PHYSIOLOGICAL STRESS OF ACIDIFICATION ON FISHES
AND ITS MANIFESTATION

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Preface

This progress report is submitted in five parts:

1. A manuscript recently submitted to Physiological Zoology, "Effects of Generation-long Temperature Acclimation on Reproductive Performance of the Desert Pupfish, Cyprinodon n. nevadensis."
2. Preliminary results of "Growth Performance of a Desert Pupfish, Cyprinodon n. nevadensis, at Different Temperatures." A finished report is expected by January, 1979.
3. Preliminary results of "The Effect of Salinity on Reproduction of the Pupfish, Cyprinodon n. nevadensis. An additional experiment needs to be completed in the next fiscal year.
4. Preliminary results of "Limits of Tolerance to Hydrogen-ion Concentration of the Desert Pupfish, Cyprinodon n. nevadensis. Larvae and adults have been tested; egg hatchability at various pH values will complete the study of resistance times to pH by various life history stages.
5. Current research in progress titled, "The Effect of Hydrogen-ion Concentration on Reproductive Success in the desert pupfish Cyprinodon n. nevadensis."

EFFECTS OF GENERATION-LONG TEMPERATURE ACCLIMATION
ON REPRODUCTIVE PERFORMANCE OF THE DESERT PUFFISH,
CYPRINODON N. NEVADENSIS¹

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ABSTRACT

The hypothesis was tested that the reproductive function would exhibit compensation to temperature if the organism was bred and reared at temperatures which produced less than optimal reproductive performance. Desert pupfish, Cyprinodon n. nevadensis, were bred and the eggs reared at the optimum temperature for reproduction, 28 C, and at two marginal temperatures, 24 and 32 C. Each brood was divided into three groups and reproductive performance was tested at the rearing temperature and at two alternate temperatures to which they had never before been exposed. The eggs laid per spawning and eggs laid per gram body weight per day showed no compensation to rearing temperature. Reproductive performance was optimal at 28 C and was poor at both marginal temperatures, regardless of the previous thermal history of the fish. Lower hatchability at 32 C was associated with a thin egg coat, small yolk diameter, and low gonasomatic index as compared with the same measurements at the lower temperatures.

INTRODUCTION

The limits of temperature for successful reproduction of the desert pupfish, Cyprinodon n. nevadensis, have recently been established (Shrode and Gerking 1977). The limits span only 6 C, from 24 to 30 C, using the number of eggs laid per unit time and 50 per cent hatchability as two major criteria. These limits are only 14 per cent of the critical thermal limits of 2 and 44 C over which the fish are able to survive after three-week acclimation to successively lower or higher temperatures. Although reproductive and lethal temperature limits vary with the species, probably all species have a severely restricted temperature range for successful reproduction as compared with the range over which they can survive.

Our objective in the present research is to learn whether the limits of reproductive temperature tolerance of the pupfish can be shifted or extended by long-term exposure to stressful temperatures. We chose generation-long exposure to simulate permanent temperature changes caused by large construction projects, such as the higher temperatures in the condenser discharge of a power plant or the lower temperatures in the tailwaters below a hydroelectric dam. Several metabolic processes, such as oxygen consumption, respond to short-term exposures to temperature above or below that to which the organism is accustomed. Upon returning the organism to the original temperature, oxygen consumption is either greater or less than it was before. These short-term exposures, called acclimation, may last for days or weeks or for seasons in the case of acclimatization in nature. Our long-term exposure of the pupfish to stressful temperatures from egg to maturity was prompted by our finding that

oogenesis is the most sensitive stage in the life cycle. Such exposure is, however, beyond the usual usage of the term "acclimation" and resembles "canalization" of Waddington (1942). He defined canalization as the adjustment of development reactions "so as to bring about an end result regardless of minor variations in conditions during the course of the reactions." Acclimation, therefore, accents effects on adults whereas canalization highlights developmental processes in the egg. We are not wholly satisfied with either term to describe generation-long exposure, because quite different effects might be expected upon exposing the developing egg and the adult to a common stress. Despite these reservations, we have used acclimation in the discussion of our results.

