COO-2498-2

REPRODUCTIVE TEMPERATURE TOLERANCE OF A
DESERT PUPFISH

Technical Progress Report
for the period July 1, 1975 to June 30, 1976

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MASTER

Prepared for the Energy Research and Development Agency
under Contract No. E (11-1)-2498

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SUMMARY

An inbred strain of C. n. nevadensis was reared in the laboratory. Reproductive performance tests were conducted with the F₂ sibling generation at age four to five months at constant temperatures 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36 C and at daily fluctuating temperatures 24-16, 32-28, 36-28, 38-28, and 39-30 C. Twelve breeding pairs were tested at each temperature for 21 days. Behavior and daily egg production were monitored and proportion of days on which spawning occurred, sequence of spawning days, eggs per spawning, eggs per day per gram of body weight, and yolk diameter of eggs were determined. At the termination of each test, ovary weights of females were measured and critical thermal maxima were determined with the males.

The effect of temperature on proportion of days laid, number of eggs per spawning, and number of eggs laid per day per gram of female weight followed a similar pattern. The range of temperatures at which egg production was greatest was at constant temperatures 24 through 34 C and at fluctuating temperatures 32-28 and 36-28 C. Production dropped sharply above and below these temperatures. The maximum daytime temperature for egg production under fluctuating temperature regimes is slightly greater than the maximum achieved under constant conditions.

The temperature at which fish were maintained affected not only the number of eggs produced, but also the quality. There was an inverse relationship between temperature and yolk diameter of eggs.

Eggs which were exposed to identical temperatures during oogenesis and incubation had greater than 50 percent normal hatch at temperatures between 24 and 30 C and at 32-28 and 36-28 C. Eggs which were exposed to 28 C during oogenesis and transferred to test temperatures at the two to eight-cell stage had greater than 50 percent normal hatch at constant temperatures 20 through 36 C and at fluctuating temperatures 28-20, 36-28, and 38-30 C. The tolerance limits for oogenesis are narrower than the tolerance limits for successful hatching of healthy eggs exposed to temperature stress during development.

Several conclusions have emerged from our research. First, the temperature range for successful reproduction is narrower than the upper and lower critical thermal limits by a considerable margin. Next, our results show, as expected, that the upper and lower thresholds (about 24 through 30 C) for optimum egg production parallel the temperature range for successful hatching. Also, the maximum daytime temperature for egg production under fluctuating temperature regimes is slightly greater than the maximum achieved under constant temperature conditions. Last, and perhaps most important, the design of our experiments revealed that oogenesis is the most temperature sensitive phase of the life cycle, whereas eggs and larvae have been viewed as the most sensitive stages heretofore.

We are in the process of preparing for publication the results of work which has been completed at this time. The following is a draft of that paper which does not include a discussion.

EFFECTS OF CONSTANT AND FLUCTUATING
TEMPERATURES ON REPRODUCTIVE PERFORMANCE
OF A DESERT PUPFISH, Cyprinodon n. nevadensis

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INTRODUCTION

Upper and lower lethal temperatures as affected by acclimation have been used as one criterion of survival for the adults of many species of fish. From this work we know that fish rarely encounter lethal temperatures either in nature or in areas of thermal pollution. This observation coupled with the ability to avoid extreme temperatures leads us to believe that the outright elimination of local populations from thermal death is rare. However, temperature affects all life processes, and survival of populations may depend on their response to a range of temperatures well within the lethal limits. Therefore, we have chosen to examine the range of temperatures over which reproduction can be accomplished successfully, because survival of populations obviously depends on their ability to reproduce. We have called this study reproductive temperature tolerance.

The effects of various constant and daily fluctuating temperature regimes on reproductive performance have been investigated in a eurythermal fish, the Amargosa pupfish, Cyprinodon n. nevadensis. This species occurs in the drainage system of the Amargosa River extending

from southern Death Valley, California, to the southwestern border of Nevada. We chose C. n. nevadensis as an experimental animal because it is hardy in the laboratory, it breeds over the whole year, and the females release their eggs individually. Thus, the pupfish provides us with the advantage of year-round observations and an unusual measure of reproductive effort, i.e., eggs laid per unit time.

