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EGG DEPOSITION OF THE DESERT PUPFISH, *CYPRINODON MACULARIUS*, IN RELATION TO SEVERAL PHYSICAL PARAMETERS [1]

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The effects of spawning mop color, salinity, and water depth on the reproduction of the desert pupfish were examined. Green spawning mops were most frequently used for egg deposition. More eggs were deposited by fish acclimated to 5 0/00 salinity than those at 15 0/00. Fish acclimated to 5 0/00 used the deepest portion (37 cm) of their tank for egg deposition significantly more than other depths. Fish acclimated to 15 0/00 utilized the intermediate depth (22 cm) for egg deposition significantly more than other available depths.

INTRODUCTION

The California Department of Fish and Game's Water Pollution Control Laboratory has been evaluating many different fish species for consideration as standardized bioassay test species. Selection criteria were based upon the physical requirements of bioassays which often include wide ranges in temperature and salinity. These considerations limited the number of species which might be suitable for laboratory bioassays to eurythermal and euryhaline species, such as the desert pupfish, *Cyprinodon macularius* (Baird and Girard). Whichever species proved most feasible for laboratory bioassay testing would also have to be available in large quantities on a year-round basis. Since the pupfish appeared to meet the selection criteria, an efficient method to propagate large members of eggs and fry was needed.

Aspects of artificial propagation for this species have been extensively studied. Kinne (1960) and Crear and Haydock (1971) have described laboratory culture utilizing individual pairs of breeding adults. Miller (1948, 1950) studied cross breeding with related species. Barlow (1958, 1961), Cox (1966), Minckley and Arnold (1969), and Arnold (1972) have all described general behavior patterns and mating activities for this species. In all these studies egg production was limited because pairs of pupfish (one male and one female) were used in each tank. The present study examines reproduction in relation to color of spawning substrate, salinity, and water depth. Reproduction of groups of pupfish rather than pairs was examined.

MATERIALS AND METHODS

Fish Stocks

Fish used for our experiments were collected from canals around the Salton

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Sea, California during January 1975. The fish were transported in Salton Sea canal water (18 0/00 salinity) by airplane to Sacramento and then by truck to our laboratory. Formulated seawater of the same salinity was utilized at the laboratory. The fish were held in 1140-liter (300-gal) circular holding tanks containing a biological filter (Spotte 1970) to reduce build-up of waste products. Frozen brine shrimp were provided as forage during the holding and test periods.

By maintaining the fish at low densitites in each of the tanks and providing continuous water filtration to reduce ammonia levels, mortalities were kept to < 1%. The fish were held in the laboratory for 2 months before the experiments were started.

Egg Collecting and Handling

Wool yarn was selected as the spawning substrate because it was available locally in a large choice of colors. Spawning maps were constructed of strands of colored wool yarn 38 cm (14.5 inches) long and consisted of 20 strands per mop. The strands were knotted as a group in the center and then slipped over a glass rod. The glass rod served not only as an anchor for the mop but also permitted retrieval of the mop and deposited eggs. Each mop was rinsed in fresh water for 24 hours before use to remove any soluble residues present on the yarn.

Mops were removed from the test tank each day and the eggs removed with tweezers, counted, and placed in a floating basket in a separate incubation tank. The basket was constructed of fiberglass window screen (Figure 1). The mesh size (approximately 1.0 mm) permitted the hatched fry to swim out of the basket into the hatching tank. This allowed easy separation of hatched fry from incubating eggs. The incubation tank water was maintained at 5 0/00 salinity and 20 ± 2 C (68 ± 3.6 F) based on results presented by Crear and Haydock (1971). Continual aeration provided water flow past the developing eggs. The incubator tanks had been filled and allowed to stand unused for several weeks prior to egg introduction. Algal growth developed in the unused tanks and provided food for developing fry. Dry, ground brine shrimp were fed as a supplement once per week.

The first series of experiments were designed to determine the preferred color of artificial spawning material. If *C. macularius* had a color preference, this could maximize spawning activity and egg deposition via visual stimulation. The second series of tests examined the relationship of depth and salinity upon egg deposition to determine if there was a preferred spawning depth at different salinities. The final experiment assessed the egg deposition rate of adult *C. macularius* held under defined laboratory conditions. For each test a new group of 10 females and 2 males was used. This ratio was maintained throughout the study and mortalities were replaced. Chi square tests were used to determine statistical significances of egg deposition rates established during the various experiments.

These experiments are designed to provide further understanding of environmental influences on the reproductive biology of *C. macularius*. This would not only evaluate the most efficient method to rear this species for the laboratory bioassay testing program, but these same methods could also find application for related pupfish species to supplement declining populations.