Studies of generation-long exposures are infrequent in the vertebrates. One of the most thorough is Kinne's (1962) work on "irreversible non-genetic adaptation" in which generation-long exposure of the pupfish, Cyprinodon macularius, to various salinities produced profound effects on growth (food conversion efficiencies). The pupfish reared at salinities to which their parents had been exposed performed much better than to some other salinity within their tolerance range. Pupfish bred and reared at 35⁰/oo salinity, for example, had higher conversion efficiencies than when eggs from the same batch were reared at 15⁰/oo. Conversely, conversion efficiencies were higher among those bred and reared at 15⁰/oo than at 35⁰/oo when conditions were reversed.

If Kinne's results are applied to reproduction, a group of fish bred and reared at one temperature should outperform a group from the same brood exposed to another temperature. To test this hypothesis,

we bred fish at 24, 28 and 32 C and reared them to maturity at those temperatures. We had previously determined that 28 C was close to the optimum for reproduction, whereas 24 and 32 C were marginal. At maturity each of the three broods was subdivided into three groups, one to be tested at its rearing temperature and the other two at temperatures which they had not encountered for an entire generation. The results of the experiment did not conform to the hypothesis; no temperature compensation was observed. Reproductive performance was optimal at 28 C, and was poor at the marginal temperatures, regardless of the previous thermal history of the fish. These results indicate that neither the optimum temperature nor the reproductive tolerance limits can be shifted upward or downward by generation-long exposure to stressful temperatures.

MATERIALS AND METHODS

A small stock of Cyprinodon n. nevadensis was collected from the marsh of Saratoga Springs, Death Valley National Monument, California, in June, 1974. From this original stock, a breeding pair was selected to produce an F_1 parental generation. Successive generations arose from breeding pairs derived from the F_1 . A pair from the F_3 generation gave rise to the experimental stock used in these experiments. The young were reared in 80 l aquaria to maturity (approximately 4 months).

The F_4 sibling stock was divided into three groups. Two groups were placed in controlled environment chambers at temperatures of 24 and 32 C (± 0.05 C), with a photoperiod of 16L 8D. The third group was reared in an aquarium room at 28 C with the same photoperiod. After maturing, breeding pairs were selected, placed in 20 l tanks

and allowed to produce an F_5 generation under the same temperature conditions that their parents had experienced. This F_5 generation had been exposed, therefore, to the same temperature from the egg maturation stage to mature adult. The standard lengths (tip of jaw to end of vertebral column) and weights of the three groups were: $24C = 28.9 \pm 5.9$ mm and 0.83 ± 0.23 g; $28 C = 33.1 \pm 2.6$ mm and 1.07 ± 0.35 g; $32 C = 32.7 \pm 3.6$ mm and 1.01 ± 0.37 g.

Reproduction of the F_5 fish was tested by placing from 6 to 10 pairs at the temperature at which they were reared and also at each of two other temperatures. Thus, the fish reared at 24 were allowed to spawn at 24, 28 and 32 C; fish reared at 28 spawned at 24, 28 and 32 C; and fish reared at 32 spawned at 24, 28 and 32 C. These reproductive tests occurred in September and October, 1976, with one exception. Due to a low survival of fish reared at 32 C, reproductive tests at 28 C were done in two series, one was run in July 1976, and it was repeated in March, 1977. The extreme temperatures were chosen because they are near the borderline of successful reproduction.

