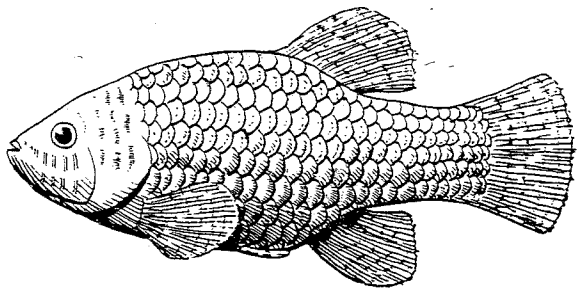
Desert Fishes Council



"Dedicated to the Preservation of America's Desert Fishes"

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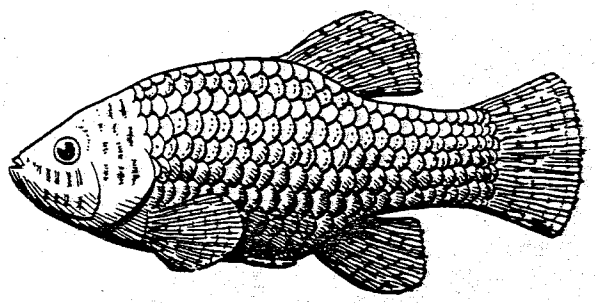

"Dedicated to the Preservation of America's Desert Fishes"

TWENTIETH ANNUAL SYMPOSIUM

Death Valley National Monument Headquarters

Furnace Creek, California

November 16-19, 1988

PREFACE

The Twentieth Annual Symposium was a nostalgic event for most members, as we recounted the Council's origin and evolution and the role it has played in protection and preservation of desert aquatic ecosystems. Nearly 200 attended the three-day meeting, representing 13 offices of the U.S. Fish and Wildlife Service, 6 offices of the Bureau of Land Management, 4 units of the National Park Service, 4 national forests, 3 offices of the Bureau of Reclamation, 2 offices of the Geological Survey, the fish and wildlife agencies of 7 western states, the Sierra Club, Naval Weapons Center at China Lake, two offices of The Nature Conservancy, 36 universities and colleges from throughout North America, and several private consulting firms (an attendance list is included in the appendix). In addition to reports of activities of government agencies, 40 research papers were presented relating to desert aquatic communities.

In commemoration of the twentieth anniversary, an extra day was added to the usual three-day symposium devoted to the general subject of "Battle Against Extinction: Native Fish Management in the American West." Twenty papers were presented in this special session, which will be published as a book by University of Arizona Press, edited by W. L. Minckley and J. E. Deacon. Publication date is scheduled for late 1989. The general program for the special symposium is included in the appendix.

Following the Thursday paper sessions, the Council held its usual barbecue. Because of extremely stormy conditions in Death Valley, National Park Service personnel allowed us to move into the sheltered area next to the auditorium. As has been their practice throughout the Council's history, the National Park Service extended every courtesy to us. For this we remain very grateful.

At the November 16 business meeting it was decided that the 1989 symposium would be held during the period of November 16-18 at Albuquerque, New Mexico, hosted by the University of New Mexico, U.S. Fish and Wildlife Service, and New Mexico Department of Game and Fish, with Steve Platania serving as general coordinator. It is currently planned that the 1990 symposium will be held in Mexico, with the exact location to be determined in 1989.

Progress Report: A comparison of morphology, thermal tolerances, and biochemical genetics of the Kendall Warm Springs dace (Rhinichthys osculus thermalis) and speckled dace (R. osculus yarrowi) of the upper Green River drainage in Wyoming.

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Abstract

Comparisons of morphology, thermal tolerance, and biochemical genetics between Kendall Warm Springs dace (Rhinichthys osculus thermalis), listed as an endangered fish by the U.S. Fish and Wildlife Service, and speckled dace (R. o. yarrowi) of the upper Green River drainage have demonstrated both similarities and differences. Tolerances for high temperatures (critical thermal maxima) do not differ significantly. Electrophoretic examination of 26 loci have shown no variants at any locus in either population, and both populations are fixed at all loci. However, five of eight restriction enzymes reveal polymorphic mitochondrial DNA (mtDNA) in R. o. yarrowi, and only one enzyme revealed a polymorphic mtDNA in R. o. thermalis. The most important morphological difference measured is in pharyngeal tooth formulas, with 86% of R. o. thermalis lacking pharyngeal teeth in at least one secondary row, compared with less than 1% of R. o. yarrowi. R. o. yarrowi have been spawned, incubated, and are being reared at 18, 24, and 28 C to determine thermal lability of meristic features, and whether those at the highest temperature will remain differentiable from R. o. thermalis.

