CHAPTER 6

Effects of pH on *Prymnesium parvum* Cell Viability and Toxicity

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Abstract

Toxic water obtained from E. V. Spence Reservoir during an ongoing *Prymnesium parvum*-related fish kill was used to evaluate the effect of lower pH levels on *P. parvum* cell integrity and toxicity. Hydrochloric and sulfuric acids were used separately to lower pH in toxic water to see if the *P. parvum* cells and ichthyotoxin would be destroyed or deactivated. The treatments ranged from pH 5.5 to pH 7.0 in 0.5-SU increments. Untreated water (control) had a pH of 8.3. The acidic pH levels were effective in reducing the density of viable *P. parvum* cells, and the percent reduction in density increased as the pH decreased. Reductions in density were 23.6% and 87.6 % for pH 6 and 5.5, respectively, 3 hours after treatment and 41.6% and 94%, respectively, 28 hours after treatment with hydrochloric acid. Sulfuric acid treatments reduced cell density by 38.3% and 61.7% for pH 6.5 and 6, respectively 1 hour after treatment and 35.8% and 82.3% 18 hours after treatment. A bioassay demonstrated that at pH 6 and 6.5, toxicity was reduced but not completely eliminated.

Introduction

The toxin-producing haptophyte *Prymnesium parvum* has been known to occur in Texas since at least 1985 when it was implicated in a fish kill in the Pecos River (James and de la Cruz 1989). In 2001, *P. parvum* was responsible for the loss of the entire crop of fingerling striped bass *Morone saxatilis* and palmetto bass (female striped bass ×male *M. chrysops*) at the Dundee State Fish Hatchery (DSFH). Additionally, the alga has been resident in Possum Kingdom Reservoir, the water supply for the Possum Kingdom State Fish Hatchery (PKSFH). The alga is reported to become endemic once established in hatchery ponds (Shilo 1967), thus successful fish culture at affected hatcheries may be dependent upon successful management of the organism or its toxins. One way to deactivate toxins may be to manipulate pH to acidic or alkaline levels, depending on the type of toxin. The activity of the *P. parvum* ichthyotoxin has been demonstrated to increase between pH 6 and 8 (Ulitzer and Shilo 1970b), and from pH 7 to 9 where it complexes better with cationic cofactors (Shilo and Aschner 1953). However, McLaughlin (1958) reported that the toxic properties of *P. parvum* culture fluids were lost at pH 6.0-6.5. The pH requirement for growth of *P. parvum* in media has been variably reported as pH 8.2-8.4 (Padan et al. 1967), pH 8.0 (Ulitzer and Shilo 1970b), and pH 9.0 (Padilla and Martin 1973). It appears slightly alkaline pH is suitable for *P. parvum* growth and ichthyotoxin activity, whereas acidic pH levels may be detrimental for toxicity.

Hatchery ponds typically experience diurnal fluctuations in pH with the lowest values occurring by just before sunrise and highest values by mid- to late afternoon (e.g. 6 p.m.; Boyd 1990). The recommended pH values for aquaculture are 6.7-8.6 (US EPA 1980). Boyd (1990) stated that optimum growth of fish occurs at pH 6-9 whereas slow growth occurs at pH 5-6, reproduction fails at pH 4-5, and acid death occurs at pH 4. At the DSFH pond pH levels fluctuated from a low of 7.2 to a high of 10.1 during the 2001 9-inch channel catfish *Ictalurus punctatus* production period in ponds treated with ammonium sulfate to control *P. parvum.* The best time to manipulate the pH to kill *P. parvum* likely would be during the mid-morning when pH values are lowest. This experiment was undertaken to evaluate the effects of pH manipulation, using hydrochloric acid or sulfuric acid, on *P. parvum* viability and toxicity.

Materials and Methods

Toxic water for the study was collected from E. V. Spence Reservoir at the Wildcat Creek Marina during an active *P. parvum*-fish kill in October 2001. Brightfield microscopy and a hemacytometer were used to determine *P. parvum* density prior to pH manipulation. Aliquots (50 mL each) of toxic water were transferred into 100-mL beakers, and either hydrochloric or sulfuric acid was added to each to achieve the final pH values of 7.0, 6.5, 6.0, and 5.5. The control (pH 8.2) received no acid. Six replicate cell counts were performed for each study beaker at 3 h and 28 h after the addition of hydrochloric acid and at 1 h and 18 h after adding sulfuric acid. Density was expressed as cells/mL. Using the mean cell density of the control 3 h post-treatment as reference point, the percent decline in cell density was calculated for each hydrochloric acid-adjusted pH treatment. Similarly, the mean cell density of the control 1 h post-treatment was used as reference point to calculate cell density for each sulfuric acid-adjusted pH treatment. The standard bioassay for toxicity testing (Appendix B) also was used 1 h after pH adjustments to determine the effect of pH on toxicity.