A reproductive test consisted of the following steps. A pair was placed in a 20 l aquarium, divided by perforated plexiglass to separate the aggressive male from the female. The divider was removed each morning for 30 minutes to allow the fish to spawn on yarn mop. The test period was 21 days. The eggs were removed from the yarn mops and counted each day. Egg diameter and yolk diameter were measured along the axis perpendicular to the animal-vegetal pole during the 2- to 8-cell stage with an ocular micrometer. Development of the eggs until hatching was followed. Rather than spawning on the 22nd day, the fish were weighed and measured and the ovaries from the females were removed and weighed.

The fish were fed Tetramin staple food and brine shrimp immediately after spawning.

RESULTS

Three criteria were used to evaluate reproductive success; eggs laid per spawning, percentage of days when eggs were laid, and eggs laid per gram of body weight per day over the 21-day span of the experiment. Egg laying was adjusted for body weight, even though no consistent correlations were found between size (standard length and body weight) as a function of each measure of reproduction for the nine temperature combinations. All three of the criteria gave the same result (Figs. 1, 2, 3). Regardless of the previous temperature experience, the optimum temperature for pupfish reproduction was 28 C. Not only did those fish which had been reared to maturity at 28 C perform optimally at that temperature, but the same can be said for those reared at 24 and 32 C.

The results were consistent inasmuch as no statistically significant difference was found among the three observations at 28 C. For example, fish reared at 28 C and tested at the same temperature laid an average of 8.8 eggs $g^{-1} day^{-1}$, whereas fish reared at 24 C and tested at 28 C laid 8.1 eggs $g^{-1} day^{-1}$ and fish reared at 32 C and tested at 28 C laid 9.0 eggs $g^{-1} day^{-1}$. All of the fish tested at 28 C laid an average of 10.2 eggs per spawning and laid them on 76 per cent of the days in the test period. In an analysis of variance, log transformed data from the nine possible test combinations showed a significant difference for each of the criteria of reproductive performance (Table 1). The differences were all attributed to superior reproductive success at 28 C. No differences were

found among the three means at 24, 28 or 32 C, regardless of the temperature to which the fish had been exposed. By coincidence, the means of two marginal temperatures were not statistically different. Such consistency among the means was not expected since the day to day variability is high (coefficient of variation ranges from 49 to 100 per cent).

Hatchability tests demonstrated some differences among the various temperatures that were not revealed by the above criteria of reproductive success (Table 2). The general conclusion was reached that a temperature of 32 C conveyed a disadvantage to hatching whether the fish were reared at that temperature or at 24 and 28 C. For example, when eggs were reared at either 24 or 28 C and testing was done at the same temperature, or its alternate, hatchability averaged 63 per cent. When testing was done at 32 C for each of these two rearing temperatures, hatchability was 22 per cent for the fish reared at 24 C and was 36 per cent at 28 C. No statistical test was conducted on these data since the observations were lumped and treated as a single determination at each temperature. Nevertheless, the differences between the optimum and marginal temperatures are striking. Hatchability was correlated with the difference between the acclimation temperature and the rearing temperature: reared at 24, tested at 32 C = 22 percent; reared at 28, tested at 32 C = 36 per cent; and reared at 32, tested at 32 C = 51 per cent. No correlation could be demonstrated when the test temperature was lower than the rearing temperature.

A smaller yolk diameter and gonosomatic index was associated with 32 C as compared with those at 24 and 28 C (see Table 1). The 1.02 mm yolk diameter at 32 C did not prove to be statistically different from the 1.05 mm diameter at 24 and 28 C, but the difference suggests

that the 32 C egg may be at a slight disadvantage in this respect. Statistical analysis clearly demonstrated, however, that at 32 C the ovary was much smaller in relation to body weight (0.07) than at either 24 (0.10) or 28 C (0.11).

DISCUSSION

Optimal reproduction. The desert pupfish lays more eggs and lays them at a greater frequency at 28 C than at either 24 or 32 C. This optimum prevails regardless of the temperature to which the fish has been exposed. Therefore, generation-long acclimation to stressful temperatures has no lasting effect on courtship behavior, egg development and the release of eggs during spawning. In our earlier study, a reproductive optimum of 30 C prevailed with an average egg production of 6 eggs $g^{-1} day^{-1}$. No tests were made at 30 C in the present study, but the 28 C average of 8 eggs $g^{-1} day^{-1}$ corresponds closely with that of the original result.

