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SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS*): AN ESTUARINE FISH SUITABLE FOR CHRONIC (ENTIRE LIFE-CYCLE) BIOASSAYS[1]

by

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ABSTRACT

The sheepshead minnow (*Cyprinodon variegatus*), an estuarine fish of the Atlantic and Gulf Coasts, is suitable for both partial chronic and chronic (egg-to-egg) bioassays. The fish is easily held at high population densities in the laboratory and, at about 30 C, produces numerous eggs. The average 30-day survival of the fish from fertile egg to fry is 75%. Generation time for this species is short (3-4 months) and its small adult size (male average standard length=48mm) provides for relatively inexpensive bioassays. This killifish's susceptibility to organochlorine toxicants is similar to that of other estuarine fishes tested and thus should produce significant information on the effects of these toxicants on the estuarine community.

INTRODUCTION

Acute, partial-chronic and chronic bioassays are necessary for setting water quality standards, according to Mount and Stephans' (1967) definition of maximum acceptable toxicant concentration and experimental definition of application factor. Partial-chronic bioassays have been accomplished on several fresh-water species such as the bluegill (Eaton, 1970) and brook trout (McKim and Benoit, 1971) in which effects of toxicants were observed on each life stage. In chronic bioassays, the test organisms are exposed to a toxicant during their entire life cycle to measure effects on survival, growth and reproduction. In this manner, the most susceptible life stage can be ascertained and the survival potential of future generations of the organism estimated. Fresh-water chronic bioassays have been completed by several investigators such as Brungs (1971), using the fathead minnow (*Pimephales promelas*). To our knowledge, no marine or estuarine fish has been used in chronic or partial-chronic bioassays.

There are several criteria to be considered when choosing a fish for a chronic bioassay:

1. The fish should be able to reproduce readily in close confinement, producing large numbers of eggs.
2. Fertility as well as survival to adulthood should be high.

[1]Contribution No. 205, Gulf Breeze Environmental Research Laboratory.

3. The organism should mature rapidly, yet be small enough at adult size to maintain large, statistically-valid numbers of fish in the bioassay.
4. The fish should be relatively sensitive to toxic pollutants.

LIFE HISTORY

The sheepshead minnow is an omnivorous killifish (Family Cyprinodontidae) that occurs in estuaries from Massachusetts to northern South America (Moore, 1968). It is important in estuarine food chains as food for commercially valuable fishes (Darnell, 1958). The adults are sexually dichromatic after attaining 27mm in standard length and, according to Hildebrand (1917), adult males average 48mm standard length and females 45mm.

Hildebrand stated that the spawning period for this species was from April to October in the Beaufort, North Carolina area. Kilby (1955) collected young fish of 15mm or less during all months except January, February and March at Cedar Key, Florida, indicating a spawning period in the warmer months.

In our laboratory studies, no spawning occured below 26 C. Eggs are approximately 1 mm in diameter, demersal and adhesive by means of minute threads. Under laboratory conditions, fry hatch in approximately five days at 30 C (Figure 1). Newly-hatched fry are 4mm in length and are able to feed on brine shrimp (*Artemia salina*) nauplii within 48 hours of hatching. Generation-time in the field has been estimated at 4 months by Holland and Coppage (1970); however, we have cultured fertile eggs to mature adults within three months in the laboratory.

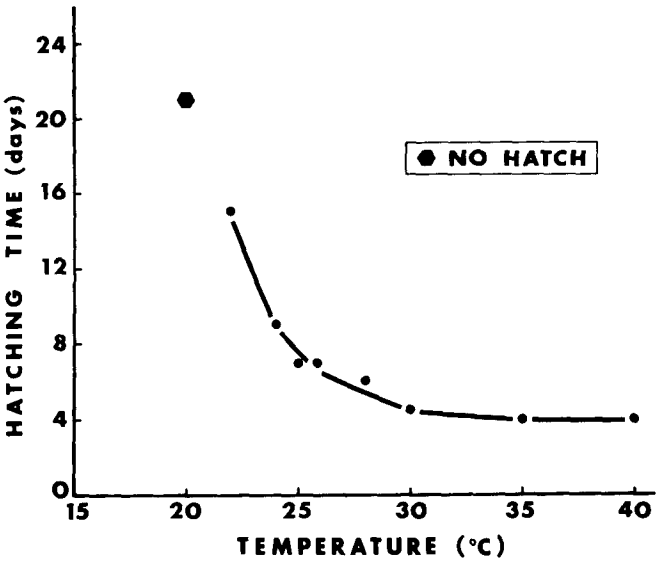

Figure 1. Relation between hatching time and temperature with *Cyprinodon variegatus* embryos and fry. Data taken from Schimmel *et al.*, 1974 (In press).

