CHAPTER 7

Use of Hydrogen Peroxide as an Algaecide for *Prymnesium parvum*

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Abstract

Hydrogen peroxide was investigated as a potential algaecide for the toxic alga *Prymnesium parvum.* The goal was to determine if hydrogen peroxide could be used to eliminate *P. parvum* in fish transportation water to prevent the incidental spread during fish stocking operations. Hydrogen peroxide concentrations of 62.5-12,500 mg/L were tested for their ability to lyse *P. parvum* cells or inhibit cell motility at 15 min, 1 h, or 24 h post-exposure. Only the highest concentration (12,500 mg/L) caused lysis of *P. parvum* cells within 15 min while concentrations ≥ 3,125 mg/L lysed all the algal cells after 1 h. At 24 h, complete lysis was observed for all concentrations. Since most cultured fish species cannot tolerate hydrogen peroxide concentrations > 500 mg/L for prolonged periods and the concentrations needed to lyse all of the algal cells within 1 h exceeded the U.S. Food and Drug Administration low regulatory treatment rates (250-500 mg/L), this chemical is not recommended as an algaecide in fish hauling water.

Introduction

Prymnesium parvum, a halophilic chrysomonad, is a small (up to 15 µm long) biflagellate phytoplankton responsible for extensive fish mortalities in brackish waters in northern Europe (Kaartvedt et al. 1991; Lindolm et al. 1999), Israel and China (Guo et al. 1996), and North America (Holdway et al. 1978a). The alga is a free-living phytoflagellate having two long flagella, a shorter haptonema, a C-shaped or saddle-shaped chloroplast, and scaly surface. *P. parvum* produces several toxins (Shilo 1981) which affect many gilled aquatic species, including fish, bivalves, and brachiated tadpoles.

In mid-April through early May 2001, the entire crop of striped bass (*Morone saxatilis*) and palmetto bass (female *M. saxatilis* × male *M. chryopsis*) was lost at the Texas Parks and Wildlife Department's (TPWD) Dundee State Fish Hatchery (DSFH). High densities of *P. parvum* were identified in Lake Diversion, the source of water for the hatchery, during active fish kills in January and February 2001. Subsequently, biologists determined the alga was responsible for the widespread mortality of striped bass and hybrid striped bass fry at DSFH. The Possum Kingdom Reservoir was similarly affected during the same period, although the Possum Kingdom State Fish Hatchery (PKSFH) was not in production at the time and no hatchery fish were lost. As of 2004, the alga continued to be present at least sporadically at both Diversion and Possum Kingdom reservoirs.

In 2002, after extensive study into chemical control methods for this alga, striped bass, palmetto bass, channel catfish, and other fish species were successfully produced at both affected hatcheries and transported to water bodies throughout Texas. Although *P. parvum*-free well water is used in hauling tanks at PKSFH to transport fish, it is virtually impossible to avoid incidental contamination of the transport water with *P. parvum*. Therefore, there is concern regarding the possible spread of this pathogen from affected hatcheries to uninfected Texas waters and, as a matter of internal policy, TPWD Inland Fisheries does not want to disperse the organism via fish deliveries.

The U. S. Food and Drug Administration (FDA) considers hydrogen peroxide a low regulatory priority drug when used as a fungicide at rates up to 500 mg/L (based on active ingredient) on all species and life stages of fish. Some species of fish are more sensitive to hydrogen peroxide than others. Rach et al. (1997) reported that several fish species such as fathead minnow *Pimephales promelas,* brown trout *Salmo trutta,* bluegill *Lepomis machrochirus*, and channel catfish *Ictalurus punctatus* tolerate hydrogen peroxide concentrations up to 1000 mg/L for 45 min. However, walleye *Stizostedion vitreum* were intolerant to exposures as low as 100 mg/L for 15 min. Rainbow trout *Oncorhynchus mykiss* fry tolerate hydrogen peroxide concentrations up to 1000 mg/L, whereas fingerlings and adults were more sensitive at concentrations ≤ 500 mg/L for the same exposure time. Rach et al. (1997) also reported that the toxicity of hydrogen peroxide increased as temperature increased. These results indicate that considerations must be made for species, life stage, and water temperature when using hydrogen peroxide in aquaculture.

The use of peroxides as algaecides is somewhat controversial. Although there are many claims regarding the efficacy of peroxides as algaecides, there are few to no formal, scientific studies that prove or disprove these claims. The mechanism by which peroxides work is corrosive oxidation which damages unicellular structures. This process is easily observed as gas formation and bubble production when hydrogen peroxide is applied to a wound infected with bacteria. Arguments against the use of hydrogen peroxide as an algaecide include that oxidation may also kill beneficial alga and bacteria, have detrimental effects on fish, and the compound is unapproved for such purposes. Despite the controversy, the lack of an effective method to remove *P. parvum* from fish hauling tanks warrants investigation into whether it can be a useful algaecide for aquaculturists.

