Research Article

Evaluation of preventative fungal treatment on fathead minnow (*Pimephales promelas*) larval survival and growth

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Abstract. The degradation of freshwater ecosystems in the last century has led to a significant loss in fish species. This, in turn, has led to a pressing need to better understand this degradation in aquatic life. The earliest stages in aquatic life are the most sensitive to these environmental changes. The use of anti-fungal treatments for increased survival in larval offspring. of fathead minnows (*Pimephales promelas*) was evaluated. The treatments used were formalin, hydrogen peroxide and tetracycline. Formalin and hydrogen peroxide were found to be lethal at concentrations of 167 mg/L and 100 mg/L respectively. Altering procedures suggested these treatments might be effective as dip treatments as opposed to continuous treatments, although no significant results were observed. Tetracycline, in particular, was found to be somewhat effective although again no significant conclusions were noted. However tetracycline may be effective in preventing fungal deaths between day 14 and day 21 in fathead minnow larvae.

Introduction

The degradation of freshwater ecosystems in the last century has led to a loss in fish species. This, in turn, has led to a pressing need to better understand the effects of this degradation in aquatic life. The earliest stages in aquatic life are the most sensitive to these environmental changes (Hutchinson and Williams, 1993). Fathead minnows are used frequently in aquatic studies because they are readily available and the procedures for maintaining and cultivating them have been well-established (Benoit and Carlson, 1977; Weber, 1993). They are also used because they are a good index to the lower levels of the food web in nature. By studying the effects of toxins on fathead minnows it is possible to predict the effects they could have on other components of the ecosystem such as larger fish and water fowl who use fathead minnows as a major food source (Denny, 1987). The larval stage is most sensitive to both environmental and laboratory influences.

There are many important factors in raising fathead minnow larvae including temperature, water chemistry, laboratory handling, and disease. Disease has the potential to destroy large populations of fish and larvae both in the wild and in captivity. Fish can acquire different types of infections such as bacterial, protozoan, and

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fungal. One of the most common infections found is fungal infections (Denny, 1987). Fungal infections are easily acquired and transferred in the wild and in captivity. In the wild they are acquired through spores which can be found in food sources, habitat, and contact with other fish and aquatic organisms (Duffy, 1997). In a controlled setting fungus can arise from an overabundance of food, algae, or cross-contamination between tanks (Denny, 1987). Fungal infections affect not only adult fish but larvae as well. These infections can prevent proper development, leading to head and spine abnormalities or death (Arthur et. al., 1994). These abnormalities can cause alterations in the food chain because, as small minnows, fatheads are prey for larger aquatic organisms, birds, and terrestrial predators. Thus, the death of fathead minnows in the wild can put one of the bases of the food chain in jeopardy.

As fungal infections continue to affect the survival of larval fathead minnows it is important to understand the consequences they may have on the fishery industry and on future aquatic research. Fungal infections in fisheries can wipe out entire populations of fish including large game fish such as salmon and other small fish such as the fathead minnow (Fitzpatrick et al., 1995). A high death rate among fish cultures is harmful from both the ecological and economical stand point.

There are two important problems in aquatic research concerning fungal infections. One is that death due to infections can prevent the study of subsequent generations of offspring or halt the study altogether. The second problem in aquatic research is inadvertently associating mortality of offspring and reproductive success with pollutant exposure and other variables when it may be more fungally related. This was the case in a study performed by Hutchinson and Williams (1993) where larval death was originally attributed to the type of food used when it was in fact due to infection. By neglecting death due to fungal infection, death of larvae could be wrongly associated with a wide number of toxins and other variables. These problems can affect accurate analysis of survival rates among larvae.

Anti-fungal agents have been widely used in treating outbreaks of infection of large game fish in hatcheries. Some effective treatments have been discovered such as Malachite green, formalin, gluteraldehyde, saline, hydrogen peroxide, tetracycline, erythromycin, potassium permangenate, and iodine (Meyer et al., 1983; Chapman et al., 1992; Fitzpatrick et al., 1995; Schreier et al., 1995). Of these treatments formalin, saline, hydrogen peroxide, and tetracycline have become the most widely accepted anti-fungal agents in fish hatcheries nationwide (Piper et al., 1982). Some of the other treatments such as Malachite green and iodine have been found to have mutagenic effects in both the fish and predators (Meyer et al., 1983). Others such as gluteraldehyde and erythromycin did not show statistically better anti-fungal effects than the accepted anti-fungal treatments and have been deemed less cost-effective (Fitzpatrick et al., 1995).

The purpose of this study was to test the effectiveness of formalin, hydrogen peroxide and tetracycline as fungal prevention treatments in relation to the survival and growth of larval offspring of the fathead minnow.

