REPRODUCTIVE TEMPERATURE TOLERANCE OF A
DESERT PUPFISH

Technical Progress Report
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TECHNICAL PROGRESS REPORT

The original breeding stock of C. n. nevadensis for this study was collected from the marsh at Saratoga Springs, Death Valley National Monument, California. From this stock three pairs of fish were selected and isolated for breeding purposes in 60-liter aquaria at 28 C, 16L 8D, under incandescent lights at an intensity of 5.5 - 6.0 electron Volts (eV). The fish were fed Tetra-Min staple diet and newly hatched brine shrimp.

One male and one female were placed in an aquarium separated by a perforated plexiglass divider to prevent the aggressive male killing the female. Each morning at 0900 hr, a yarn spawning mop was placed on the aquarium bottom and the divider was removed for 30 minutes. Eggs were quickly deposited one at a time in the mops. The yarn was removed after 30 minutes and maintained at 28 C while the eggs were gently removed with a watchmaker's forceps and examined under a binocular microscope. The eggs were placed in chiffon-lined plastic mesh incubation baskets, 9 x 9 x 8 cm deep, never more than 30 eggs per basket. The baskets were suspended in aerated 22-liter fiberglass tanks. A 600-watt thermostatically controlled heater in each tank was adjusted to maintain 28 ± 0.1 C in the eggs baskets. Eggs were raised in dechlorinated Tempe tap water to which 2 mg/1 methylene blue was added to retard fungus. The photoperiod was 16L 8D under incandescent lights at an intensity of 5.5 - 6.0 eV. Newly hatched larvae were transferred to aquaria and reared on a diet of Tetra-Min staple diet and live brine shrimp at 28 C, 16L 8D.

One of the three sibling stocks was chosen as the parent stock for subsequent experiments. Seventeen breeding pairs were selected from this parent stock; each pair was placed in a 20 l aquaria at 28 C, 16L 8D. The male and female in each tank were allowed to breed, as described above, daily for a three-week period each month from October to the present time, except for December, when the breeding period was two weeks. During each breeding period the following observations were made daily for each pair:

1) Reproductive behavior was observed. Intensity of breeding coloration and chasing by the male was noted. The response of the female was observed, either to swim toward the surface away from the male or into the mop, often followed by spawning.

2) The number and sequence of days on which eggs were laid by each pair was recorded and the eggs were counted. The yolk diameters of eggs were measured with an ocular micrometer at 25X. They were measured at the two- to four-cell stage across the axis perpendicular to the animal-vegetal pole axis.

3) On the middle of the fourth week of each month when no breeding took place, each fish was measured, total and standard length to the nearest mm, then weighed to the nearest .01 gr on a balance in a pre-weighed beaker of water. All fish were weighed in the morning before feeding.

The mean size of the females in the parent stock over the five-month period of October through February was 29.0 ± 2.7 mm (SL $\pm$ 1 SD, n = 77), range 24 to 35 mm. The coefficient of condition $\frac{Wt(mg) \times 100}{TL^3}$

ranged from 1.53 to 2.28. The variation is partly due to the variation in the gravid condition of females at the time of weighing. At 28 C these females laid eggs an average of 44% of the total days they were allowed to breed, the mean number of eggs/day/female was 3.2 ± 3.6 , or 4.6 ± 5.2 eggs/day/gram. All breeding pairs produced successfully; the lowest maximum egg production for any one female was 0.65 eggs/day.

With these parent stock experiments we have accomplished two goals; we have produced the sibling generation fish for thermal reproductive experiments and we have answered some fundamental questions which will be important in interpretation of the results of those experiments. Is number of eggs laid per day correlated with female size? At 28 C, the correlation between standard length (SL) of the female and eggs produced/day is not significant ($r = 0.14$, $P > 0.50$, $n = 77$). There is also no significant correlation between egg size as measured by yolk diameter and SL of females ($r = 0.29$, $P > 0.05$, $n = 44$). However, there may be a difference in egg size between females. For example, yolk diameters for eggs of seven four-month-old females, SL 28 to 29 mm, which laid more than 40 eggs during the three-week breeding period, were compared in a completely randomized analysis of variance. Differences in egg size were significant ($P < 0.05$ with 6 and 200 DF). In a Student-Newman-Keuls multiple range test, one female was significantly different from the rest. This female was the smallest of the group, but laid the largest eggs and the most eggs during the time period.

The sequence of days on which eggs were laid was tested for a pattern, such as occurs in some fish such as the zebra fish, Brachydanio

rerio, which lay eggs approximately every five days. When the sequences of days on which eggs were laid by pupfish were tested in a one-sample runs test, 60 of 62 3-week sequences were random, only two were non-random.

We conclude that egg size is not related to female size but may be affected by differences in individual females. Neither is the number of eggs laid per unit time related to female size. This may be compounded, however, by the affects of age and season which cannot be excluded since all fish tested so far have been the same age during the same season. We also conclude that there is no pattern to the sequence of days on which eggs are laid. The collection of this data on the parent stock is continuing at the present time.

During February, 1975, two walk-in controlled environment chambers were installed in the laboratory and tested. These were ready for use in March, at which time the reproductive temperature tolerance tests were begun.

The first tests were conducted at the expected low end of the breeding range, 20 and 22 C. These tests are still in progress. Ten males and ten females were selected from the second generation sibling stock for each of the two tests and acclimated for three weeks at the test temperature under a photoperiod of 16L 8D. Males and females were kept in separate aquaria. At the end of the acclimation period, breeding pairs were placed in divided 20 l aquaria in the controlled environment room at the test temperature, 16L 8D. After an adjustment period of three days breeding tests were begun as described above and continued daily for three weeks. Reproductive behavior and coloration

are observed daily. The number and sequence of days on which eggs are laid is recorded. The number of eggs laid each day is counted, the yolk diameters are measured, and the eggs are returned to the chamber for incubation to determine percent survival at the test temperature. At the end of the three-week period, the breeding fish are weighed and measured (TL and SL). From the females the ovaries are dissected, weighed, and preserved in Simpson's modification of Gilson's fluid. The yolked follicles are counted and a sample (50) of the largest measured with an ocular micrometer.

The results of the tests in progress at 20 and 22 C to this point indicate very minimal breeding success. No fish at 20 C show breeding colors and only three pair have produced one or two eggs each. The rest demonstrate no chasing or other indication of reproductive behavior. At 22 C a total of nine eggs have been produced in two weeks by two of the breeding pairs. One male shows moderate breeding color. Two males consistently chase the females, who respond negatively by swimming toward the surface. All other pairs remain passive when the dividers are removed.

The acclimation and breeding procedure described above will be repeated for a range of constant and fluctuating temperature regimes. By June 30th, constant temperature tests will have been conducted at 20, 22, 24, 28, 32, 34, 36, and 38 C.