ENVIRONMENTAL FACTORS

Field observations indicate that the sheepshead minnow can tolerate a wide range of environmental stress. Populations of the fish have been observed at water temperatures from 11 to 35 C and salinities from 0 to 120 o/oo (Copeland, 1967). Population densities of the adult fish in shallow marsh ditches have exceeded 20 individuals per m^2 despite aggressive territorial activities of the males during spawning.

Our laboratory studies corroborate field observations on tolerances to temperature, salinity and population density. Embryos and fry can be efficiently cultured in water from 24 C to 35 C and 15 to 30 o/oo (Figs. 2 and 3). Our studies also indicate that holding fish at high population densities is not difficult. For example, twenty-five adult fish have been held in 50-liter aquaria under acceptable flow-through conditions (A.P.H.A., Standard Methods, 1971) without appreciable loss due to aggressiveness of the fish or other factors.

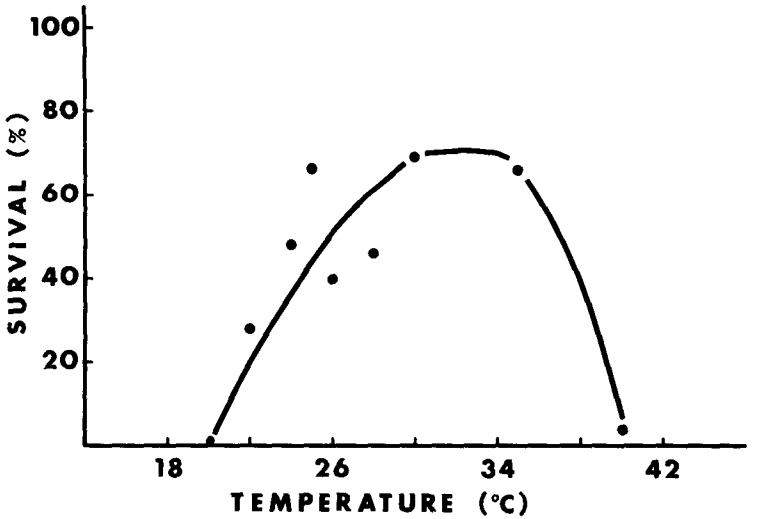

Figure 2. Relationship between temperature and survival with *Cyprinodon variegatus* embryos and fry. Data taken from Schimmel *et al.*, 1974 (In press).

FERTILITY, GROWTH AND SURVIVAL

To determine the quantity of fertile eggs that would be produced by a pair of sheepshead minnows during a 28-day period (at 30 C), two fish were placed in a small spawning chamber (10 X 12 X 18cm) constructed of acrylic plastic. The spawning chamber was small enough to be placed in an aquarium, but large enough to permit the female to avoid the aggressiveness of the male. Two sides of the spawning chamber were made of 2mm-square mesh nylon screen that allowed water to exchange between the chamber and the larger bioassay aquarium. The bottom of the chamber was made of 4mm-square mesh nylon screen through which eggs could pass, thus reducing chance for predation of eggs by adult fish. Eggs falling below the adult chamber landed on a 0.25mm-square mesh screen drawer which was removed daily for counting of eggs and

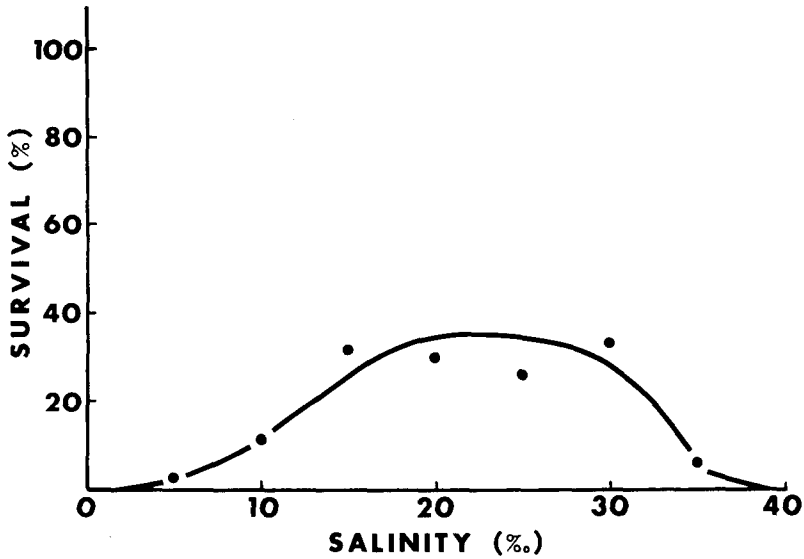

Figure 3. Relationships between salinity and survival with *Cyrpinodon variegatus* embryos and fry. Data taken from Schimmel *et al.*, 1974 (In press).

