 18–20 C. Sample size is five fish per test in replicate tests.

	Mean chemical characteristics			Fish		
Mean test temperature (C)	Number of measurements	Dissolved oxygen (mg/l)	pH	Mean weight (g)	Number dead (96 hr)	Percent mortality (10 fish)
30.0	9	6.4	8.75	1.82	0	
30.0	”	6.5	8.20	1.46	0	0
Control (21.6)	”	7.3	8.50	2.14	0	
30.6	8	6.8	8.76	2.08	2	
30.6	”	6.8	8.17	2.40	3	50
Control (22.7)	”	7.6	7.98	1.62	0	
30.7	18	6.5	8.54	2.24	4	
30.7	”	6.6	8.36	2.28	4	80
Control (21.3)	”	7.6	8.15	2.08	0	
31.0	7	6.1	8.39	1.68	5	
31.1	”	6.1	8.51	3.20	5	100
Control (21.7)	”	7.3	8.03	1.70	0	

the main effects (P<0.001). An a-posteriori comparison showed that the Butte Valley (Atwood Ranch) fish were significantly more resistant to concentrated alkaline-saline waters than the Goshute Valley (Phalan Spring) population (P<0.05). The resistance of time of Atwood Ranch fish was about twice that of the Phalan Spring fish at both the 15,759 and 20,475 mg/liter levels (Table 5).

There exists an apparent interaction between temperature and salinity tolerances of relict dace (Table 4). No mortality occurred at low temperatures (21–22 C) over a range of TDS from 157 to 5652 mg/liter. But at an elevated temperature (30 C) differential mortality occurred: 0, 20, and 60 percent at TDS levels of 157, 2845, and 5652 mg/liter, respectively.

Discussion

Temperature

Compared to other families of freshwater fishes, the Cyprinidae generally occupy an intermediate rank of upper thermal tolerance. Through acclimation, an upper lethal temperature exceeding 30 C is achieved by most cyprinids; at the highest tested acclimation levels eight species ranged from 29.3 (*Rhinichthys atratulus*) to 38.6 C (*Carrassius auratus*) (Brett 1956).

The upper lethal TL50 of 30.6 C herein determined for relict dace acclimated at 18–20 C falls within the range exhibited by other cyprinids. If the test fish were acclimated to higher temperatures, it is likely that the upper lethal temperature would be significantly greater.

The maximum water temperature I measured in a relict dace habitat was only 23 C; however, Hubbs et al. (1974) recorded summer temperatures as high as 25 C. Different populations of relict dace are subjected to a wide variety of temperature regimes. Nonthermal springs and shallow ponds may have great daily and seasonal changes with minimum temperatures near freezing (e.g., Franklin Lake). At the other end of the spectrum are thermal springs that never vary more than a few degrees in terms of diel, seasonal, and annual, presumably over extremely long time periods. For example, the head of Phalan Spring exhibited less than a 1 C diel change in February when air temperature ranged from 7.5 to -9.0 C, and seasonal water temperature variation was only about 3 C. In addition, there are various types of intermediate gradations with respect to the temperature emanating from the springs and

TABLE 3. Environmental characteristics of selected relict dace, *Relictus solitarius*, habitats, April 1980–February 1981.

Valley/Site	Date	Temperature (°C)	Total dissolved solids (mg/l): Summation of all constituents	Total dissolved solids (mg/l): Summation (USGS 1979)	Total alkalinity (mg/l $CaCO_3$)
Ruby					
Alkali Pond	12/80	2.0	1721	1157.8	912
Franklin Lake	12/80	3.5	683.8	444.3	387
Steptoe					
Steptoe Ranch	12/80	20.8	388.5	255.6	215
Cardano Ranch	12/80	11.5	415	265.3	242
Goshute					
Phalan Spring	04/80*	22	–	216	115
	07/80*	23	–	175	118
	09/80*	22	–	190	132
Butte					
Atwood Ranch	04/80*	19	–	375	172
	07/80*	21	–	325	182
	09/80*	20	–	325	158
	12/80	16.5	469.3	325.7	232
Odgers	04/80*	12	–	216	120
	07/80*	20	–	205	115
	09/80*	14	–	190	118

*Conducted by: Water Analysis and Consulting, Inc. 1980. Water Quality Study Wells Environmental Statement Area. Bureau of Land Management, Elko District.

the changes that occur in the length of streams and/or depths of ponds.

