1.0 OBJECTIVE

To describe the procedures used in maintaining a reproducing colony of fathead minnows (Pimephales promelas).

2.0 HEALTH AND SAFETY

All personnel must be enrolled in the Clemson University Medical Surveillance Program. Personnel must wear lab coats, or waterproof apron, and additional PPE as appropriate to the task.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

All personnel must have completed the required Animal Care and Use Committee on-line training.

3.1 PERSONNEL

3.1.1 Only those individuals trained and having the documentation of such training will be allowed to assume the responsibility of the fish colony's maintenance.

3.2 TRAINING

3.2.1 All personnel should read and understand the USEPA methods paper (EPA/505/8-89/002b) as well as view the supplemental video prior to taking on the responsibility of colony maintenance.

3.2.2 All personnel should complete a tour and a procedure training seminar presented by the "colony manager".
3.3 RESPONSIBILITY

3.3.1 The primary responsibility of maintaining the colony belongs to the "colony manager", and then falls to someone named by the manager, who has been deemed adequately trained, in case of the manager's absence.

4.0 REQUIRED AND RECOMMENDED MATERIALS

Aquaria or troughs
Biological filter (optional)
Air pump with stone
4" Spawning tiles (PVC)
Separatory funnels
6" Fish net
Cleaning utensils
Brine shrimp cysts
Frozen brine shrimp
Tetramin® fish food
Assorted pipettes
Nalgene® Autoclave tub
Form No. 172 - Aquatic Toxicology Data Sheet

5.0 PROCEDURE

5.1 CULTURE WATER PREPARATION

5.1.1 Natural water, drinking water, or reconstituted water may be used in the culturing of fathead minnows if it is deemed to be toxicant free.

5.2 NATURAL WATERS

5.2.1 Surface waters such as those from a river, lake, well or spring may be used if passed through a carbon and/or sand filter followed by a fine filter (~5µm). If resident fish populations exist in the source then some measures must be taken to remove potential fish pathogens. An ultraviolet sterilizer or ultrafiltration are acceptable methods.

5.3 DRINKING WATERS

5.3.1 Tap water may be used if dechlorinated first. Dechlorination can be accomplished by three common methods, aeration, passage through a carbon filter, or the use of sodium thiosulfate. Some care must be taken if using sodium thiosulfate as it exhibits some toxicity to fathead minnows.
5.4 RECONSTITUTED WATERS

5.4.1 Deionized water from an adequate ultrafiltration system may be combined with reagent grade chemicals in a recipe manner according to those guidelines set forth by EPA and ASTM.

5.4.2 Commercial mineral water may be mixed with ultrafiltered water (1:4) as a source of culture water.

5.4.3 Water stored in carboys should be covered and out of direct light. Shelf life is approximately 14 days.

5.2 WATER DELIVERY

5.2.1 If using a flow-through system turnover should be at least 1 time per day.

5.2.2 Static tanks should have either an under-the-gravel filter or an external filter. Renew the water by replacing 25%, every 2 or 3 days, with distilled water.

5.3 FEEDING

5.3.1 Feeding the Larvae

5.3.1.1 From hatching to the age of 30 days the larvae should be fed live, less than 24-h old, brine shrimp, Artemia spp.

5.3.1.2 Fish should be fed twice daily, Monday-Friday, and once a day on weekends.

5.3.1.3 Observe consumption so as to estimate an adequate amount of brine shrimp.

5.3.1.4 Siphon out any excess food each day.

5.3.2 Feeding Juveniles and Adults

5.3.2.1 Fish older than 30 days and less than 90 days should be fed partially frozen, adult brine shrimp.

5.3.2.2 Fish should be fed twice daily, Monday-Friday, and once a day on weekends.

5.3.2.3 Feed an amount that will be consumed in 10-30 minutes.

5.3.2.4 Naturally occurring periphyton in the tanks serve as a good dietary supplement.
5.3.2.5 Commercial flake food, such as Tetramin® may be used to feed fish older than 90 days.

5.4 CULTURING

5.4.1 Initiating Cultures

5.4.1.1 Embryos may be obtained from a reliable research laboratory.

5.4.1.2 Once acclimated to 25°C the embryos will hatch in 4-5 days and may be treated as described earlier (Section 5.3.1).

5.4.2 Development

5.4.2.1 Fish 30 days old may be used for acute toxicity testing or grown out as brood stock.

5.4.2.2 Decreased stocking densities and the addition of spawning tiles will speed up maturation.

5.4.2.3 Signs of maturity begin to appear at 3-4 months of age.

5.4.2.3.1 Males will develop a wide head with tubercles along with black coloration on their sides.

5.4.2.3.2 Females will be smaller with an olivaceous color, and will exhibit an ovispositor when mature enough to spawn.

5.4.3 Spawning

5.4.3.1 There are three options to separating adults for breeding.

5.4.3.1.1 Spawning pairs may be set up with one spawning tile.

5.4.3.1.2 Place a group of mature fish in a 20 gallon aquaria with up to 4 spawning tiles, resulting in a female:male ratio of 8:3.

5.4.3.1.3 Place a group of mature fish into a trough system with ratio of spawning tiles and males:females based on the surface area of the bottom and compared to the acceptable ratios in section 5.4.3.1.2.

5.4.3.2 Females may release 100-400 eggs on the underside of the spawning tile whereupon they are fertilized by the male.
5.4.3.3 Fathead minnows spawn approximately every 4-5 days in the laboratory.

5.4.3.3.1 If no embryos are produced in a 3 week period, replace the pair or the entire group, unless the fish are young adults.

5.4.3.3.2 In trough system, replace the mature adults at approximately one to one and a half years of age, dependant upon egg production.

5.4.3.4 Collecting Embryos

5.4.3.4.1 Tanks should be checked mid-morning each day for embryos.

5.4.3.4.2 Remove spawning tiles with tongs.

5.4.3.4.3 If embryos are to be used in toxicity testing then they may be removed from the tile with a gentle, circular, rubbing motion while keeping the tile submerged.

5.4.4 Hatching

5.4.4.1 There are two methods plausible for the hatching of fathead minnow embryos.

5.4.4.1.1 Roll the embryos off the tiles into a culture dish containing culture water and pipet them into a separatory funnel filled with aerating culture water. After 2 days empty the separatory funnel into a pan (white preferably), at a temperature of 25°C. The embryos will hatch in 2-3 days.

5.4.4.1.2 Place the entire spawning tile into a pan filled with aerating culture water, at 25°C, and the embryos will hatch in 4-5 days. Arrange the tiles so that the bubbles from the air stone flow over the eggs so as to keep algal and fungal colonies from forming. These can entrap the larvae as they hatch. Check the tiles on days 1 and 2 for fungus or lack of viability and remove any such with forceps. Minimize disturbances on days 3-5, as it may cause early hatching. If 50% of the embryos from any one tile have to be removed because of lack of viability, then discard all remaining eggs.

5.4.4.2 Disinfect all used tiles by soaking in a chlorine bath (1ml/L) for 1 hour, followed by rinsing with tap water. Neutralize any residual chlorine with sodium thiosulfate (100 mg/L) for 10 minutes, and finally rinse with culture water and allow to air dry.
6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

The Quality Assurance Unit will periodically review these procedures.

7.0 LITERATURE CITED
