LABORATORY REARING OF THE DESERT PUPFISH, *Cyprinodon macularius*

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ABSTRACT

The desert pupfish, *Cyprinodon macularius*, may be reared in the laboratory for use in the study of embryology, genetics, physiology, and behavior. It is euryhaline (0-70 ‰) and eurythermal (8°-44.6° C) and may be useful as a bioassay for either freshwater or marine pollutants. In the Salton Sea area of California, the recent introduction of exotic species and the encroachment of civilization have drastically reduced the formerly abundant pupfish populations. Laboratory rearing eliminates the need for continuous exploitation of a rapidly contracting natural population and could supply adequate stocks for sanctuaries, thereby preserving the species from extinction. Laboratory apparatus and conditions are described for maintaining larval and adult pupfish. Parasites and diseases encountered are discussed and successful treatments described. Methods for spawning and rearing the desert pupfish in the laboratory are detailed. These methods may also be applicable to many other species of pupfish that are in danger of extinction.

The desert pupfish, *Cyprinodon macularius* Baird and Girard, is a killifish (Cyprinodontidae) native to the Lower Colorado River Basin from southern Arizona to southern California and the Sonoyta River of northern Sonora, Mexico (Miller, 1948). It thrives under the harsh conditions of the desert environment. It lives in fresh water as well as highly saline pools that few other vertebrates can tolerate. Its ability to survive in such environments, plus other important biological characteristics listed in Table 1, renders it an exceptionally hardy laboratory animal potentially valuable for research in many fields.

POTENTIAL FOR RESEARCH

Desert pupfish has many characteristics favorable for embryological research. It can be spawned with relative ease and can be maintained in the laboratory throughout the year to supply large eggs (approximately 2 mm in diameter), suitable for vital marking and grafting experiments. Other favorable characteristics are the transparent chorion and the long developmental period which can be temperature-controlled (New, 1966). The sticky filaments that are attached to the chorion of its demersal egg can be partially removed by rolling the eggs gently on filter paper 4 to 8 hr after fertilization. Any remaining filaments are matted together and can be easily removed with small forceps.

Desert pupfish, since they mature quickly, could be used for research in fish genetics and on the aging process. Barlow (1961) reported that pupfish reach maturity in the field in 3 months. F_1 pupfish, reared from eggs and maintained at 27° C, were observed spawning in the laboratory approximately 4 months after hatching.

The desert pupfish, a euryplastic species, also possesses physiological and behavioral traits that make it valuable for scientific research. The juveniles can tolerate salinities ranging from fresh water to 90 ‰ (Barlow, 1958). The adults, although less euryhaline, are known to spawn in salinities as high as 70 ‰ (Kinne and Kinne, 1962). The salinity tolerance of newly hatched pupfish render them potentially useful for comparative bioassay of freshwater and marine pollution. The extreme temperature tolerance is an additional asset. Desert pupfish

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TABLE 1.—Biological characteristics of the desert pupfish, *Cyprinodon macularius*, and other important fish in research.[1]

Items	Desert pupfish, *Cyprinodon macularius*	Trout: *Salmo trutta*, *S. gairdneri*	Killifish, *Fundulus heteroclitus*	Medaka, *Oryzias latipes*
Salinity tolerance:				
Adults	0-70 ‰	Euryhaline	Marine-estuarine	Fresh water
Eggs	0-90 ‰	Fresh water	Wide tolerance	— ? —
Natural spawning season	April to October[2]	Spring or fall	June to August	April to October
Artificial spawning	Spawns naturally all year, cannot be stripped	Seasonal spawning, eggs are stripped	Seasonal spawning, eggs are stripped	Spawns naturally all year, eggs can be stripped
Egg type	Demersal; transparent	Demersal; opaque	Demersal; transparent	Demersal; transparent
Egg diameter	Ca. 2 mm	Ca. 5 mm	Ca. 2 mm	Ca. 1 mm
Incubation temperature Range	13°-36° C; at 20° C, 10 days	3°-13° C; at 10° C, 34-37 days	15°-27° C; at 20° C, 9.5 days	13°-25° C; at 25° C, 8 days
Length of adult	4-5 cm	15-30 cm at first spawning	8-12 cm	2-4 cm
Age at maturity	3-4 months	2-4 years	1-2 years (?)	1-2 months

[1] Data compiled from: Barlow, 1958, 1961; Frost and Brown, 1967; Kinne and Kinne, 1962; Miller, 1970 (personal communication); New, 1966; Rugh, 1962; Trinkaus, 1967; Yamamoto, 1967.
[2] Desert pupfish has not been recorded spawning throughout the year in nature as have other species of pupfish but presumably would do so under the proper conditions (Bunnell, 1970).

have been found in the field at temperatures ranging from 8° to 44° C (Lowe and Heath, 1969; Kinne, 1960).