Limited data exist on thermal tolerance levels of cyprinid species inhabiting the Great Basin. Speckled dace (*Rhinichthys osculus*) taken from intermittant Arizona streams at < 25 C had ultimate incipient upper lethal levels of 33 C for juveniles and 32 C for adults (John 1964). The Borax Lake chub, *Gila boraxobius*, is endemic to a thermal lake in southeastern Oregon; the lake typically exhibits temperatures of 29–32 C, with extremes of 17–35 C (Williams and Williams 1980). Another cyprinid from the northwestern Great Basin, the desert dace (*Eremichthys acros*), is endemic to thermal springs ranging from 18.5–40.5 C; this Nevada species tolerated temperatures of 2–37 C in the laboratory when acclimated at 23 C (Nyquist 1963). Virgin River spinedace (*Lepidomeda mollipinis*), native to the Colorado River System, had a 14-hr upper lethal temperature of 31.2–31.4 C when acclimated at 20 C (Espinosa and Deacon 1978).

In contrast, extensive research has been done on the thermal requirements of Cyprinodontidae inhabiting the Death Valley region of the southern Great Basin. Pupfish (*Cyprinodon*) acclimated at 10–20 C can tolerate temperatures of 39–40 C (Brown and Feldmeth 1971, Otto and Gerking 1973, Feldmeth et al. 1974).

Representatives of another cyprinodont genus (*Crenichthys*) are remnants of Pluvial river systems in the southeastern Great Basin. *Crenichthys baileyi* inhabits springs in the Moapa Valley at constant temperatures of 32.2 C (Kopec 1949). In the Moapa River *C. baileyi* and *Moapa cariacea* (Cyprinidae) occupied habitats at temperatures of 27–32 C and 19.5–32 C, respectively (Deacon and Bradley 1972). *Crenichthys baileyi* and *C. nevadae* live in various spring outflows in the White River Valley and Railroad Valley, respectively, at temperatures ranging from 21 to 37.3 C (Hubbs et al. 1967). At Lockes Ranch spring complex *C. nevadae* were observed at temperatures of 18.3–37.8 C (Baugh and Brown 1980).

Although individuals may survive for short periods of time at extremely high temperatures, the maximum constant temperature occupied by a reproducing population of desert fish is rarely greater than 35 C (Soltz and Naiman 1978). This may be attributed to the differential tolerance of various life stages; e.g., Amargosa pupfish (*Cyprinodon nevadensis*) juveniles are most tolerant of extreme temperatures, with adults intermediate, and eggs least tolerant (Shrode 1975, Shrode and Gerking 1977). The reproductive tolerance range (≤50 percent hatch) of *C. nevadensis* was 24–30 C or one-seventh the critical thermal tolerance range (Shrode

TABLE 4. Salinity tolerance tests (96 hr) of relict dace *Relictus solitarius*, conducted during December 1980 and March 1981. The first series of bioassays tests interaction with temperature; the second series tests differences by fish population. Sample size is five fish per test.

		Mean chemical characteristics				Fish		
TDS (mg/ℓ)	Population: valley (habitat)	Number of measurements	Temperature (C)	D.O. (mg/ℓ)	pH	Mean weight (g)	Number dead	Percent mortality
157 (Control)	Butte (Atwood)	9	21.6	7.3	8.50	2.14	0	
	”	”	30.0	6.4	8.75	1.82	0	0
	”	”	30.0	6.5	8.20	1.46	0	
2,845	Butte (Atwood)	”	21.9	7.4	9.11	1.78	0	0
	”	”	30.0	6.4	9.20	1.18	1	20
5,652	Butte (Atwood)	”	20.9	7.3	9.21	1.60	0	0
	”	”	29.9	6.4	9.20	2.22	3	60
11,043	Butte (Atwood)	12	21.4	7.6	9.24	2.14	0	0
	Goshute (Phalan)	”	21.4	7.6	9.24	2.00	0	0
15,759	Butte (Atwood)	7	21.8	7.6	9.25	2.22	5	100
	Goshute (Phalan)	4	22.0	7.4	9.13	1.92	5	100
20,475	Butte (Atwood)	2	22.3	7.1	9.09	1.90	5	100
	Goshute (Phalan)	”	22.3	7.1	9.09	1.80	5	100
157	Butte (Atwood)	12	21.4	7.6	8.35	1.98	0	0
(Control)	Goshute (Phalan)	”	21.4	7.6	8.30	6.02	0	

and Gerking 1977). This example illustrates that multiple criteria are necessary to establish the thermal requirements of a fish species. Thus, the TL50 determined in this study at a single acclimation regime is a useful baseline datum but is not comprehensive.