confirmation of fertility. The reproduction of 34 pairs of fish was monitored for 28 days. Pairs of fish were selected so that males and females ranged in size from 23 to 52mm standard length.

The pairs survived well and spawned readily, most producing enough fertile eggs for a chronic bioassay. All of 34 females produced eggs and 66% of the females survived the full 28 day spawning period in the chamber. The number of eggs produced per female ranged from 2 to 1,028 and averaged 186. Eighty-eight percent of the 6,339 eggs produced were fertile. The number of eggs produced each day varied (Figure 4), and increased with time. Fish produced an average of 22 eggs during the first week, 57 eggs and 83 eggs in the next two weeks. These data indicate that there is a 1-2 week acclimation period prior to optimum production. Once the fish began spawning, however, most spawned daily.

Total egg production was not related to the size of the fish but frequency of spawning and egg fertility appeared size-dependent. Females began producing eggs at about 27mm standard length. Nineteen fish less than 35mm long produced an average of 8.2 eggs per day and 15 fish 35mm and larger produced 7.8 eggs per day. The smaller fish produced eggs more consistently (50% of the days versus 31%) with greater fertility than the larger fish (94% fertile versus 79%).

Data analyses of control and no-effect experimental groups in *C. variegatus* bioassays reveal that most deaths occur among embryos and newly-hatched fry, with negligible deaths among juveniles and adults. Survival of embryos and fry averaged 75% over the first four weeks (Fig. 5). Most of this mortality occurred during embryonic development. Survival of 250 juvenile fish held under laboratory conditions for four weeks was 97%. Ninety-three percent of 250 adult fish, including territorial males, survived the four-week bioassays.

A 5-month chronic bioassay using *C. variegatus* exposed to endrin has recently been completed and the data are now being analyzed.

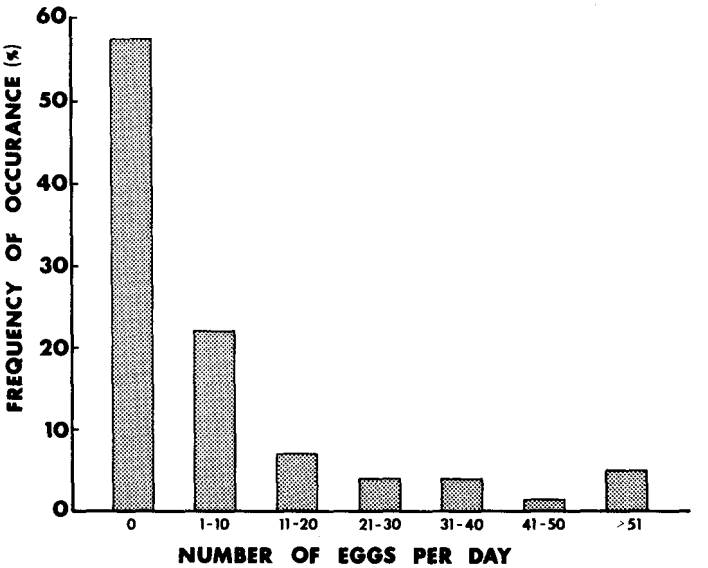

Figure 4. Daily egg production by 34 female *Cyprinodon variegatus* during a 28-day period.