Since it can be relatively easily spawned in the laboratory, the desert pupfish is a good model for the study of reproductive behavior. Numerous and rapid behavioral sequences precede the actual spawning act. On occasion, however, fish in high spawning readiness eliminate many behavioral sequences and commence spawning immediately. When properly stimulated the fish swim parallel to one another, the male slightly behind the female, twist into an S-curve, and spawn. Release of the gametes is accompanied by a quivering movement of both fish. The male wraps his anal fin under the female's vent and fertilizes each egg as it is extruded. The female then dips, leaving the fertilized egg attached to the substrate. This process is repeated until the female is spent, having spawned 50 to 200 eggs in about 2 hr under laboratory conditions. In nature the female only rarely spawns more than one or two eggs in succession (Barlow, 1961). The mature male is easily recognized by his aggressiveness and his brilliant, blue coloration. The pugnacious male pupfish must be separated in the laboratory from females and other males. The determination of the male to spawn, regardless of circumstances, makes the pupfish a potentially valuable species for classroom demonstrations. Barlow (1961) has presented a complete and detailed description of the social and reproductive behavior of the desert pupfish.

LABORATORY REARING THE DESERT PUPFISH FOR CONSERVATION

Another value in rearing desert pupfish lies in the preservation of the species. Today the pupfish faces elimination from many areas of its natural range due to predation and competition from exotic species and the modification or destruction of its habitat. Large populations of desert pupfish, once prevalent around the Salton Sea, have been alarmingly reduced. At the present rate of population reduction, the species may well become extinct in this area in the near future unless steps are taken to insure its survival. Artificial rearing is one of the possible means.

Coleman (1929) conducted an ecological survey of the Salton Sea for the California Depart-

ment of Fish and Game and judged that the numbers of mosquitofish, *Gambusia affinis*, and desert pupfish were sufficient to support a large population of carnivorous game fish. Cowles (1934) reported the pupfish populations to be exceedingly large in and around the Salton Sea. In 1956 Barlow (1961) observed schools of juvenile pupfish of nearly 10,000 individuals. He estimated that one large, isolated, shore pool at the Salton Sea contained 150 adults per square meter. Today, in the Salton Sea area desert pupfish are almost totally confined to a few tributaries. In response to the severe reduction of pupfish populations in the Salton Sea area, Jack Hesemeyer, Supervisor of the Anza-Borrego State Park, has built a pupfish sanctuary near the Park headquarters at Borrego Springs. This small sanctuary was stocked on June 24, 1970, with 48 laboratory-reared fish produced by the techniques outlined below. Several hundred additional fish were placed in the Palm Canyon pools nearby.

The authors hope that this article, in addition to demonstrating the value of the desert pupfish as a teaching and research animal, will help in its preservation by describing laboratory-spawning methods that can provide adequate stocks for sanctuaries in natural and artificial habitats. In addition, the rearing techniques described here for desert pupfish may be useful for the preservation of many other species of pupfish that are in danger of extinction.

MATERIALS AND METHODS

The desert pupfish used to develop spawning techniques were seined from an irrigation ditch emptying on the northwestern shore of the Salton Sea in Riverside County, Calif. Specimens were transported in plastic garbage pails filled with aerated ditch water to the Fishery-Oceanography Center at La Jolla, Calif. We found that the water temperature during transport should not be allowed to fluctuate radically from that at which the fish are found.