TDS-Alkalinity

The relict dace has experienced a variable environment during the past two million years in the Great Basin. Numerous cycles of Pluvial filling and interpluvial desiccation of large Pleistocene lakes occurred. Concomitant changes in the salinity of the lacustrine habitats undoubtedly took place. The variable content of TDS presumably has been a factor of considerable importance in the survival or extinction, and probably in the speciation of the populations of endemic fishes (Hubbs et al. 1974). During the past 10,000 years the lacustrine habitats of the relict dace have completely dried up and the species persists in remnant springs. The vast reduction of surface water to isolated springs is the outstanding feature of the habitats of native fishes in the Great Basin (Hubbs et al. 1974).

The elevated salinity level that relict dace tolerated (11,043 mg/liter TDS) is over six times the maximum TDS level of any current relict dace habitat measured. The corresponding total alkalinity level (2,580 mg/liter) represents nearly three times that in any current habitats. The spring habitats are generally <250 mg/liter total alkalinity and <500 mg/liter TDS (Table 3); the most saline habitats are ponds in the Ruby Valley, which exhibit total alkalinity levels as high as 912 mg/liter (TDS = 1,721 mg/liter). I hypothesize that relict dace evolved the physiological mechanisms to withstand highly saline-alkaline waters during the desiccation of their lacustrine environment.

In contrast to relict dace habitats, Cyprinodont environments in the southern Great Basin can be extremely saline—as much as 4.5 times that of sea water, composed predominantly of NaCl (Hunt et al. 1966). The composition of one saline habitat, Cottonball Marsh, was about 78 percent NaCl, with alkalinity not listed as a constituent (LaBounty and Deacon 1972). During laboratory studies with the ionic composition approximating that of sea water, the Cottonball Marsh pupfish (*Cyprinodon milleri*) survived at 88,000 mg/liter TDS, with a few individuals tolerating 130,000 mg/liter for several weeks (Naiman et al. 1976). Numerous field observations of various *Cyprinodon* species living at salinities greater than 90,000 mg/liter are documented in the literature (Barlow 1958, Deacon and Minckley 1974).

Thus, relict dace can tolerate only a fraction of the TDS at which Cyprinodonts exist. This is probably a reflection of the differences in their respective evolutionary environments. Precursors of the Cyprinodontidae had marine affinities (Smith 1981), and the family is apparently preadapted to harsh physical and chemical conditions; this enabled the group to further evolve tolerance to extremes of salinity and temperature (Miller 1981). In contrast, Cyprinidae is a primary freshwater family (Miller 1958). However, the ionic composition of their present environments and test media may also be a critical factor; i.e., the alkalinity component could affect the TDS tolerance of both taxa.

Salinity bioassays on relict dace at normal and elevated temperatures demonstrated a synergistic effect. Brett (1960) points out that with the multiple role of temperature bringing increased attention to the problem of interaction, emphasis will shift away from the singular effects of temperature to synergistic effects within the overall characteristics of environments that permit survival of the species. In his work with the euryplastic *Cyprinodon macularius*, Kinne (1960) showed that

TABLE 5. Mean resistance time of Atwood Ranch (A) and Phalan Spring (P) relict dace, *Relictus solitarius*, to highly alkaline-saline waters. Sample size is five fish per test.

TDS (mg/*l*)	Population	Mean resistance time (minutes)	95% confidence interval (minutes)
11,043	A	Indefinite (>5760)	–
	P	Indefinite (>5760)	–
15,759	A	1907	1204–2610
	P	848	280–1416
20,475	A	213	121–305
	P	111	70–152

the combination of temperature and salinity was of basic physiological importance; the effects of a given temperature depend on the salinity and vice versa. Similarly, when dealing with the habitat requirements of relict dace, the synergistic relationships of salinity and temperature are important with respect to their physiology and ecology.

ACKNOWLEDGMENTS

This research was sponsored by the Desert Research Institute (DRI) Project Assignment Committee. Dr. Clifford J. Murino, DRI president, was very supportive of the work. Consultation with Dr. James E. Deacon (University of Nevada, Las Vegas) was helpful in initiating this research on relict dace. Dr. Robert R. Miller (University of Michigan, Ann Arbor) kindly reviewed my proposal and manuscript for accuracy of content. Mr. David L. Galat (Colorado Cooperative Fishery Research Unit) and Dr. Thomas P. Lugaski (University of Nevada, Reno) critically read the manuscript and made helpful suggestions. DRI Water Center Analytical Laboratory conducted water chemical analyses.

LITERATURE CITED

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION), AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater, 14th ed. New York, New York, USA. 1193 pp.