The genus Cyprinodon has attracted attention because it is widely distributed throughout the deserts of the southwestern United States and several of its species are endangered. As a result, the temperature relations of several Cyprinodon species have been investigated. The critical thermal maximum (CTM), or short-term temperature tolerance, of adult pupfish from several springs and marshes from Arizona and the Death Valley region has been determined; C. macularius (Lowe and Heath 1969), C. nevadensis, C. salinus and C. diabolis (Brown and Feldmuth 1971) and C. milleri (Otto and Gerking 1973). Studies have also been conducted on the production of a pupfish population (C. nevadensis amargosae) in a thermal stream (Naiman 1976), social organization and life history (Stoltz 1975), and temperature effects on growth and metabolic efficiency in C. macularius by Kinne (1960) and in C. n. nevadensis by Raush (in prep). The effect of temperature on development rate and upper and lower limits for egg incubation have been determined under constant temperature conditions for C. macularius (Kinne and Kinne, 1962), C. rubrofluvialis (Echelle, Hubbs, and Echelle 1972) and under constant and diel cycling temperatures, in C. n. nevadensis (Shrode 1975).

Several conclusions have emerged from our research. First, the temperature range for successful reproduction is narrower than the upper and lower critical thermal limits by a considerable margin. Next, our results show, as expected, that the upper and lower thresholds (about 24-30 C) for optimum egg production parallel the temperature range for

successful hatching. Also, the maximum daytime temperature for egg production under fluctuating temperature regimes is slightly greater than the maximum achieved under constant temperature conditions. Last, and perhaps most important, the design of our experiments revealed that oogenesis is the most temperature sensitive phase of the life cycle, whereas eggs and larvae have been viewed as the most sensitive stages heretofore.

MATERIALS AND METHODS

A breeding stock of Cyprinodon n. nevadensis was collected from the marsh of Saratoga Springs, Death Valley National Monument, California. A breeding pair from the field stock produced the sibling F_1 parental stock. Breeding pairs of this sibling parental stock were placed in 17, 20-liter aquaria. The male and female in each tank were separated by a perforated plexiglas divider to prevent the aggressive male from chasing the female. The divider was removed for 30 minutes each morning to allow spawning. Eggs were laid on a yarn mop from which they were removed and incubated in sterile petri dishes. The dishes were checked daily for hatched fish. These F_2 sibling generation fish became the experimental stock. The young fish were separated by sex as soon as males were identifiable by behavior and coloration.

Breeding pairs, eggs, and larvae were all maintained at 28 C under a photoperiod of 16L 8D under a combination of incandescent and fluorescent lights. Fish were fed Tetra-Min fish food and brine shrimp daily.

Reproductive performance tests were conducted with the F_2 sibling generation at age four to five months. For each test males and females were acclimated at least 21 days to the test temperature in separate tanks. Breeding pairs were then selected from the acclimated group and placed in divided 20-liter aquaria in a controlled environment chamber which maintained the test temperature within 0.5 C and a photoperiod of 16L 8D. After a three-day adjustment period, the divider was removed from each aquarium at 0800 hr for 30 minutes each day for 21 days to allow spawning.

Breeding coloration and behavior of males during the spawning period was noted. Dividers were then replaced, fish fed, spawning mops removed, and aquaria checked for rare eggs not laid on yarn mop. Eggs were counted and the yolk diameter was measured with an ocular micrometer at the two to eight-cell stage along the axis perpendicular to the animal-vegetal

pole. The eggs were then placed in covered petri dishes and returned to controlled environment rooms for incubation. The dishes were checked daily and the number of larvae hatched was recorded.

Egg laying was completed within the allotted daily spawning period. When females were not ready to spawn they swam toward the water surface as males approached.

On the morning of the 22nd day, before spawning, fish were anesthetized in MS-22, blotted dry, weighed and total length (TL) and standard length (SL) were measured. The ovaries were then removed, quickly weighed and preserved in Gilson's fluid. Yoloked oocytes in each ovary were measured with an ocular micrometer.