Introduction

The purpose of this study is to determine differences or similarities between the Kendall Warm Springs dace (Rhinichthys osculus thermalis) and speckled dace (R. osculus, also known as R. o. yarrowi) of the upper Green River drainage through comparisons of several categories of characteristics: morphology and meristics, physiological thermal tolerances, and biochemical genetics. These comparisons are intended to evaluate the validity of the present subspecific designation of the Kendall Warm Springs dace, which is listed as an endangered fish by the U. S. Fish and Wildlife Service. These dace were first designated as a separate subspecies by Hubbs and Kuhne (1937), on the basis of morphological differences from other R. osculus and on their isolation in a small, thermal spring creek. Since it is possible that some of these morphological differences could be environmentally induced by elevated temperatures during early development of the young, as recognized by Hubbs and Kuhne (1937), the validity of the subspecific designation has remained in question.

Methods

Specimens of R. osculus thermalis were collected from Kendall Warm Springs and R. o. yarrowi from three sources: the Green River upstream from the mouth of Kendall Warm Springs; Boulder Creek, a tributary downstream from its mouth, and Duck Creek, a tributary to the Green River near Pinedale, Wyoming. Collections were made in accordance with permits issued by the U. S. Fish and Wildlife Service (FA/SE/Blanket Permit, Subpermit 86-16) and the Wyoming Game and Fish Department (Permit No. 91). Collection dates were October 3, 1987 and September 6, 1988 in Kendall Warm Springs and Duck Creek, and September 7, 1988 in the Green River and Boulder Creek. Dace were taken with dip nets in Kendall Warm Springs and with a backpack electrofisher in the other waters. A total of about 50 dace from each source were preserved in formalin for morphologic measurements. About 150 from Kendall Warm Springs, 75 each in 1987 and 1988, and about 350 from Duck Creek, 250 in 19877 and 100 in 1988, were transported live to Montana State University in Bozeman. All references to live specimens of speckled dace (R. o. yarrowi) in this report refer to those taken from Duck Creek.

Morphologic and meristic measurements followed standard procedures and concentrated on numbers of fin rays, lateral line scales, and pharyngeal teeth.

For tests on thermal tolerances, Kendall Warm Springs dace and speckled dace were acclimated to 27 C, and speckled dace also to 24 C, before being subjected to standard measurements (Paladino et al. 1980) of their critical thermal maxima (CTM). Dace were tested individually in 14.5 liter tanks. Temperature started at the acclimation level and was raised at a rate of .45 C per minute until the endpoint was reached of first loss of equilibrium by the fish.

Electrophoretic analyses of protein variation and mitochondrial DNA were conducted both on specimens sacrificed from among those maintained live, and specimens frozen after dying in captivity. Protein variation of both liver and muscle tissue was studied through methods similar to that described in Brussard et al. (1981), while techniques for comparisons of mitochondrial DNA (mtDNA) restriction endonuclease cleavage sites were similar to that described by Avise et al. (1984).

For attempts at captive breeding, speckled dace were maintained at temperatures of 15 to 24 C, and Kendall Warm Springs dace at 24 and 28 C, under 14 hour photoperiod, until specimens appeared to become gravid. Specimens tentatively identified to sex by external appearance were placed in 75-liter spawning tanks in groups of three, two males and a female, or five, three males and three females. Spawning substrates were either gravel, small rocks, or a contoured, woven, plastic material (Tensar Mat, Tensar Corporation, Morrow, Georgia) held down with rocks and gravel.

Results and Discussion

Morphology

Selected morphological features are presented in Table 1. The number of lateral line scales is significantly lower in Kendall Warm springs dace than in speckled dace from the other three sources, and the number of pectoral fin rays is significantly different from those of speckled dace

Table 1. Selected morphological features of Rhinichthys osculus thermalis and three nearby populations of R. o. yarrowi. Mean counts and (standard deviations) are given for lateral line (LL) scales and pectoral fin (PF) rays. Number of individuals and (% of total) are given for fish lacking pharyngeal teeth in at least one secondary row.

	R. o. thermalis	R. o. yarrowi		
	Kendall Warm Spr.	Duck Creek	Green River	Boulder Cr.
Number	50	50	21	40
LL scales	56.0 (4.7)*	64.8 (3.9)	68.6 (3.5)	68.0 (4.2)
PF rays	13.3 (0.7)	13.4 (0.8)	14.2 (0.9)	13.3 (0.7)
No. without teeth in one secondary row	43* (86%)	0	0	1 (3%)

* Significantly different ($P<.05$) from all R. o. yarrowi populations

from the Green River, but not from Duck Creek or Boulder Creek. These results are in general agreement with the earlier descriptions by Hubbs and Kuhns (1937), except that the present pectoral fin ray counts are higher, and the Kendall Warm Springs dace are not significantly different from speckled dace of all sources. The most important finding is the difference in pharyngeal tooth formulas, which have not previously been studied for Kendall Warm Springs dace. Of 50 specimens examined, 43 (86%) lacked pharyngeal teeth in at least one of the secondary rows, whereas this condition was extremely rare (1 out of a total of 111) in speckled dace from the three sources. Since pharyngeal tooth counts appear not to be thermally labile during early development, this suggests a genetically based morphological differentiation of the Kendall Warm Springs dace.