BARLOW, G. W. 1958. High salinity mortality of desert pupfish, *Cyprinodon macularis*. Copeia. 1958:231-232.

BAUGH, T. M., AND B. G. BROWN. 1980. Field observations on the response of the Railroad Valley springfish (*Crenichthys nevadae*) to temperature. Great Basin Nat. 40(4):359-360.

BRETT, J. R. 1956. Some principles in the thermal requirements of fishes. Quart. Rev. Biol. 31(2):75-87.

———. 1960. Thermal requirements of fish—three decades of study. Pages 110-117 *in* C. M. Tarzwell, ed., Biological problems of water pollution. United States Department of Health, Education, and Welfare. Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, USA.

BROWN, J. H., AND C. R. FELDMETH. 1971. Evolution in constant and fluctuating environments: thermal tolerances of desert pupfish (*Cyprinodon*). Evolution. 25(2):390-398.

DEACON, J. E., AND W. G. BRADLEY. 1972. Ecological distribution of fishes of Moapa (Muddy) River in Clark County, Nevada. Trans. American Fisheries Soc. 101(3):408-419.

DEACON, J. E., G. KOBETICH, J. D. WILLIAMS, AND S. CONTRERAS. 1979. Fishes of North America endangered, threatened, or of special concern. Fisheries (Bethesda) 4(2):29-44.

DEACON, J. E., AND W. L. MINCKLEY. 1974. Desert fishes. Pages 385-487 *in* Desert Biology, Vol. 2. G. W. Brown, Jr., ed. Academic Press, New York, New York, USA.

ESPINOSA, F. A., AND J. E. DEACON. 1978. Rearing bait fish in the desert southwest. State of Nevada. Final Report to Federal Aid in Commercial Fisheries Research and Development Project No. 6-9-D. University of Nevada, Las Vegas. USA. 29 pp.

FELDMETH, C. R., E. A. STONE, AND J. H. BROWN. 1974. An increased scope for thermal tolerance upon acclimating pupfish (*Cyprinodon*) to cycling temperatures. J. Comp. Physiol. 89:39-44.

HARDY, T. 1979. The Inter-Basin Area Report—1979. United States Department of the Interior and the State of Nevada. Pages 5-21 *in* Proceedings of the Desert Fishes Council. Vol. XI. Bishop, California, USA.

HUBBS, C., R. C. BAIRD, AND J. W. GERALD. 1967. Effects of dissolved oxygen concentration and light intensity on activity cycles of fishes inhabiting warm springs. Am. Midl. Nat. 77(1):104-115.

HUBBS, C. L., AND R. R. MILLER. 1972. Diagnosis of new cyprinid fishes of isolated waters of the Great Basin of western North America. Trans. San Diego Soc. Nat. Hist. 17(8):101-106.

HUBBS, C. L., R. R. MILLER, AND L. C. HUBBS. 1974. Hydrographic history and relict fishes of the north central Great Basin. Mem. California Acad. Sci. Vol. 7. 259 pp.

HUNT, C. B., T. W. ROBINSON, W. A. BOWLES, AND A. L. WASHBURN. 1966. Hydrologic basin, Death Valley, California. U.S. Geol. Surv. Prof. Paper No. 494-B.

JOHN, K. R. 1964. Survival of fish in intermittent streams of the Chiricahua Mountains, Arizona. Ecology. 45:112-119.

KINNE, O. 1960. Growth, food intake, and food conversion in a euryplastic fish exposed to different temperatures and salinities. Physiol. Zool. 33(4):288-317.

KOPEC, J. A. 1949. Ecology, breeding habits, and young stages of Crenichthys baileyi, a cyprinodont fish of Nevada. Copeia 1949(1):56-61.

LABOUNTY, J. F., AND J. E. DEACON. 1972. *Cyprinodon milleri*, a new species of pupfish (family Cyprinodontidae) from Death Valley, California. Copeia 1972(4):769-780.

LUGASKI, T. P. 1980. Comparative chemotaxonomy of selected Great Basin native cyprinid fishes. Unpublished dissertation. Univ. of Nevada, Reno, USA.

MILLER, R. R. 1958. Origin and affinities of the freshwater fish fauna of western North America. Pages 187-222 *in* Carl L. Hubbs, ed., Zoogeography. American Association for the Advancement of Science, Baltimore, Publ. No. 51.

———. 1981. Coevolution of deserts and pupfishes (Genus Cyprinodon) in the American Southwest. Pages 39-94 *in* R. J. Naiman and D. L. Soltz, eds., Fishes in North American deserts. Wiley & Sons, New York, New York, USA.