Many of the specimens collected were infected by a freshwater parasitic copepod of the family Lernaeidae (possibly introduced with home-aquarium fish). The large egg cases of this copepod were clearly visible on the fish, usually at the base of the fins. Individuals carrying this parasite were weak and commonly died during or soon after transport. Reichenbach-Klinke and Elkan (1965) recommend a salt bath (NaCl, 0.76-1.1%) to eliminate such copepods. To treat this infection, all fish on arrival at the laboratory were converted to seawater over a 5-day period. Kinne (1960) reported that a 1-month-old pupfish can survive sudden salinity changes up to 35 ‰, whereas 1-year-old adults cannot survive sudden salinity changes of more than 10 to 15 ‰. Robert R. Miller (1970, personal communication), however, reports that in 1937 he found that this species could be shifted with ease directly from fresh water to seawater and back. After conversion to seawater all traces of the parasitic copepod vanished. Toward the end of the experiment, several fish died from a devastating protozoan infection in the epithelial tissue surrounding the mouth. The tissue appeared bloody and often had completely disintegrated. Many apparently healthy fish died with little or no warning in less than 12 hr. The marine parasitic protozoan, *Cryptocaryon irritans*, prevalent in the Scripps water system was suspected (Wilkie and Gordin, 1969). The surviving fish were transferred back to fresh water and, fortunately, the parasite failed to make the transition.

LABORATORY CONDITIONS

The fish were maintained in 20-gal tanks with subsurface filters covered with crushed oyster shell. The water was changed completely and the shell washed approximately every 6 weeks. Four individuals were isolated in each aquarium by plastic, perforated dividers. The tanks were maintained at room temperature, 20° to 22° C. Standard aquarium heaters were used whenever higher temperatures were needed. No attempt was made to control pH other than the use of the crushed oyster shell substrate.

The fish were fed frozen adult brine shrimp, *Artemia salina*, three times daily during the week and once a day on weekends. Kinne (1960) and Kinne and Kinne (1962) used two species of

worms (*Enchytraeus albidus*[3] and *Tubifex* sp.), two species of crustaceans (*Daphnia* and *Cyclops*), beef liver, fresh lettuce, spinach, and several brands of commercial fish food.

The fish were subjected to a daily 16-hr light and 8-hr dark cycle. The lighting used was a combination of daylight fluorescent bulbs and mercury vapor arc lamps. Most fish were ready to spawn under these conditions within 3 weeks of capture, although there was marked seasonal and individual variation. Observations made suggest the length of the light period is more important than light intensity for the induction of spawning. Kinne and Kinne (1962) reported using a 14L-10D cycle with a combination of fluorescent tubes and natural daylight.

SPAWNING METHODS

The fish were spawned on a varied schedule, depending on the readiness of the female. The average female spawned 50 to 200 eggs, depending upon her size, approximately once a week. One large, exceptionally prolific female spawned 200 eggs twice a week for 2 months. Subsequently, this female was not spawned on schedule, became eggbound, and was unable to extrude her eggs. After her death the large, single ovary contained over 800 eggs and accounted for 44 % of the total body weight. Females that would not spawn at room temperature were spawned at 27° C. Females that could not spawn at either room temperature or 27° C were removed from isolation to a community tank (4 females, 2 males) on a 12L-12D photoperiod, wherein they quickly spawned out their eggs at 27° C. Kinne (1960) reported that the fish do not spawn in the laboratory at temperatures below 20° C and seem to spawn optimally between 28° and 32° C. He also noted that the temperature in the field varies between 25° and 35° C for most of the spawning season.

Kinne and Kinne (1962) reported that pupfish embryos have one period of low thermal stability between fertilization and gastrulation and a second period just before hatching. Observations made during our study indicate another period of low thermal stability just before fertilization. When a female maintained at room temperature is transferred to a higher temperature to induce spawning, most of the eggs either are not fertilized or do not develop. We found that females should be maintained and spawned at one temperature. If maintained at a temperature above 25° C, they will need to be spawned regularly; otherwise, the eggs are dropped to the bottom of the aquarium unfertilized. A more flexible spawning schedule is possible if the female is kept at a lower temperature.

The fish were spawned in white, plastic, food containers measuring 27 × 20 × 10 cm, containing 2.5 liters of water at 22° C. Immediately after the spawning these containers were suspended in a water bath at 27° C. This technique produced good hatches in spite of the reported low thermal stability between fertilization and gastrulation (Kinne and Kinne, 1962). The water bath was a 20-gal tank with a standard aquarium heater.