Variability. Day-to-day egg laying is highly variable. To illustrate this variability, the 21-day performance of a single female at 28 C is reported: 16,0,0,3,1,3,6,0,9,8,1,14,2,0,0,0,16,31,20,5,7. The presence of several days when no eggs were laid contributes heavily to the high coefficients of variation for the measures of reproductive performance (eggs $g^{-1} day^{-1}$ = 70 per cent; percent days laid = 51 per cent; eggs per spawning = 69 per cent). We had hoped to reduce this variability by continued in-breeding, but we have seen no improvement between the F_3 and F_5 generations.

Hatchability. In an earlier paper we remarked that the egg coat was thin and tended to break when the eggs were transferred from the spawning mop to the hatching dishes at 32 C. This situation also

prevailed in the present research and undoubtedly contributed to the lowered hatchability at that temperature. The smaller yolk diameter and lower gonosomatic index at 32 C than at 24 and 28 C may also have contributed to lower hatching success. The higher metabolic rate at that temperature evidently siphoned away energy for maintenance requirements that was apportioned to ovarian development at the other two temperatures.

Temperature compensation. This is the first study of temperature compensation of the reproductive function, and our results show no adaptation to stressful temperatures. Hazel and Prosser (1974) review acclimation effects on activity, performance, neural and endocrine function, digestive processes, oxygen consumption, and cellular metabolism. They cite a long list of enzymes and studies similar to ours from the coelenterates to the vertebrates which show no temperature compensation. Our findings are analogous to those on the metabolism of the Atlantic salmon (Salmo salar). Specimens were acclimated to 6 and to 18 C and then placed in the alternate temperature. Oxygen consumption, after a two-week adjustment period, was typical of the test temperature and was independent of the direction of temperature change (Peterson and Anderson 1969). Among studies of specific tissues, Evans, et al (1962) found that the gill tissue of rainbow trout (Salmo gairdneri) was able to extract oxygen and perform its osmoregulatory functions at different temperatures without undergoing metabolic compensation to these temperatures. On the other hand, brain and liver tissues showed varying degrees of adaptation to the acclimating temperatures.

Other physiological functions in a broad phylogenetic sampling which exhibit no temperature compensation are: oxygen consumption in

the coelenterates, Aurelia aurita and Chrysaora quinquecirrha (Mangum et al 1972); in the annelid, Diopatra cuprea (Mangum 1972); and in the echinoderms, Strongylocentrotus purpuratus and Allocentrotus fragilis (Albricht 1973). No temperature compensation has been found in the ciliary resistance times of the molluscs, Anodonta anatina and A. cygnea (Senius 1975); in the critical thermal resistance of the tarantula, Aphonopelma sp. (Seymour and Vinegar 1973); or in the heart rate of the frog, Rana temporaria (Harri and Talo 1975). This list does not exhaust the possibilities in the literature. We conclude that no temperature compensation (Type 4 of Precht 1958) is unusual but not rare among the multitude of temperature compensation studies that have been performed. Thus, our finding that the reproductive function exhibits no temperature compensation is not entirely unexpected, although it could not have been predicted on the basis of past research on other metabolic parameters in fish or other animals.

Practical significance. Our results bear on several questions relating to the survival of fish populations in stressful thermal environments. First, although survival tests on eggs, larvae or adults will be valuable to measure the effects of short exposures, they will not yield critical information on the ability of a population to persist in a stressful thermal regime. This results from the fact that thermal limits for successful reproduction are much narrower than those for survival. Second, the thermal limits for pupfish reproduction cannot be shifted upward or downward. If this same result applies to other species, fish trapped in water of unaccustomed temperature, such as a thermal effluent from a power plant, will not accommodate their reproductive function to the new regime, even though they live a whole

generation in the altered conditions. Third, we conclude that the reproductive function is regulated in such a complex manner that the multiple physiological mechanisms involved--neural, endocrinological, genetic--are not subject to alteration by generation-long acclimation.