Results and Discussion

Acidic pH was effective in reducing the density of viable *P. parvum,* and percent reduction in viable cells increased as the pH decreased. Percent reductions in density for pH 6 and 5.5 were 23.6% and 87.6 %, respectively, 3 h after treatment and 41.6% and 94%, respectively, 28 h after treatment with the hydrochloric acid (Table 1). A similar pattern was observed for the sulfuric acid treatments. One hour after treatment the cell density was reduced by 38.3% and 61.7% for pH 6.5 and 6, respectively, and 35.8% and 82.3%, respectively, after 18 h of treatment (Table 2). These results suggest that decreasing pH to 6 or less would destroy *P. parvum* and cause considerable reduction in cell density in 1-28 h.

The pH among the treated beakers returned to basic conditions when they were tested at 18 h and 28 h post-treatment for the sulfuric acid and hydrochloric acid treatments, respectively (Tables 1 and 2). The temporary nature of the acid treatments may enhance the feasibility of using pH to control *P. parvum* in fish culture systems. Since fish can tolerate pH 5-6 but suffer slow growth at these conditions (Boyd 1990), the temporal nature of the acidic treatments would allow *P. parvum* to be treated without significant loss of fish growth.

Also, infested culture systems could be treated with pH 6 or less to reduce *P. parvum* densities and then pH allowed to rise to neutral or basic conditions before fish are stocked or effluents are discharged. The minimum pH for aquaculture effluents is pH 6, thus some minimum post-treatment holding time following acid treatments may be required before effluents are discharged.

The bioassays revealed that pH 6-6.5 reduced the toxicity but did not eliminate it completely. Fish mortality in the undiluted water without cofactor was 33.3% for the untreated control (pH 8.3) and 0% for pH 6.5 or 6 (Table 3). However, when cofactor was added, fish died (33-100% mortality) at all pH levels. The pattern of mortalities suggest that toxicity was reduced by the lower pH, which support the findings of McLaughlin (1958) who reported that the toxicities of *P. parvum* culture fluids were lost at pH 6.0-6.5.

This was a pilot study intended to provide a cursory view of the potential effects of acidic conditions on *P. parvum* and its toxicity. The study design was limited with no replicates within treatments. Although the results of this study appear promising, it is unknown if the toxin returns with the increasing pH. Also, it was calculated that approximately 875 L of concentrated hydrochloric acid would be required to effectively treat a typical 1-acre pond, which is a major impediment to the efficacy of such treatments. If further studies are undertaken, they should determine at what rate pH returns to basic conditions and evaluate longer-term effects on cell density and toxicity, as well as how pH deactivates the toxin. Further studies also should use water from affected hatcheries, which may have different buffering capacities and require different volumes of acid for efficacious treatment.

TABLE 1.—Mean *Prymnesium parvum* densities (percent reduction in parenthesis) in toxic water from E. V. Spence Reservoir, Texas following additions of concentrated hydrochloric acid to reduce pH.

		Mean cell density (cells/mL)*	
Treatment pH	Final pH	3 h post-treatment	28 h post-treatment
8.2	8.6	22,250 (0)	26,000 (-16.8)
7.0	8.3	22,000 (1.1)	22,000 (1.1)
6.5	8.2	20,750 (6.8)	18,000 (19.1)
6.0	8.1	17,000 (23.6)	13,000 (41.6)
5.5	8.1	2,750 (87.6)	1,333 (94)

* $N = 6$ replicates
** Untreated control

TABLE 2.—Mean *Prymnesium parvum* densities (percent reduction in parenthesis) in toxic water from E. V. Spence Reservoir, Texas following addition of sulfuric acid to reduce pH.

		Mean cell density (cells/mL)*	
Treatment pH	Final pH	1 h post-treatment	18 h post-treatment
8.3 (control)	8.3	24,300 (0.0)	19,300 (20.5)
7.0	8.1	17,300 (28.8)	17,000 (30.0)
6.5	8.0	15,000 (38.3)	15,600 (35.8)
6.0	7.6	9,300 (61.7)	4,300 (82.3)

* $N = 6$ replicates

TABLE 3.—Bioassay results for toxic water (due to *Prymnesium parvum*) from E. V. Spence Reservoir, Texas 1 h following pH adjustment using sulfuric acid.

	Dead fish/total fish		
pH	Undiluted water	Undiluted water + cofactor	1:5 dilution + cofactor
8.3 (control)	1/3	3/3	3/3
6.5	0/3	3/3	3/3
6.0	0/3	3/3	1/3