Materials and Methods

Tank Apparatus

Three twenty-gallon aquaria with charcoal filters were divided into three compartments to house the fathead minnows. The dividers were made from mesh fabric, plastic edging and tygon tubing to allow maximum water exchange and aeration between compartments. Each tank division contained gravel-ground covering and a wooden spawning substrate. The wooden spawning substrate skeletons were constructed from plexiglas and dowels to which two by three-inch rectangular pieces of wood were attached using rubber bands, providing a place for the eggs to adhere.

Breeding pairs

Thirty fathead minnows between the ages of six and eight months were obtained from Chesapeake Cultures Haynes, Virginia. The fish were split into breeding groups consisting of two females and one male. Each breeding group was placed in one of the compartments of the large aquaria.

Environment

The tank apparatus was kept in a controlled growth chamber. The temperature was maintained between 22-24°C and on a 16 hour light : 8 hour dark photoperiod. These conditions were maintained to mimic summer breeding months. Water chemistry of these tanks was also checked twice weekly to monitor changes in dissolved oxygen, conductivity and alkalinity using a YSI multimeter. pH was also monitored using a Texas Instrument pH meter.

Feeding

The adult fish were fed twice daily, once in the morning and once at night. They were fed frozen brine shrimp, daphnia and Zeigler Brother's brand protein enhanced trout starter. The weight and length of each fish were measured biweekly.

Eggs

The spawning substrates were checked twice daily (morning and afternoon) for eggs. When eggs were discovered they were carefully rolled off the wooden squares and into a Syracuse dish. Eggs were counted and divided into four equal clutches. Each clutch was removed to its own floater and placed into one of four five-gallon aquaria to receive one of four fungal prevention treatments. The day that the eggs were laid and placed in treatment was considered Day 0.

Treatments

Tank 1: Clutches were treated with pulsed doses of a 167mg/L formalin solution every Monday (Fisher Scientific).

Tank 2: Clutches were treated with pulsed doses of 250mg/5 gal of tetracycline (Lambriar's Animal Care Catalog) every Monday.

Tank 3: Clutches were treated with pulsed doses of 100mg/L Wegman's Brand hydrogen peroxide solution every Monday.

Tank 4: Clutches were left untreated as a control group. Eggs were counted on day 2 to determine the number of live, dead and infected eggs. Egg diameter measurements were measured for five eggs (a representative sample of the entire clutch) using an ocular micrometer.

Larvae

The eggs hatched into larvae on or near day 4 at which point they were counted and measured in the same manner as described for the eggs and transferred into a larval incubation chamber. The larval incubation chambers were constructed using clear plastic containers, 200-micron mesh, wooden dowels, rubber bands and silicon. The larval incubation chambers were placed back into the treatment tanks. During the 24 days of the study, larvae were counted and measured as described on days 7, 14, 21, and 28.

Results and Discussion

The first conclusion of this study is that the initial concentrations of hydrogen peroxide and formalin, 100mg/L and 167 mg/L respectively, were found to be lethal to fathead minnow eggs. Formalin was found to harden and fix the egg yolks while hydrogen peroxide was found to dissolve the egg casing, leaving only the yolk. Both of these defects led to the observed mortalities.

The procedures were modified by reducing the concentrations of hydrogen peroxide and formalin by 50%, to 50 mg/L and 83 mg/L respectively. These concentrations of hydrogen peroxide and formalin, however, were also lethal.

The study procedure was again modified to a 10 min dip in 50 mg/L hydrogen peroxide or 83 mg/L formalin on days when survival and growth were measured (Figure 1). Nothing conclusive can be determined from these results. This is because the procedure was changed during the study, and only two clutches were treated. The survival rates observed (40% - 60%) are similar to those observed in other studies (Benoit et. al., 1995). Because these treatments are not uniformly lethal, further work can be performed.

Results were collected on the effectiveness of tetracycline (250mg) throughout the study (Figure 2). The results were not significant, however it was noted that survival at day 21 was higher than the untreated group (Figure 2). The period

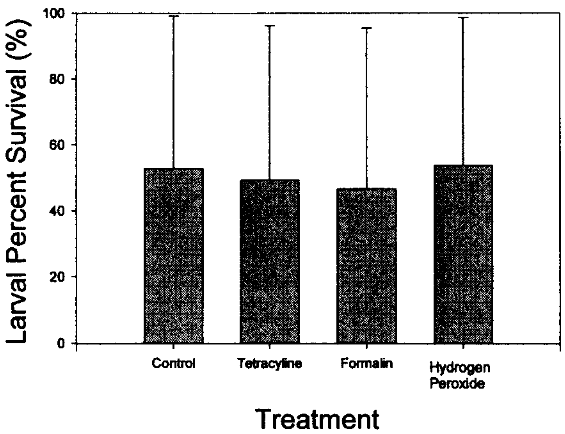

Fig. 1. Overall effectiveness of dip treatments. The effectiveness of formalin, hydrogen peroxide, and tetracycline as preventative fungal treatments in fathead minnows (*Pimephales promelas*) was evaluated. The treatments show larval survival rates similar to that of the EPA (Benoit et al., 1977).

between days 14 and 21 is crucial for fathead minnow development (Hutchinson and Williams, 1993). Future work could be done testing the effectiveness of antibiotics during this stage. The results of tetracycline, however, varied between clutches (data not shown). In some clutches abnormalities such as lordiosis, a spinal defect, were observed.