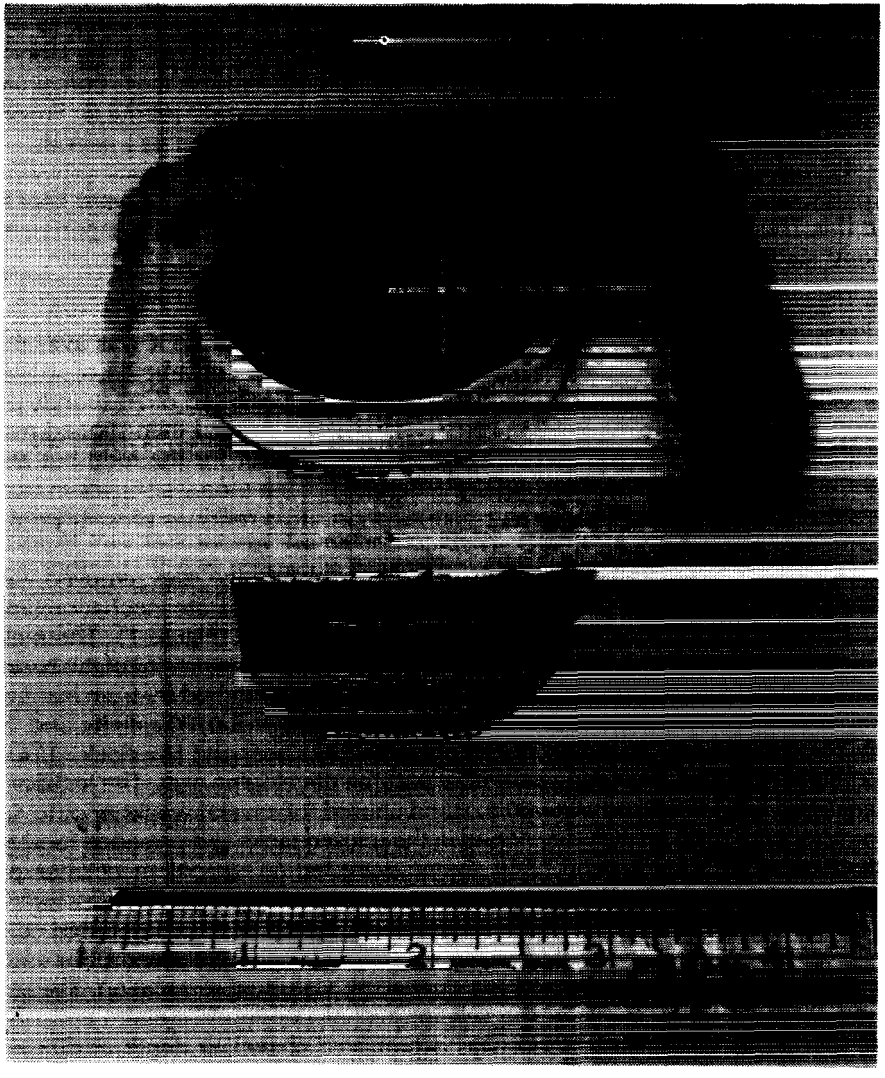

FIGURE 1. Floating incubation basket

Color Preference

Ten female and two adult male pupfish were placed in a 95-liter (25-gal) substrate filtered aquaria. Salinity was 5 0/00 and temperature 20 ± 2 C (68 ± 3 F). Two mops of different colors (green, gold, grey, or beige) were placed in the tank at different locations. Each day the mops were removed, the eggs collected, and the mops returned to the test tank at reversed locations. A heat-light stimulus approximating summer conditions was applied to the tank to initiate egg deposition, This consisted of 14-hour light cycle and slow elevation of tank temperature from 20 C up to 28 C (82 F). At the end of the light period, the heater and light were slowly turned off to simulate sunset. The water cooled

to 20 C by the start of the next cycle. At the end of each week the total number of eggs per color was determined, and the mop color with the highest egg deposition was utilized again the following week against either a new color or a repetition of the previous week's color.

Salinity and Water Depth

Techniques similar to those presented above were utilized with the following modifications. Two 226-liter (70-gal) tanks equipped with a biological filter (Spotte 1970) were used for this experiment. Concrete blocks stacked at one end, created different spawning depths. The eggs appeared to adhere to the spawning wool, but as a precaution, the arrangement of the blocks prevented eggs deposited on a higher level from drifting down to a lower level. Green spawning mops were placed at each of the three depths measured from the surface (2 to 6, 18 to 22, and 33 to 37 cm) (0.8 to 2.4, 7.1 to 8.7, and 13 to 14.6 inches). One tank was maintained at 5 0/00 and the other at 15 0/00 salinity throughout the study. These salinities were selected based on previous results of Kinne (1960).

Egg Production

The experimental design used for salinity and water depth was modified as follows: The water in both tanks was maintained at 5 0/00 salinity and the daily range in temperature was reduced to 15 to 24 C (59-75 F) to simulate the fall conditions present in the Salton Sea Canals. Mops were placed on the bottom of the tanks and checked every other day. A new group of 10 female and 2 male pupfish were used for this experiment.

