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Project Summary

Guidelines for the Culture of Fathead Minnows, *Pimephales promelas* for Use in Toxicity Tests

Jeffrey S. Denny

Fathead minnows, *Pimephales promelas* Rafinesque, have been cultured at the Environmental Research Laboratory - Duluth (ERL-D) for use in aquatic toxicity tests since the establishment of the laboratory in 1967. The techniques and apparatus described in this report were developed over the years by many researchers. This paper sets forth the conditions and procedures now being used to produce research quality fathead minnow embryos, larvae, juveniles, and adults. These guidelines can be modified to adapt to existing circumstances and needs.

Information on the physical system includes water supply, construction materials, water temperature, photoperiod, and the water delivery system. The biological section addresses the selection of spawning fish, incubation of embryos, larval and adult feeding, disease, and gene pool considerations. This document is meant to be a guide for those interested in culturing fathead minnows for use in fish toxicology research.

A fathead minnow culture facility can provide a continuous supply of embryos or fish of known age, raised under known conditions, for aquatic toxicity testing. The use of laboratory reared animals is advantageous since age and genetic background are known, diet is controlled, fish are free from disease, and are available year-round.

The life stages of fish in greatest demand for testing are less than 24 hour old embryos, 0-24 hour old larvae, and 30 day old juveniles. The ERL-D system is designed to produce these three life stages, plus adults for future brood stock. Ninety-six pairs of adult spawners provide 1,000-2,000 embryos/day. These can be used immediately for testing, or incubated to provide larvae or juveniles for testing at later dates.

This Project Summary was developed by EPA's Environmental Research Laboratory, Duluth, MN, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Physical System

Water Supply

Lake Superior water is supplied to the ERL-D culture system at constant flow volumes of about 150 ml/min per tank. If available, springs, wells, or controlled surface waters are recommended. Dechlorinated tap water from a municipal supply should be used only as a last resort (Benoit, 1982). The supply should be examined for contamination by pesticides, heavy metals, sulfides, disease vectors, or any other suspected contaminants.

The quantity of water necessary depends on the size of the intended culture unit. The ERL-D system of over 150 tanks consumes 15-20 liters/minute when in full operation. Smaller systems, or systems in areas of limited water supply could operate on a reduced flow. Though less desirable, static

renewal or recirculating systems may be effective.

Tanks

Tanks are 57 liter (15 gallon) glass aquaria, with standpipe drains adjusted to provide approximately 40 liters of water. Tanks are supported on racks of slotted angle iron and 3/4 inch plywood, both of which are painted with epoxy paint to resist moisture. Stainless steel screen can be used to divide tanks into sections for spawning pairs of fish. White plastic dishpans, commonly available in department stores, are used to hold spawning tiles on which the embryos incubate.

Water Delivery System

The ERL-D water delivery system is a constant temperature (25°C), flow through system. It is gravity fed, with stainless steel mixing boxes positioned in an open ceiling approximately 3.5 m overhead. A standard toilet float valve maintains water level in the headbox, while a solenoid valve adds heated water to maintain temperature at 25°C. The solenoid valve responds to a temperature probe in the headbox via a solid state electronic controller. Air stones or some other type of agitator must be used in the headbox to assure complete mixing and to prevent supersaturation.

Water flows to the tanks through a 1/2 inch PVC pipe manifold. Above each tank is a tee with a 1/2 to 3/8 reducer, with a 3 ml disposable syringe barrel glued into it. This allows the use of different sizes of hypodermic needles to control flow rates.

Construction Materials

Construction materials which come into contact with the water must not contain leachable substances. Rubber, copper, brass, or plastics containing fillers, additives, stabilizers, plasticizers, etc., must not be used (Mount, 1971). Glass, stainless steel, Teflon, and PVC are the preferred construction materials. All piping should be of rigid PVC. *Threadable* PVC must be used, to avoid the danger of toxicity from PVC glue. Silicone glue is safe to use as long as enough curing time is allowed. Ground fault interruptors are necessary on all electrical components due to the close proximity of electricity and water in these systems.

Photoperiod

Photoperiod should be constant at 16 hours light/8 hours dark. The ERL-D system uses lights that simulate the wavelength spectra of sunlight. However, fathead minnows will spawn under "cool white" lighting. Temperature adjustments may be necessary to offset the warming effects of fluorescent lighting.

Aeration

Provide continuous gentle aeration to the tanks to maintain dissolved oxygen concentrations above 5.0 mg/l at all times, but avoid vigorous aeration with newly hatched larvae. If a level of 5.0 mg/l cannot be maintained, remove some fish from the tank (Mount, 1971). Check the location of air intakes and efficient operation of laboratory air compressors to avoid introducing contaminants. An oil trap or filter may be necessary on some systems.

Spawning Substrates

Four inch diameter PVC pipe, cut into 4 inch pieces, and halved lengthwise, provide a semicircular arch under which fathead minnows will readily spawn. The inside can be roughened with a wire wheel to improve egg adhesion. Another type of substrate can be made by halving small clay flower pots.

Biological System

Obtaining Brood Stock

Fish that are free of disease and adapted to laboratory conditions make the best initial brood stock. For the least risk of disease, and greater ease of shipment, begin with embryos. Use of embryos also avoids any bioaccumulation of toxicants that may occur with adults. Less desirable is the use of fish caught in the wild, or purchased from a bait dealer. Take care that the animals are *Pimephales promelas*, and not a related species. Examine all fish, especially wild caught or bait dealer fish, for signs of disease.