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Table 1. Summary of the tests of reproductive performance by the desert pupfish, Cyprinodon n. nevadensis. The means and standard deviations at each test temperature are composed of three observations, one from each acclimation temperature.

Criteria of reproductive success	Test temperature		
	24	28	32
Eggs per spawning	4.5 $\pm$ 3.7	10.2 $\pm$ 5.0*	2.9 $\pm$ 2.2
% days when eggs were laid	39 $\pm$ 19	75 $\pm$ 22*	38 $\pm$ 21
Eggs g ⁻¹ day ⁻¹	2.0 $\pm$ 2.0	8.6 $\pm$ 5.2*	1.4 $\pm$ 1.4
Yolk diameter	1.05 $\pm$ 0.04	1.05 $\pm$ 0.03	1.02 $\pm$ 0.04
Gonosomatic index	0.10 $\pm$ 0.03	0.11 $\pm$ 0.03	0.07 $\pm$ 0.03**

*F value significant at $P < 0.05$ with 8 and 67 d. f.; superior performance at 28 C indicated.

**F value significant at $P < 0.05$ with 2 and 72 d. f.; smaller diameter at 32 C indicated

Table 2. Hatchability of desert pupfish, Cyprinodon n. nevadensis
eggs from fish reared under different temperature conditions.
Hatchability was measured at the test temperature.

Rearing Temperature °C	Test temperature °C	Egg number	Hatchability %
24	24	62	63
24	28	665	61
24	32	97	22
28	24	215	64
28	28	549	63
28	32	163	36
32	24	319	56
32	28	119	55
32	32	106	51

Figure 1. The percentage of days on which eggs were laid by pupfish, Cyprinodon n. nevadensis, in 21-day reproductive tests in the laboratory. Mean and standard deviation are given for each point on the graph.

Figure 2. The number of eggs laid per spawning by the pupfish, Cyprinodon n. nevadensis, in 21-day reproductive tests in the laboratory. Mean and standard deviation are given for each point on the graph.

Figure 3. The number of eggs laid per gram of body weight per day by the pupfish, Cyprinodon n. nevadensis, in 21-day reproductive tests in the laboratory. Mean and standard deviation are given for each point on the graph.

PRELIMINARY RESULTS

Growth Performance of a Desert Pupfish Cyprinodon n. nevadensis, at Different Temperatures

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Abstract

This report brings up to date the progress in the analysis of pupfish growth at 20, 24, 28, 32 and 36C. Wet weight and dry weight gains or losses have been observed on 8-10 specimens at each temperature after feeding daily rations of 1.5, 3.0 and 4.5 percent of the body weight per day. At 36C weight loss was so severe at 1.5 percent that it was discarded in favor of a high rate of feeding, 6 percent of the body weight per day. The following body content analyses have been made: caloric content, ash, protein, and fat. Feces have been weighed for determination of assimilation rates. Since fish weights were not exactly alike in each experiment, weight and temperature effects are confounded at each ration. The relative contribution of each to the total variation will be analyzed by analysis of covariance and proper adjustments will be made.

Work Accomplished Since 1977 Report

Feeding experiments were conducted at 20 and 36C, completing a series that now comprises 20, 24, 28, 32 and 36C. The details of the experiments are given in the 1977 report. One major unanticipated finding caused a shift in our original plans. The weight loss at the 1.5 percent body weight daily ration at 36C was so severe that several specimens died before completing the 7-week growth test. Weight loss was pronounced at 3.0 and 4.5 percent also. Therefore, a high rate of feeding, 6 percent per day, replaced the original 1.5 percent per day but even this was insufficient food to prevent weight loss. Apparently metabolism is very high at 36C, and the fish are not able to assimilate enough energy to match the loss.