RESULTS AND DISCUSSION

The order of increasing egg deposition/mop color was grey, beige, gold, green (Table 1). Preference for green spawning substrate is not unexpected since plants are customarily used by this species for egg deposition. Random choices would result in similar numbers of eggs being deposited on each color. Perhaps the particular wave lengths of light which cause green coloration (490–575 mμ) act as a visual stimulus to the pupfish. The variability in the number of eggs deposited during any particular week was probably related to the number of females actively spawning. Crear and Haydock (1971) reported large variations in not only the fecundity of individual female pupfish, but also the frequency of spawning.

TABLE 1. Spawning Mop Color Preference of Desert Pupfish, *Cyprinodon macularius*

Week	*Color choice*	*Total eggs deposited*
1	green/gold	56/24
2	green/grey	37118
3	greenlgrey	43/22
4	green/beige	117/75
5	green/gold	41/15
6	green/gold	161/71
7	green/grey	61/20

The second experiment demonstrated increased egg deposition at almost all depths in lower salinities (Table 2). The distribution of eggs at each depth differed significantly from the expected distribution of 33:33:33 for each treat-

ment group (5 0/00 and 15 0/00) (X^2 = 772.6, P = 0.01, d.f. = 2, and X^2 = 619.4, P = 0.01, d.f. = 2, respectively). Results indicate pupfish preferred the 33-37 cm level for egg deposition at 5 0/00 and the 18-22 cm level at 15 0/00.

TABLE 2. Effects of Depth and Salinity on Egg Deposition of the Desert Pupfish, *Cyprinodon macularius*

Week	Depth *	Salinity 5 0/00	Salinity 15 0/00
1	A	364	73
	B	221	56
	C	537	53
2	A	155	66
	B	316	478
	C	823	153
3	A	14	152
	B	707	425
	C	739	127
4	A	294	57
	B	179	143
	C	256	26
Subtotal	A	027	348
	B	1423	1102
	C	2355	359
Totals		4605	1809

* A = 2-6 cm
B = 18–22 cm
C = 33-37 cm

This difference in spawning rates at different salinities is unknown but may be related to energy requirements. Rao (1968) evaluated the energy expenditure for osmoregulation at different salinities. He demonstrated increased expenditure with increasing salinity. If a female pupfish had to shift more of her available energy stores to osmoreguiatory processes, less energy would be put into egg production. This could account for the overall reduced egg production at 15 0/00 compared to the production at 5 0/00.

The final study recorded egg production of replicate groups of pupfish held at low salinities (5 0/00) utilizing green spawning mops placed at 33 to 37 cm (13 to 14.6 inches). Egg production during the 31 -day test period yielded an average of 1554 eggs/week. Crear and Haydock (1971) reported that female pupfish spawned 50 to 200 eggs once per week. The present study shows a similar level of production. However, the female pupfish in the present study survived for a longer time period (4 to 6 months) than the 2 months reported by Crear and Haydock (1971). The methods reported here increase the survival of spawning female pupfish and provide an efficient method for egg propagation. The hatching system described permitted fry survival and growth. Approximately 70% of the eggs hatched and 50% of the resulting fry lived for a period of 3 months. Longevity of the fry could not be determined because testing was terminated. The fry were used for standardized bioassay testing.

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REFERENCES

Arnold, E. W. T. 1972. Behavioral ecology of two pupfish (Cyprinodontidae, Genus *Cyprinodon*) from Northern Mexico. Ph.D. Thesis, Arizona State University.

Barlow, G. W. 1958. High salinity mortality of desert pupfish, *Cyprinodon macularius*. Copeia, 1958(3): 231-232.

———. 1961. Social behavior of the desert pupfish, *Cyprinodon macularius*, in the field and in the aquarium. Amer. Midl. Nat., 65(2): 339-359.

Cox, T. J. 1966. A behavioral and ecological study of the desert pupfish (*Cyprinodon macularius*) in Quitobaquito Springs, Organ Pipe Cactus National Monument, Arizona. Ph.D. Thesis, Univ. Arizona, Tucson. 120 p.

Crear, D., and I. Haydock. 1971. Laboratory rearing of the desert pupfish (*Cyprinodon macularius*). U. S. Fish and Wildlife Service Fish Bull., 69(1): 151-156.

Kinne, 0. 1960. Growth, food intake, and food conversion in a euryplastic fish exposed to different temperatures and salinities. Physiol. Zool., 34(4): 288–317.

Miller, R. R. 1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. Misc. Publ. Mus. Zool. Univ. Mich., 68: 1-155.

———. 1950. Speciation in fishes of the genera *Cyprinodon* and *Empetrichthys*, inhabiting the Death Valley region. Evolution, 4: 155–163.

Minckley, W. L., and E. A. Arnold. 1969. "Pit digging": a behavioral feeding adaptation in pupfishes (Genus *Cyrpinodon*) Ariz. Acad. Sci., J. 5 (4): 254–257.

Rao, G. M. M. 1968. Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. Can. J. Zool, 46: 781-786.

Spotte, S. H. 1970. Fish and invertebrate culture. Wiley-Interscience, New York.