Reproductive performance experiments were conducted at constant temperatures of 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36 C and at daily fluctuating temperatures 24-16, 32-28, 36-28, 38-28 and 39-30 C. The fluctuating regime temperature started to increase at 0800 hr and decreased starting at 2000 hr (Fig. 1). Spawning occurred at 0900 hr.

Thermal tolerance of adult fish was determined as the critical thermal maximum (CTM). CTM tests were conducted on male fish from the reproductive performance tests. At the termination of the reproductive experiment, the male fish were transferred to 22-liter test tanks at the acclimation temperature. At 1000 hr the next day, the temperature was increased 0.3 C/min with a 600-watt thermostatically controlled heater. The temperature at which each fish lost equilibrium was recorded; the mean represented the CTM for each acclimation temperature.

RESULTS

An attempt was made to use fish within as small a size range as possible for the reproductive tolerance tests; however, during acclimation and testing, fish at intermediate temperatures, 28 and 30 C, grew faster and were slightly larger by the end of the test period (Table 1). In a

control experiment, egg production of 57 four-and five-month old fish ranging in weight from 0.31 to 1.45 g (SD = 0.22) and TL from 28 to 42 mm (SD = 3.0) was monitored for three weeks at 28 C. Within this size range there was no correlation between weight and percent days on which eggs were laid ($r = 0.16$, $P = 0.2$), eggs per spawning ($r = 0.02$, $P = 0.9$), or mean number of eggs laid per day ($r = 0.07$, $P = 0.6$).

At test temperatures 24 through 34 C males were in vivid breeding color with bright blue sides, yellow forehead, and strong black bands on posterior margins of anal, caudal and dorsal fins. (See Table 2 for length and weight). They immediately began pursuing females when the divider was removed and maintained their interest throughout the allocated spawning period. At 22 C both male and female were often hidden under the spawning mop. Occasionally both would swim around and at times the males chased the females. Males were in pale breeding color except one which was medium blue. At 20 C pale breeding colors were seen only on one male; the others showed no breeding coloration. All activities were slow at 20 C, and the fish often remained on the bottom or hidden in the mop and showed little interest in each other. At 36 C the color of the males usually was very pale but they pursued the females vigorously.

There was no pattern to the sequence of days on which spawning occurred at any test temperatures. The proportion of days on which any eggs were laid ranged from 2 percent at 36 C to 72 percent at 30 C, with a mean of 38 percent (Fig.2). Differences between temperatures in the proportion of days on which eggs were laid were significant by an analysis of variance test with arcsin transformation ($F = 4.3$; 14 and 134 df; $P < 0.01$). Performance was optimum at constant temperatures 24 through 34 C and fluctuating regimes 32-28 and 36-28 C.

The number of viable eggs per spawning was also affected by temperature. An analysis of variance on log transformed data was

highly significant ($F = 9.7$; 14 and 134 df; $P < 0.01$). The mean number of eggs per spawning was four for all temperatures while the maximum was ten at 30 C (Fig. 3). The number of eggs per spawning was greatest at constant temperatures 24 through 32 C and 32-28 and 36-28 C.

The number of eggs laid per day per gram of female weight followed a pattern similar to that of number of eggs per spawning (Fig. 4). An analysis of variance on log transformed data was highly significant ($F = 14.8$; 14 and 134 df; $P < 0.01$). The range of temperatures at which egg production per gram body weight was greatest was constant temperatures 24 through 34 C and fluctuating temperatures 32-28 and 36-28 C. Production dropped sharply above and below these temperatures. When egg production among the first, second and third week of each constant temperature experiment was compared, no significant differences were observed at 20, 22, 26, 28 and 30 C. At 24 and 32 C, the third week was better than the second which in turn was better than the first (analysis of variance, $F = 6.1$; 2 and 27 df; $P < 0.01$). At 34 C the order was second, third and first ($F = 7.8$; 2 and 21 df; $P < 0.01$).

Egg production under a daily fluctuating temperature regime was comparable to egg production at constant temperatures when the maximum or minimum temperature of the regime did not exceed the optimal constant range of 24 to 32 C (Figs. 2, 3 and 4). However, at temperature regimes in which the maximum or minimum fell above or below this range, egg production was reduced in comparison with performance at constant temperature equivalent to the mean temperature of the fluctuating regime. Performance was better than that at the constant temperature equivalent to the maximum temperature of the fluctuating regime.