Analysis of weight loss and gain. All of the basic data on weight gain and loss have been scrutinized carefully (Table 1) and show expected responses to daily ration and temperature. With some minor variations,

weight loss, where it occurs, is progressively more severe as the temperature increases at each daily ration. Weight gain is reduced under the same conditions. This is true whether wet weight or dry weight is used as a criterion of growth response. The results are even more consistent with respect to daily ration at each temperature. Without exception gain in weight increases (or weight loss is reduced) as more food is offered. Therefore, we conclude that the data basically reflect the expected responses and are amenable to more detailed treatment.

Adjustment for beginning body weight. The beginning body weights vary from one feeding trial to another. Since body size is generally correlated with a reduced growth potential, the growth responses need to be adjusted in order to equalize this effect over the whole experiment. The experiments at 20C were obviously run with smaller fish than at the other temperatures and the greatest adjustment will be made in this series of growth responses. The equalization can be accomplished by an analysis of covariance, and this will be the next step.

Assimilation. The feces were weighed each week for each fish when the water was changed. The water was filtered through glass discs under suction and the difference in weight of the disc before and after feeding was regarded as undigested food. We are aware of three potential sources of error in this procedure.

1. All of the food may not have been consumed. Special effort was made to observe food particles which were caught in the surface tension. It was our conclusion that all of the food was consumed in a vast majority of cases. Therefore, we are treating the data as if the pupfish ate all of the food that was offered to them.
2. Algae growth in the beakers. On some occasions the presence of undigested food stimulated algal growth. The growth was not consistent from beaker to beaker and it never became heavy during the one week between changes of water. Nevertheless, we do know that some of the fecal samples were contaminated with algae. No method of correcting for algal growth was found. Therefore, this error was disregarded.
3. Coprophagy. No significant error would be caused if the food passed through the gut a second time unless the feces particles were colonized by heavy growths of bacteria. We do not know

the extent of bacterial colonization and have reluctantly disregarded this possible source of error.

All of the data have been assembled in order to compare the feces with the amount of food offered to the pupfish (assimilation efficiency) but they have not yet been analyzed.

Proximate body composition. Ash contents, protein and fat analyses have been completed. Ash was measured by conventional techniques, but we resorted to the Folch wash for the small sample available for fat content. Protein was also measured by a microtechnique based on the Lowry analysis. From these analyses we expect to obtain two kinds of results: the change in body composition with daily ration and the estimation of caloric content. A preliminary inspection of the data indicate that fat increases with daily ration as expected. This will affect the relative proportions of ash and protein in the body composition.

Caloric content can be estimated closely from the caloric values of fat and protein in the body. Such estimates compare closely with bomb calorimeter measurements. By comparing the caloric value of the fish at the beginning and end of a feeding trial and knowing the maintenance level (standard metabolic rate) and growth in calories, we will be able to partition the total metabolic cost into some of the important metabolic expenditures.

Preliminary Results

The Effect of Salinity on Reproduction of the Desert Pupfish, Cyprinodon n. nevadensis

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Abstract

Reproductively active pupfish (Cyprinodon n. nevadensis) pairs were allowed to spawn throughout a salinity range from 0.5°/oo to over 40°/oo. Utilizing eggs per gram body weight per day and percentage of days laid and eggs per spawning as reproductive success criteria, 10 and 20°/oo were the optimal salinities tested. Successful reproduction decreased below 10°/oo and decreased markedly above 20°/oo. The range of salinities at which this subspecies reproduces in nature is 2 to 8°/oo.

Introduction

This study of the salinity effect upon reproduction, as well as the upper and lower lethal limits for salinity, will complement previous work done with the desert pupfish. With the addition of these salinity results to the thermal data previously obtained, we will have considerable knowledge of the reproductive limitations of this unique animal, which has adapted to such an extreme environment.