Selection of Spawning Fish

Stock juvenile fish 3-4 months old at a density of 35-40 fish per 15 gallon tank, and provide with approximately 4 spawning substrates. The presence of substrates hastens the maturation process. In 1-2 weeks some males in the tank will show signs of maturing. Females become gravid soon after the males exhibit spawning color. For observation, net fish from the tank and place individually into a 400 ml beaker with appx 3 cm of water. Sexual maturity can then be determined as follows: Breeding males develop a conspicuous gray pad of spongy tubercles on the dorsal surface anterior to the dorsal fin, and two rows of tubercles across the snout. The sides of the body become almost black except for two wide vertical bars which are light colored. Another characteristic of the breeding male is the presence of a dark spot at the anterior insertion of the dorsal fin. Females remain quite drab (Eddy and Underhill, 1974). The female fathead minnow exhibits an ovipositor at least a month before spawning (Flickinger, 1969). Backlighting makes the female ovipositor easier to see.

Removal of the mature fish for spawners will stimulate maturation of subordinate fish to replace the dominant fish that were removed from the hierarchy. This method will provide a continuous source of mature fish.

Spawning

The fathead minnow is an intermittent, multiple spawning species with an extended breeding season, possibly spawning intermittently all summer (Hasler, 1946; Radcliff, 1931). Under controlled culture conditions fathead minnows will spawn throughout the year.

The buoyant, adhesive embryos stick to one another and to the undersurface of the nesting object. After deposition is complete, the male remains at the nest site tending and defending the embryos until hatching occurs (Andrews and Flickinger, 1973). More than one female may spawn in a male's nest, and up to 12,000 embryos have been found in one nest (Markus, 1934), indicating that several females contribute to the embryo mass. Unpublished data indicate an average of 258 eggs/spawn and an average of 3,095 total eggs/female during 100 days of spawning activity (Olson, 1974).

Separation into spawning pairs reduces competition between males, and allows the fecundity of individual pairs to be monitored. Sterile, or "spawned out" fish can then be replaced to maintain egg production. Other investigators have reported success with male to female ratios of 2/4 (Olson, 1974), 3/6 (Benoit and Carlson, 1977), and 4/10-15 (Mount, 1971). The males are territorial, so at least as many spawning substrates must be provided as there are males in the tank to achieve optimum egg production.

Embryo Incubation

Spawning tiles containing embryos are removed from the tanks daily, and new empty tiles provided. Embryos are incubated by placing tiles on edge in a dishpan of culture water, with an airstone placed between two tiles. Nonviable embryos must be removed daily with a tweezers, to prevent spread of fungus. At 22-23°C embryos will begin to hatch in 5 days. Tiles can be disinfected between uses by soaking in chlorine and then neutralizing with sodium thiosulfate.

Larval Handling

Larvae can be handled using a large bore, 50 ml volumetric pipette. For rearing in 15 gallon aquaria, a stocking density of 250 larvae per tank is recommended. All fathead minnow larvae less than 30 days old are fed live brine shrimp twice each day. It is important that the brine shrimp nauplii be small enough for the fathead larvae to ingest (Norberg and Mount, 1985). Gape size of larval fathead minnows is in the range of .24-.28 mm, so the width of the nauplii offered must be slightly less than this for the nauplii to be ingested.

Conditions for hatching brine shrimp are usually provided by the supplier. It is best to harvest nauplii as soon after hatch as possible for smallest size and best nutritional value. A rule of thumb is to provide no more or less shrimp than the fish can consume in 15-20 minutes. The quantity will vary widely depending on size and number of fish in the tank.

Adult Feeding

All fish over 30 days old are fed frozen brine shrimp twice per day. Shrimp should be allowed to thaw slightly (not fully) for easy handling. The same rule applies to quantity, i.e., no more or no less than the fish will eat in a short period of time.

Disease

Discard any diseased lots of fish and disinfect the tanks with hypochlorite. Two hundred ppm active chlorine for 1 hour is sufficient to disinfect tanks and to use as a bath for nets, sponges, brushes, etc. Numerous references are available concerning diagnosis and treatment of fish diseases, but treated fish are rarely, if ever, used in bioassays.

Gene Pool

The Environmental Research Laboratory - Duluth mixes existing brood stock with healthy wild stock every two years to eliminate the risk of developing a homogenous strain. Homogenous genetic stock may provide smaller variance in test results, but it correspondingly reduces the strength of possible inferences to ambient conditions. Proper genetic management depends on the goals of the overall research program.

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The EPA author, ***J. S. Denny*** *is with the Environmental Research Laboratory, Duluth, MN 55804.*
R. L. Spehar *is the EPA Project Officer (see below).*
The complete report, entitled "Guidelines for the Culture of Fathead Minnows, Pimephales promelas *for Use in Toxicity Tests," (Order No. PB 87-165 247/AS; Cost: $14.95, subject to change) will be available only from:*
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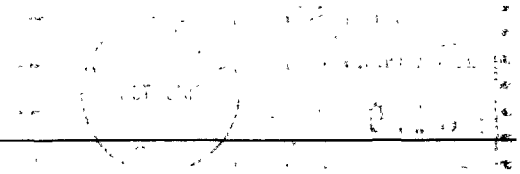

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