This study was conducted to determine the effectiveness of hydrogen peroxide as an algaecide for *P. parvum*. The specific objectives were to investigate if selected concentrations of hydrogen peroxide would kill or lyse *P. parvum* cells in 15-60 min and to determine the effects that 15-min to 24-h exposures of concentrations of hydrogen peroxide considered tolerable by fish may have on *P. parvum*.

Materials and Methods

An axenic culture of *P. parvum* was obtained from the University of Texas – UTEX Culture Collection of Algae in Austin, Texas. The concentration of the hydrogen peroxide solution was determined by a standard test using potassium permanganate

(Jeffery et al. 1989). Aliquots of this solution were diluted with sterile, glass-filtered deionized water to 10-mL volumes to produce the target concentrations of hydrogen peroxide. The test containers were 15-mL disposable tubes.

Three concentrations (781.25, 3,125, and 12,500 μg/mL) of hydrogen peroxide were used to determine the acute (immediate) effects of high concentrations to *P. parvum* (Table 1). A 1-mL aliquot of the algal culture was added to each of these concentrations, as well as a control consisting of sterile, glass-filtered deionized water. The density of the *P. parvum* cells was determined to be 8,000 cells/mL using a hemacytometer. The experiment consisted of two replicates per treatment (hydrogen peroxide or control) and was repeated three times for each of two dates at room temperature (25°C). The cells were observed at 15 min and 60 min post-treatment for cell integrity (i.e., lysed or intact) and movement (i.e., motile, slow motility, and flagella motion) using an Olympus CH-2 brightfield microscope.

The second part of the experiment used hydrogen peroxide concentrations reported to tolerated by fish (Rach et. al 1997). The experimental design and conditions were similar to the one above except that the treatment concentrations were 0 (control), 62.5, 125, 250, and 500 μg/mL and the *P. parvum* cells were observed at 15 min, 1 h, and 24 h post-treatment for cell integrity and movement as described above.

Results and Discussion

Among the hydrogen peroxide concentrations tested, only 12,500 μg/mL lysed all *P. parvum* cells within 15 min while 250-3,125 μg/mL inhibited cell motility (Tables 1 and 2). For 1-h exposures, hydrogen peroxide concentrations ≥ 3,125 μg/mL lysed all cells whereas no lysis was observed for concentrations ≤ 781.25 μg/mL. Motility was inhibited at 1-h exposure and concentrations ≥ 500 μg/mL (Tables 1 and 2). Complete lysis of cells was observed in all 24-h hydrogen peroxide treatments while no lytic effect or changes in motility were observed in the control (Table 2).

These results suggest that hydrogen peroxide exposures from 62.5 to 500 μg/mL for 24 h would lyse *P. parvum*, but this exposure time is greater than typical fish transport times in Texas. Treatment regimes demonstrated safe for fish vary by species. Largemouth bass *Micropterus salmoides* tolerate hydrogen peroxide at <150 μg/mL for 60 min; walleye, bluegill and channel catfish tolerate <100 μg/mL for 60 min; and fathead minnow fry tolerate <50 μg/mL for 60 min (Gaikowski 1999). Lumsden et al. (1998) demonstrated hydrogen peroxide effective against bacterial gill disease in rainbow trout when used at concentrations between 100-250 μg/mL for 1 h, although significant gill damage was observed at treatment rates greater than 175 μg/mL. Although Rach et al. (1997) showed that some fish species could tolerate hydrogen peroxide at concentrations up to 1000 μg/mL for 15-45 min for four consecutive days, the present study indicates that hydrogen peroxide is ineffective as an algaecide against *P. parvum* at concentrations ≤ 3,125 μg/mL for 15-min treatments and at concentrations ≤ 781.25

μg/mL for 60-min treatments. Since hydrogen peroxide exposures tolerated by fish are much less than those required to lyse *P. parvum* cells, the chemical is not recommended for this purpose.

TABLE 1.—Motility and integrity of *Prymnesium parvum* cells at 15-min and 1-h exposures to various concentrations of hydrogen peroxide.

Exposure time	12,500 µg/mL	3,125 µg/mL	781.25 µg/mL	0 µg/L
15 min	lysis	no lysis no motility	no lysis no motility	motility motility
1 h	lysis	lysis	no lysis no motility	motility motility

TABLE 2.—Motility and integrity of *Prymnesium parvum* cells at 15-min, 1-h, and 24-h exposures to various concentrations of hydrogen peroxide (µg/L).

		H_2O_2 (µg/L)				
Date	Exposure	500	250	125	62.5	0
13 Aug 2002	15 min	no lysis no motility	no lysis FM	no lysis slow motility	no lysis motility	motility
	1 h	no lysis no motility	no lysis FM	no lysis FM	no lysis slow motility	motility
	24 h	lysis	lysis	lysis	lysis	motility
14 Aug 2002	15 min	no lysis no motility	no lysis FM	no lysis slow motility	no lysis motility	motility
	1 h	no lysis no motility	no lysis FM	no lysis FM	no lysis slow motility	motility
	24 h	lysis	lysis	lysis	lysis	motility

FM = flagella motion, no motility observed