Either a green plastic mat or white cheesecloth was used as a substrate on which the females could attach the adhesive eggs. On several occasions, when the plastic mat was used, the pupfish were observed eating the previously spawned eggs, which were readily visible against the green background. The substitution of cheesecloth with L-shaped glass-rod weights at its periphery successfully reduced parental egg consumption. The cheesecloth was a superior substrate because many of the eggs were buried in the material and thus were inaccessible to the parents. Furthermore, the combination of a white spawning bin, white cheesecloth, and nearly transparent eggs made the latter virtually invisible to the experimenters and, presumably, to the fish.

The parent fish were well fed prior to spawning to help reduce the number of eggs consumed. The spawning bin, containing the female, was placed in a quiet location. Five to fifteen min later the male was introduced to the spawning bin which was then left undisturbed for 1 to 2 hr. Barlow (1961) reported that spawning

[3] See Kinne (1960) for details on mass culturing *Enchytraeus albidus*.

lasts from 30 min to 2 hr, depending on the size of the female and the number of eggs laid. In order to prevent the serious injury or death of the female, the male was not left in the spawning bin for longer than 2 hr. After termination of spawning the fish were returned to their aquaria and the feces were removed from the container. The spawning bin was then suspended in a 27° C water bath with aeration. Kinne and Kinne's (1962) data on hatching shows that any incubation temperature between 24° and 30° C should produce hatches of at least 80 % in 100 % air-saturated seawater. Pupfish eggs left at room temperature in the laboratory at La Jolla suffered extremely high mortalities owing to daily temperature fluctuations between 18° and 24° C. Kinne and Kinne (1962) supplied data on egg mortalities and incubation periods at different temperatures from 10° to 37° C.

Hatching success of different breeding pairs varied unexplainably under constant conditions. A large sample of eggs from one breeding pair showed, however, that salinity and temperature markedly effected the hatching success of pupfish eggs (Table 2). Eggs in small clusters, apparently laid at nearly the same instant, seldom hatched. Kinne and Kinne (1962) also reported reduced development and increased mortalities for conglomerated eggs.

Pupfish larvae are large enough (5.5 mm total length at 27° C in 50 % seawater) to feed from the day of hatching on brine shrimp nauplii (Salt Lake variety), *Artemia salina*. The larvae when handled were drawn with a bulb into a long glass tube. Kinne and Kinne (1962) gave extensive data on the growth, food intake, and food conversion for pupfish larvae at different temperatures and salinities.

TABLE 2.—*Hatching success of desert pupfish eggs.*

Salinity	Temperature	Eggs			
		Developing		Dead	Total
	° C	*No.*	*%*	*No.*	*No.*
Fresh water	22	74	54.0	66	140
	27	4	5.4	70	74
	27	3	5.0	57	60
Half seawater	27	92	85.1	16	108
Seawater	27	17	29.6	40	57
	27	13	29.5	31	44
	27	7	16.6	35	42

CONCLUSIONS

The desert pupfish may be reared in the laboratory over a wide range of temperatures and salinities. Half-strength seawater at 27° C provided the best hatch observed during this study. The pupfish is a hardy laboratory animal that does well in captivity if proper attention is paid to food, space, and hygiene. However, a note of caution should be added, since we reared only two generations of pupfish. Bunnell (1970) states that when a pupfish stock is bred in captivity from a single pair, the fish do well at first, but gradually die out over several generations. This possibility should be further substantiated before committing experimental studies to a single line of descent.

The authors believe that the desert pupfish is an excellent experimental animal for many types of biological research. Studies of the systematics (Miller, 1948), behavior (Barlow, 1961), and physiology (e.g., Kinne and Kinne, 1962) provide a wealth of background information on the basic biology of pupfish which will prove valuable to investigators interested in using this species in teaching and research. Other reports (e.g., Bunnell, 1970) indicate the critical status of some species of *Cyprinodon*, including *C. macularius*, and point out the need to provide sanctuaries to avoid extinction of this unique species. Laboratory rearing of pupfish will not only provide material for scientific observation and experimentation, but will also remove some of the pressure on an already rapidly contracting natural population by providing adequate stocks for present and future sanctuaries. Both measures should enhance the value of the desert pupfish and emphasize the importance of saving the species from extinction.

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