This study found that continuous use hydrogen peroxide and formalin as fungal treatments cause 100% mortality to fathead minnow larvae. Although these two treatments have been used effectively on larger fish (Fitzpatrick et. al, 1995), they are clearly not suitable for larval fathead minnows at concentrations of 100mg/L and 167 mg/L, respectively, or at half these strengths. The adverse effect of these treatments may be due in part to the difference in larval or adult size between the species. Tetracycline, however, may show promise for reducing infections in larval fish, especially between days 14 and 21. Future work using antifungal treatments between day 14 and 21 of larval growth could reveal appropriate procedures for the dip treatment.

The larval stage is the most sensitive in fathead minnow; survival rates are highly variable, but are typically not extremely high. From day 0 through 7 survival rates are high (between 90% and 100%), but at day 7 survival drops off continually until day 28 (Benoit et al., 1995). The survival rates observed in this study (56%) were found to be lower than the range of 67%-93% overall survival achieved by the EPA (Benoit et al., 1995). The lower rates of survival observed in this study can be partially attributed to other laboratory variables such as stocking density, feeding and handling, which are all known causes of lethality.

However, our progress made in the increase of larval fathead minnow survival allows for effective toxicity testing and the observance of the second generation of individuals tested. This increase in survival also decreases the number of deaths wrongly attributed to toxicity testing

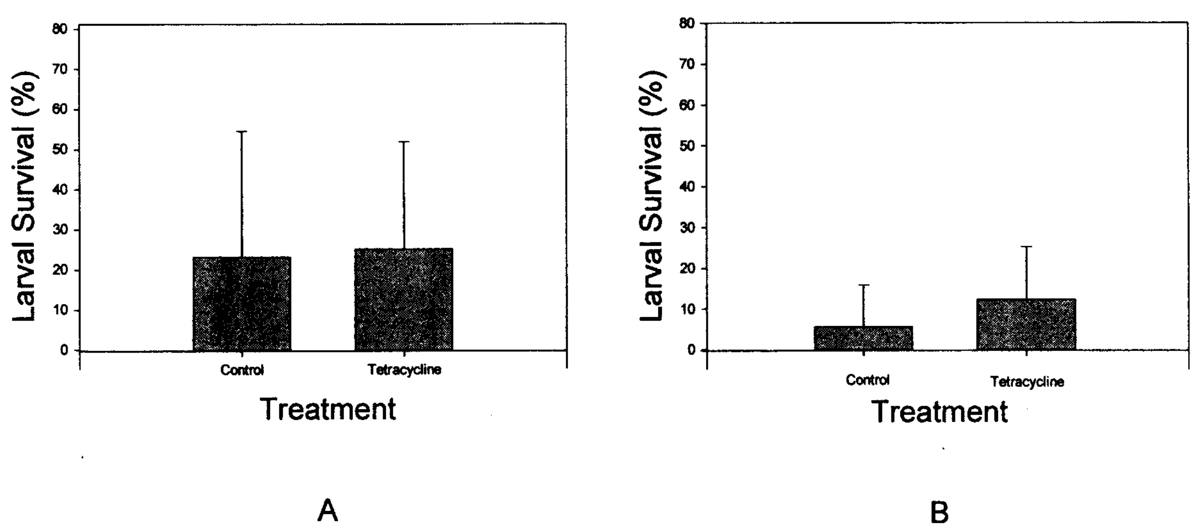

Fig. 2. A) Overall effectiveness of tetracycline at day 14. B) Overall effectiveness of tetracycline at day 21. The use of tetracycline between days 14 and 21 may increase fathead minnow larval survival during its sensitive early life stages.

which may be due to fungal infections (Hutchinson and Williams, 1993). Furthermore, developmental abnormalities associated with the antibiotics used in this study could also serve as an index for environmental emissions from pharmaceutical companies and their effects upon aquatic ecosystems.

This research has refined our understanding of preventative antifungal treatments and acceptable concentrations for use on laboratory-reared fathead minnows. The results from this study have important implications for use in increasing the accuracy of toxicity testing using fathead minnow larvae and for the effects of pharmaceuticals in the environment.

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