Ovary weights (Table 3) and number of yolked oocytes per ovary were determined at the end of each breeding experiment. There was high individual variation in both measures at all but extreme temperatures. There was no indication of accumulation of eggs or of atretic eggs under any of the

experimental conditions.

The temperature at which fish were maintained affected not only the number of eggs produced, but also the quality. There was an inverse relationship between temperature and the yolk diameter of eggs; the differences in the yolk diameters at the various temperatures were significant (analysis of variance, $P < 0.01$) (Table 3). At 32 C and above a great number of small, soft eggs which easily collapsed were laid. Since it was impossible to count or measure these eggs they were not included in the results (Fig. 2), which were biased in favor of larger yolk diameters at these temperatures.

Eggs which were exposed to identical temperatures during oogenesis and incubation had greater than 50 percent normal hatch at test temperatures between 24 and 30 C and at 32-28 and 36-28 C (Table 4). This corresponds to the optimum range for egg production, except at 32 C where large numbers of eggs with low viability were produced. The range for successful hatching in eggs which were exposed to identical temperatures during oogenesis and incubation was much narrower than the range for successful hatching of eggs which underwent oogenesis and were spawned at 28 C and were then transferred to test temperatures from 18 to 39 C (Table 4). Under these conditions greater than 50 percent normal hatch occurred at constant temperatures 20 through 36 C and at fluctuating temperatures 28-20, 36-28 and 38-30 C. The tolerance limits for oogenesis were narrower than the range of temperatures for successful hatching of healthy eggs subjected to temperature stress at an early stage (2-8 cell) of development.

Critical thermal maximum (CTM) was related to acclimation temperature (Fig. 5). The highest CTM attained, at acclimation temperature 36 C, was 43.5 C. The critical thermal minimum for fish acclimated at 28 C was 4.5; at 20 C it was 2.8. The reproductive tolerance limits fell toward the upper range of these acutely-determined limits under which fish can escape unfavorable conditions.

Figure 1.

Daily cycle of fluctuating temperature regimes.
10 C daily temperature change, dashed line, 8 C,
solid line, 6 C, dotted line. Spawning occurred at 0900 hr.

Figure 2.

Mean ($\pm$ SD) proportion of days on which spawning occurred for breeding pairs acclimated, then bred for 21 days at test temperatures 18 through 39 C. N in Table 1.

Figure 3.

Mean ($\pm$ SD) number of eggs per spawning for breeding pairs, acclimated, then bred for 21 days at test temperature 18 through 39 C. N in Table 1.

Figure 4.

Mean ($\pm$ SD) number of eggs per total number of days
bred per gram of body weight for fish acclimated,
then bred at test temperatures 18 through 39 C for
21 days. N in Table 1.

Figure 5.

Critical thermal maximum ($\pm$ SD) of male fish from reproductive tolerance tests acclimated at temperatures 18 through 39 C.

Table 1. Length and weight of females used in reproductive tolerance tests.

TEMP	N	MEAN WEIGHT (g)	SD	MIN	MAX	MEAN TL (mm)	SD	MIN	MAX	MEAN COEFF CON ¹
18C	7	0.875	0.301	0.487	1.294	30.4	2.9	27	36	1.93
20C	14	0.818	0.278	0.424	1.351	30.1	3.1	24	35	1.82
22C	7	0.617	0.101	0.431	0.724	23.9	1.3	27	31	1.75
24C	10	0.794	0.095	0.622	0.930	30.3	1.1	28	31	1.80
26C	10	0.896	0.178	0.619	1.142	31.3	1.6	29	34	1.81
28C	12	1.059	0.158	0.882	1.450	32.5	1.9	30	37	1.96
30C	10	1.194	0.215	0.806	1.623	33.6	1.9	30	36	2.07
32C	10	0.832	0.250	0.587	1.369	29.2	1.8	27	33	2.13
34C	9	0.734	0.182	0.402	1.036	28.8	2.7	24	33	1.94
36C	10	0.759	0.108	0.591	0.896	29.4	1.0	28	31	2.01
24-16C	10	1.013	0.245	0.699	1.458	32.7	2.3	30	37	1.81
32-28C	11	0.754	0.082	0.614	0.882	29.5	1.4	28	32	1.93
36-28C	11	0.773	0.110	0.634	1.028	30.2	1.1	29	32	1.86
38-28C	8	0.763	0.094	0.555	0.860	29.5	1.5	26	31	1.96
39-30C	10	0.793	0.184	0.400	1.043	29.8	2.6	24	32	1.96

¹ Weight (g) x 10⁵/TL (mm)³

Table 2. Length and weight of males used in reproductive tolerance tests.