Critical Thermal Maxima

There was no significant difference in critical thermal maxima between Kendall Warm Springs dace and speckled dace from Duck Creek, with both acclimated to 27 C (Table 2). For the Kendall dace, this acclimation

Table 2. Critical thermal maxima of Rhinichthys osculus thermalis and R. o. yarrowi.

Source and Acclimation	N	Mean length in mm	Mean CTM
Kendall, 27 C	14	43.1	34.8 (a)*
Speckled, 27 C	13	48.4 (a)	34.3 (a,b)
Speckled, 24 C	7	48.8 (a)	33.9 (b)

* Common letter indicates $P>.05$ (Newman-Keuls)

temperature approximates the lower limit that might be experienced at any time in the thermal spring creek. The CTM values for these dace acclimated

to 27 C are about 2 C lower than measured by Deacon et al. (1987) for speckled dace from the Virgin River in Utah acclimated to 25 C. This difference may be related in part to the much larger specimens (average of 72.2 mm) used in that study. Speckled dace used in the present trials were deliberately selected for small size so that they would be similar to the Kendall Warm Springs dace, however, they were still significantly larger.

Electrophoretic Studies

The 26 loci examined for 12 specimens each from Kendall Warm Springs and Duck Creek are listed in Table 3. No differences were discernable at

Table 3. Enzymes examined for comparisons of R. o. thermalis and R. o. yarrowi.

Locus	Enzyme	Buffer*
AAT-1,2	Aspartate aminotransferase	R
AGP	Alpha-glycerophosphate dehydrogenase	4
EST-1,2	Esterase	R
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	C
GDH	Glutamic dehydrogenase	C
GP-1,2,3	General protein	C
GPI-1,2	Glucosephosphate isomerase	C
G6PDH	Glucose-6-phosphate dehydrogenase	R
HBDH	Hydroxybuteric dehydrogenase	C
IDH-1,2	Isocitrate dehydrogenase	C
LDH	Lactate dehydrogenase	4
MDH-1,2	Malate dehydrogenase	C
ME	Malic enzyme	R
MPI	Mannosephosphate dehydrogenase	R
PGD	Phosphogluconate dehydrogenase	4
PGM	Phosphoglucomutase	C
SOD-1,2	Superoxide dismutase	R
XDH	Xanthine dehydrogenase	C

* R = Ridgeway, G. J. et al., 1970, Trans. Am. Fish. Soc. 99:147-151;
4 = Selander, R. K. et al., 1971, Univ. Texas Publ. 7103:49-90;
C = Clayton, J. W. and D. N. Tretiak, 1972, J. Fish Res. Bd. Can. 29:1169-1172.

these loci between Kendall Warm Springs and speckled dace. No variants were observed at any locus in either population, and both populations were fixed for the same electromorphs at all loci.

Mitochondrial DNA Studies

Initial results from four Kendall Warm Springs dace and seven speckled dace from Duck Creek are depicted in Fig. 1. Five of the eight restriction enzymes reveal polymorphic mtDNA in fish from Duck Creek, and one enzyme revealed a polymorphic mtDNA in fish from Kendall Warm Springs. On the basis of this small sample analyzed to date, Kendall Warm Springs dace appear to have inherited only one form of the mtDNA found in speckled dace in this nearby tributary of the Green River.