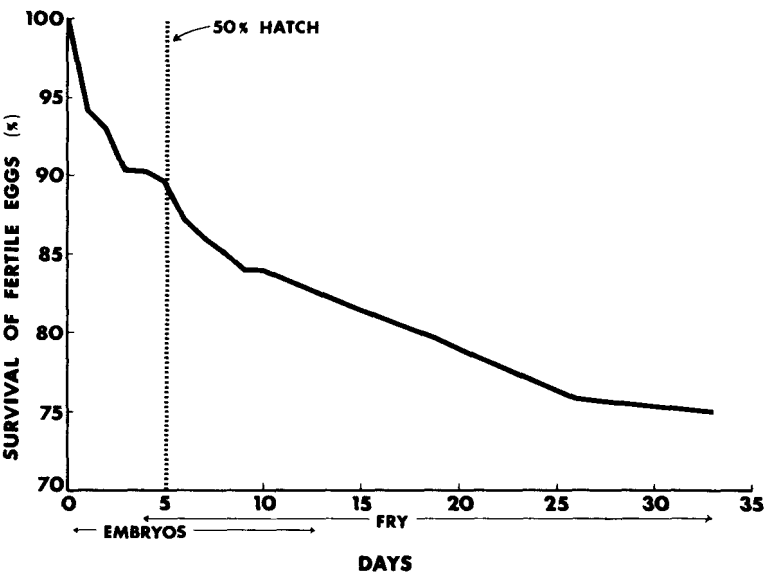

Figure 5. Survival of embryos and fry of *Cyprinodon variegatus* at 30 C (range 28 to 32 C).

A fish for chronic bioassay should be at least as susceptible to toxicant poisoning as other fishes in a similar ecosystem. Studies performed at the Gulf Breeze Laboratory (Lowe, unpublished data[2]) showed that susceptibility of *C. variegatus* to endrin, dieldrin and DDT was comparable to that of three other estuarine fishes - *Fundulus similis, Leiostomus xanthurus* and *Mugil cephalus* (Table 1). Schimmel *et al.* (1974) have found that embryos and fry of the sheepshead minnow are more susceptible to the polychlorinated biphenyl, Aroclor®1254 (effect at 0.32 ug/l, P 0.01) than other juvenile or adult species in any taxonomic group studied at this laboratory (Nimmo *et al.* 1974). Since the above-mentioned chemicals, being organochlorines and probably similar in their activity, do not aptly reflect how all toxicant types will affect the fish, our data do indicate that the animal is not prohibitively resistant to some major insecticides and related compounds.

Table 1. Comparative 48-hour EC-50's (in ug/l) of four organochlorines for *Cyprinodon variegatus* and three other estuarine fishes. Jack I. Lowe, unpublished data, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida.

Chemical	*Cyprinodon variegatus*	*Mugil cephalus*	*Leiostomus xanthurus*	*Fundulus similis*
Endrin	0.32	2.00	0.32	0.23
Dieldrin	24.	0.66	–	5.5
DDT	3.2	0.50	1.8	5.5

CONCLUSION

In our view, *Cyprinodon variegatus* would fill the need for an estuarine fish suitable for chronic bioassays, producing significant information on the effects of toxic compounds on estuarine fishes. This small fish is ubiquitous on the Atlantic and Gulf Coasts of the United States, survives well in the laboratory, is fecund and has a short generation time. Susceptibility of this fish to some commonly used pesticides is comparable to that of some other fish species in the estuarine system.

ACKNOWLEDGEMENTS

We wish to thank Stephen Foss for preparing the Figures (1-5).

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STATUS OF FAUNAL RECOVERY IN THE NORTH FORK HOLSTON RIVER, TENNESSEE AND VIRGINIA

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ABSTRACT

Two years after pollution abatement, faunal communities in the North Fork Holston River below Saltville, Virginia, remain in a perturbed condition. The number of fish species is increasing at the lower downstream stations, but show little change immediately downstream of Saltville. Two species which occur regularly above Saltville were not taken at any downstream station during this study.

The abundance and diversity of aquatic insects at downstream stations are extremely variable, but are generally less below Saltville than they are upstream. Populations of mussels have not been successful in recolonizing downstream areas.

Water quality parameters fall within acceptable ranges for healthy aquatic communities, but an analysis of river sediments documents the existence of high mercury concentrations.

The relatively slow rate of natural recovery processes can probably be attributed to the continuing input of toxic materials from the abandoned waste lagoons.

INTRODUCTION

For decades increasing numbers of streams throughout the United States and other countries have been subject to municipal, agricultural, and industrial waste discharges. The degree of damage suffered varies according to a complex of interrelated factors which include the characteristics of the receiving stream and the nature, magnitude, and frequency of the stress or stresses applied. Often the degradation has been so severe and of such long duration that the streams are no longer considered valuable in terms of their biological resources.

With the advent of the current environmental movement, significant numbers of streams are experiencing pollution abatement, and, consequently, have the potential