Kinne (1966) stated that the cleavage, embryonic and larval stages were those stages most sensitive to salinity extremes. These were followed in order of sensitivity by breeding and fertilization.

Gunter (1956) concluded that animals distribute themselves over definite

salinity ranges and appear to be quite sensitive to salinity differences near the lower end of their tolerance ranges. Deacon and Minckley (1974) recorded the salinity of Saratoga Springs as 7.94‰. Other salinity measurements, by Deacon and the present authors, have been in the 2-4‰ range. Very high evaporation rates during summer months may change these values considerably, especially in the shallow waters preferred for spawning.

Materials and Methods

A laboratory stock (F_6) of Cyprinodon n. nevadensis from Saratoga Springs, Death Valley National Monument, California, was used. Breeding pairs from this stock were placed in 20 l aquaria, the fish separated by a perforated plastic divider. This divider was removed for 30 minutes daily to allow spawning. Eggs were laid on a yarn mop from which they were removed and incubated in petri dishes. Yolk diameters were measured along the axis perpendicular to the animal-vegetal pole during the 2- to 8-cell stage with an ocular micrometer.

Salinities of 10, 20, 30 and 40‰ were produced with Instant Ocean salts. Lower salinities were obtained by diluting the standard conditioned tap water with distilled water. Salinities were measured as conductivity values determined with a YSI T-C-S meter.

The experiment was performed in two series; the lower salinities and a control as one series and the upper salinities and a control as the other. The fish were maintained at 26 ± 0.5 °C for the lower salinities and at 28 ± 0.5 °C for the higher salinities, with a photoperiod for both of 16 L and 8 D. Immediately after spawning each day, the fish were fed Tetra Min staple food.

Results

The results of this experiment, shows that 20°/oo is the optimal salinity for egg production, falling off precipitously beyond this point (Table 1). It appears that egg quality is maximum at 10°/oo, with a more gradual decline in either direction.

Pupfish are unable to live beyond a mean of 8.5 days at salinities below 0.05°/oo; while they could tolerate salinities up to 85°/oo, when the salinity was increased gradually (Table 2). Egg production occurred from 0.14 to over 43°/oo. Using 50% hatch as the success criterion, a range from 0.47 to 20°/oo would enclose reproductive capabilities.

The differences between the two control series may be attributed to the small difference in temperature (26 and 28 C), between the two series of experiments, the small change in salinity of the tap water between experiments (0.05 to 0.06), and the time the two series were conducted.

A full analysis of the data has not been completed, but certain interpretations can be suggested. Due to the high evaporation rates found in the Death Valley area and the pupfish's predilection to spawn in the shallowest portions of its marsh habitat, it is conceivable that the fish may encounter salinities in excess of the 7.94°/oo reported earlier. The pupfish may be 'selecting' these higher salinity situations for the enhanced value it gives to the eggs. Eggs produced at these higher salinities have a much harder chorion, one less susceptible to injury or penetration. We expect to explore this possibility in the field during the coming year.

The results would indicate that, contrary to Gunter's (1956) findings, high salinities were at least as critical, if not more so, than lower salinities for reproductive success or failure. If the cleavage and embryonic stages are most sensitive to salinity, hatching success would be low at salinities where egg production was still occurring. This is borne out at the 30 and 40⁰/oo levels, where the eggs were fertilized, yet cleavage stages were arrested at some point prior to hatching.