TEMP	N	MEAN WEIGHT (g)	SD	MIN	MAX	MEAN TL (mm)	SD	MIN	MAX
18C	7	1.27	0.50	1.06	1.85	40.3	6.2	40	46
20C	14	1.16	0.49	0.53	2.10	38.9	4.1	33	45
22C	7	0.57	0.16	0.37	0.81	31.6	3.0	28	36
24C	10	0.91	0.21	0.61	1.21	36.7	2.7	33	41
26C	10	1.24	0.31	0.86	1.97	40.2	2.6	36	46
28C	12	1.12	0.25	0.76	1.49	39.0	2.4	35	43
30C	10	1.34	0.34	0.75	1.86	41.1	4.3	31	46
32C	10	0.84	0.18	0.58	1.18	35.9	1.9	34	40
34C	9	0.93	0.19	0.69	1.33	36.4	3.1	32	42
36C	10	1.94	0.24	0.66	1.31	37.1	2.0	35	40
24-16C	10	0.90	0.25	0.60	1.43	36.5	4.4	31	47
32-28C	11	0.99	0.26	0.69	1.49	37.9	3.3	33	44
36-28C	12	0.95	0.29	0.63	1.46	37.0	3.0	33	42
38-28C	8	0.76	0.25	0.47	1.31	34.5	3.1	31	41
39-30C	10	0.83	0.23	0.56	1.35	35.5	1.6	32	37

Table 3. Yolk diameter of eggs laid and Gonosomatic Index (GSI)²

TEMP	YOLK DIAM MEAN	SD	N	GSI MEAN	SD	N
18C	1.07	0.09	40	0.05	0.02	7
20C	1.08	0.09	64	0.08	0.04	14
22C	1.07	0.04	16	0.09	0.03	7
24C	1.06	0.05	335	0.13	0.06	10
26C	1.02	0.08	212	0.14	0.05	10
28C	1.01	0.05	187	0.10	0.03	12
30C	1.00	0.05	375	0.07	0.04	10
32C	1.02 ¹	0.07	373	0.09	0.05	10
34C	1.04 ¹	0.08	126	0.05	0.02	9
36C				0.02	0.02	10
24-16C				0.09	0.03	10
32-38C	1.01	0.06	266	0.09	0.04	11
36-28C	1.00	0.06	203	0.09	0.04	11
38-28C	0.98	0.07	44	0.04	0.02	8
39-30C	0.93	0.08	51	0.04	0.02	10

¹Does not include measurements of large numbers of small, fragile eggs laid at these temperatures.

²Gonosomatic Index = ovary weight/(body weight - ovary weight).

Table 4. A comparison of percent hatch between (1) eggs exposed to identical temperatures during oogenesis and incubation, and (2) eggs exposed to 28 C during oogenesis and transferred at spawning to incubation temperatures ranging from 18 to 39 C (Shrode 1975).

INCUB TEMP.	OOGENESIS TEMP. SAME AS INCUBATION TEMP.		OOGENESIS TEMP. 28 C	
	Percent Hatch	N	Percent Hatch	N
18C	0	61	20	270
20C	0	164	67	270
22C	3	52	1	
24C	52	276	90	270
26C	84	386		
28C	69	300	95	270
30C	78	1400	-	
32C	13	400	87	270
34C	7	283	-	
36C	0	7	69	270
38C	-		4	270
39C	-		1	270
26-14C	0	40	-	
28-20C	14		64	270
32-28C	62	90	-	
36-28C	50	92	60	270
38-30C	0	130	655	270
39-30C	0	132	0	270

¹No test.

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