Captive Breeding and Rearing

Speckled dace have spawned repeatedly in our laboratory tanks, but

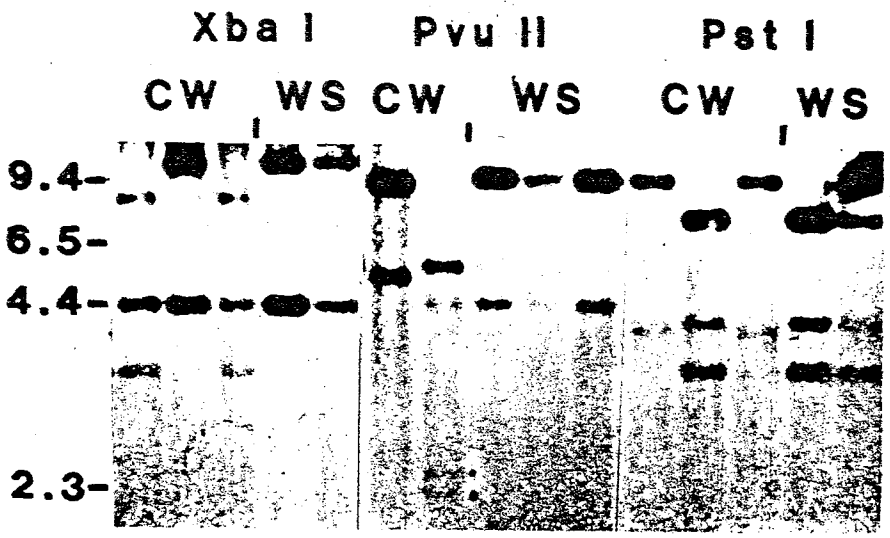

Figure 1. Mitochondrial DNA polymorphisms in Kendall Warm Springs (WS) and speckled dace (CW). Restriction endonuclease Xba I and Pst I show a polymorphism in speckled dace (CW), one form of which is also found in the Kendall Warm Springs dace (WS). Pvu II polymorphisms found in speckled dace (CW) have not been found in Kendall Warm Springs dace (WS).

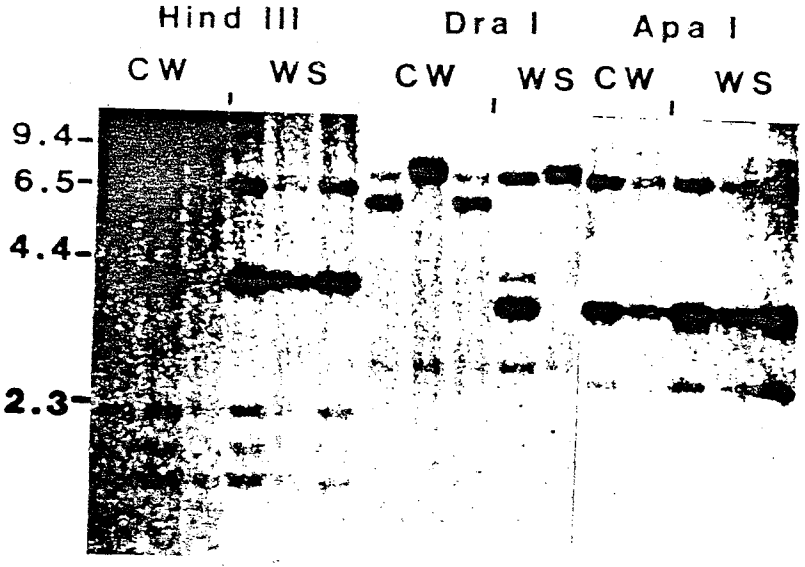

Figure 2. Mitochondrial DNA polymorphisms in Kendall Warm Springs (WS) and speckled dace (CW). Restriction endonuclease Apa I is monomorphic in all fish tested. Hind III and Dra I are both polymorphic in speckled dace (CW) but only one of the forms for each enzyme is found in the Kendall Warm Springs dace (WS).

most eggs from earlier spawnings, over a gravel substrate, were eaten by the adults. Eggs from later spawnings were saved from predation by lining the bottom of the tanks with the artificial plastic substrate, through which the eggs would sink beyond the reach of the adults. Young were produced from spawnings of four separate groups of two females and three males each which spawned between 30 August and 9 September 1988, one group at 18 and three at 24 C. Temperature for one batch of eggs spawned at 24 C was increased to 28 C. Fry are thus presently being raised that have been incubated, hatched, and reared at 18, 24, and 28 C. The young speckled dace will be maintained at these three temperatures until they are large enough for morphologic measurements. This could determine the extent to which meristic features of R. osculus yarrowi are affected by temperature during development, and whether specimens developing at a level (28 C) similar to those experienced by R. o. thermalis will remain differentiable from the latter.

Despite the relative ease with which we have been able to induce spawning in speckled dace, we have not yet been able to do so with Kendall Warm Springs dace. Various combinations of conditions in the spawning tanks, including flowing vs. static water, different substrates, and presence or absence of vegetation, have not yet produced results.

Summary

Results to date provide new evidence in support of the differentiation of Kendall Warm Springs dace, R. o. thermalis, from speckled dace of the upper Green River drainage, R. o. yarrowi. The most important differences have come from pharyngeal tooth counts and mitochondrial DNA analyses, neither of which have been compared previously in these fish. Mitochondrial DNA analyses are continuing with additional specimens. Further morphologic comparisons will be conducted on young speckled dace presently being reared at 18, 24, and 28 C, the latter similar to temperatures within Kendall Warm Springs. If results of these ongoing investigations continue to show differences between Kendall Warm Springs dace and speckled dace, this would provide important evidence to support the original description of R. o. thermalis by Hubbs and Kuhne (1937) as an identifiable variant of R. osculus.

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