TABLE 1

	Salinity ‰							
	0.140	0.214	0.470	0.620	11.000	19.700	30.900	43.300
Reproductive Measures								
eggs/gram/day	.74	2.78	3.34	6.90	8.04	14.65	1.13	.86
% days laid	24.7	51.4	48.8	58.9	63.6	68.8	6.6	8.5
eggs/spawning	2.12	4.92	4.63	5.70	5.70	9.07	2.84	2.42
eggs/day	.79	2.54	2.48	3.71	3.70	6.14	.55	.85
Egg Quality Measures								
% hatch	43.6	41.7	68.9	77.0	81.9	80.5	0.0	0.0
% bad eggs	44	34	14	11	4	9	0	2
egg diameter	2.78	2.81	2.80	2.77	2.71	2.65	2.73	2.80

Preliminary Results

Limits of Tolerance to Hydrogen-ion Concentration of the Desert Pupfish, Cyprinodon n. nevadensis

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Abstract

Pupfish were placed in acidified tanks with pH values ranging from 3.5-5.0 to determine a 96-hour LC-50. A linear relationship occurred between pH (3.90 to 4.70) and mean survival time. The 96-hour LC-50 was 4.67; above 4.70 the fish lived indefinitely, below 3.90 the fish lost equilibrium in less than 8 hours. Fish behavior appeared to be normal above pH 4.15. Below pH 4.20 a mucoid substance formed on the fins.

Introduction

To be able to determine the effects of acid upon reproduction, it is helpful to know the lower limits of survival for the test organism. This aids in the design of reproductive experiments to be performed at more moderate pH levels. It would also be of interest to determine whether sex or age differences cause variation in the lethal limits.

Literature Cited

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Materials and Methods

The system used to monitor pH was a Corning Model 12 pH meter connected to a YSI Model 80A recorder. A Thermomix Junior Immersion Thermostat was used to keep the water well circulated at 28 C. pH values were maintained within ± 0.15 pH units by manual addition of dilute sulfuric acid.

For each test, the system was brought to the target pH and allowed to equilibrate. 10 fish, from a laboratory stock (F_6) of Cyprinodon n. nevadensis, were added to the acid/conditioned tap water in a 201 aquaria. The time of equilibrium loss for each fish was recorded over a 96-hour period. The mean survival time for each test was then plotted against pH. Fish were maintained in a 16L 8D photoperiod and were fed Tetra Min staple food every 24 hours.

Five pH test runs were performed; 3.88, 4.15, 4.28, 4.40 and 4.67.

Results

The regression generated from plotting mean survival time against pH provides the following results: (1) LC-50 for 96-hours occurs at pH 4.67, (2) LC-50 for 48-hours occurs at pH 4.25, and (3) LC-50 for 24-hours is pH 4.04. Fish kept above pH 4.70 were apparently unaffected by the ion concentrations. Fish kept below pH 3.90 lost equilibrium in less than 8 hours. Below pH 4.20, excess mucous formed on the fins and it sloughed from the fish during the experiments. Normal behavior, feeding, sexual display and fighting occurred as low as pH 4.15. Below this point the fish appeared to be seriously stressed. The fish would alternate active surface swimming with resting motionless on the bottom.

Loss of equilibrium was regarded as the time at which a fish would continually lay on its side despite being touched with a glass stirring rod, while still exhibiting opercular movements. Approximately 75 per cent of the fish would recover completely after this point, if they were placed in normal tap water.

Males and females appear to be equivalent in their response to acidification. Larval fish, like the adults, appear to have a 96-hour LC-50 at 4.70, but they will succumb more rapidly than the adults to pH's at the 4.20 level and below (LC-50 for 48 hours occurs at pH 4.31 and LC-50 for 24 hours occurs at pH 4.19).

Preliminary Results

The Effect of Hydrogen-ion Concentration on
Reproductive Success in the Desert Pupfish,

Cyprinodon n. nevadensis

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Current Research

At the present time, reproductive tests are being performed at pH 5.5 and 6.0. The initial results indicate that a comparison can be made with a control group with respect to the number of eggs laid and hatchability. These test fish lay eggs at only 25 per cent of the rate that fish at more moderate pH's (ca. 8.0) produce eggs. These experiments will be repeated at pH 5.25, 5.00 and 4.75.