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Laboratory tests of ammonium and barley straw extract as agents to suppress abundance of the harmful alga *Prymnesium parvum* and its toxicity to fish

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ABSTRACT

Prymnesium parvum is a harmful alga whose blooms can cause fish kills in brackish waters. Two potential suppressants of this alga were tested, ammonium and barley straw extract (BSE), at temperatures of 10, 20 and 30 °C. Laboratory batch cultures were grown for 3 weeks at each temperature, with weekly doses of ammonium or BSE at either low or high levels, or a no-dose control treatment. The growth rate of *P. parvum* during exponential phase was highest at 20 °C and lowest at 10 °C, and was stimulated by the highest ammonium dose. Only cultures grown at 20 °C were toxic to fish. The highest ammonium dose abolished such toxicity and reduced the endpoint population density of *P. parvum*. BSE did not reduce the exponential growth rate, endpoint density, or toxicity to fish of *P. parvum*. The results support the use of ammonium additions, but not BSE, to suppress harmful blooms of *P. parvum* in those circumstances where the possible disadvantages can be managed.

1. Introduction

In the past two decades, blooms of harmful algae have received increasing attention from scientists and water resource managers (Smayda, 1990; Hallegraeff, 1993). *Prymnesium parvum* is one of many harmful algal species. It was first described from a coastal pond (Carter, 1937), and has been recorded in widespread locations from coastal and inland waters of varying salinity (Moestrup, 1994; Edvardsen and Paasche, 1998). Tolerance of low salinity has enabled its spread to many inland waters. In the mid-twentieth century *P. parvum* was found, often in abundance, in fish ponds in Israel (Reich and Aschner, 1947) and the Ukraine (Krasnoshchek and Abramovich, 1971). Later in the 1980s, blooms were first noted in the brackish inland waters of the southwestern US, at several locations in the Pecos River (James and De La

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Cruz, 1989). Since then, *P. parvum* has been found in an increasing number of rivers and reservoirs in Texas and other states of the American southwest (Texas Parks and Wildlife Department, 2003).

Blooms of *P. parvum* have long been associated with fish kills (Otterstrøm and Steeman Nielsen, 1940). Fish kills attributed to *P. parvum* have occurred in small, enclosed bodies of water such as fish ponds (Reich and Aschner, 1947; Krasnoshchek and Abramovich, 1971), and in larger bodies of water such as reservoirs and stretches of rivers (James and De La Cruz, 1989; Rhodes and Hubbs, 1992). Historically, the toxins responsible for fish kills associated with *P. parvum* have been difficult to identify (Shilo, 1967, 1981). These toxins also appear harmful to aquatic invertebrates (Koski, et al., 1999; Barreiro et al., 2005) and microorganisms (Fistarol et al., 2003). To date, the only characterized toxins are two closely related, large molecular-weight cyclic polyethers (Igarashi et al., 1996; Murata and Yasumoto, 2000), but the presence of other toxins remains possible.

In small, enclosed bodies of water, it is possible to treat *P. parvum* blooms with chemical agents to reduce abundance and toxicity. Ammonium treatments successfully controlled harmful blooms in fish ponds in Israel (Reich and Aschner, 1947). The efficacy of ammonium treatment increased with temperature and pH, suggesting deprotonation of ammonium (NH_4^+) to free ammonia (NH_3), which is toxic to *P. parvum* (Shilo and Shilo, 1953, 1962). More recently, abundant *P. parvum* populations in samples from a fish hatchery were reduced with ammonium treatments to below detection with hemacytometer counts ($<10^5$ cells/mL) within 48 h (Barkoh et al., 2003). The lowest concentrations of ammonium causing such eradication were found at high temperature (25 °C) and pH (>9.0). Ammonium sufficient to produce 10 μmol/L of free ammonia was recommended for such treatment. The concentrations of ammonium and free ammonia sufficient to control *P. parvum* populations under these conditions are known to not be acutely toxic to many freshwater fish, including the fathead minnow (USEPA, 1991).

Plant litter materials such as barley straw have been reported to suppress algal blooms in small water bodies (Welch et al., 1990; Newman and Barrett, 1993; Everall and Lees, 1996), presumably by generating phenolic compounds toxic to algae (Pillinger et al., 1994; Everall and Lees, 1997). Additions of commercially available barley straw extract (BSE) were recently tested in field enclosures containing populations of *P. parvum* (Roelke et al., 2006; Errera et al., unpublished). No suppression of either the abundance of *P. parvum*, or its toxicity was found in these experiments.

These previous tests of ammonium and BSE as suppressants of *P. parvum* all treated field populations or recently collected field samples with a single dose. Uncontrolled conditions and the presence of many additional organisms could thus have obscured or altered effects on *P. parvum*. In this study, weekly doses of ammonium and BSE at two levels were examined for their effect on *P. parvum*. Using laboratory cultures, two dosing levels of each agent were crossed with three temperatures in a factorial experiment examining the growth, abundance and toxicity of *P. parvum* to fish. Using laboratory cultures also enabled determining the nutrient element composition of *P. parvum* cells during the experiment, which could have affected their toxicity (Dafni et al., 1972; Johansson and Granéli, 1999; Granéli and Johansson, 2003; Barreiro et al., 2005; Uronen et al., 2005).

2. Materials and methods

2.1. Culturing

Stock cultures of a Texas strain of *P. parvum* (UTEX LB ZZ181) were grown in a defined medium of artificial sea water (Kester et al., 1967), diluted to 5.8 psu salinity with ultrapure water (18 MΩ/cm, Millipore) and enriched with f/2 levels of nitrogen, phosphorus, trace metals, and vitamins (McLachlan, 1973). In the f/2 trace metals solution, ferrous ammonium sulfate was substituted with ferric chloride equimolar in iron. Stock cultures were maintained at 20 °C and on a 12:12 photoperiod with photon flux of about 150 μmol/m^2/s. Cultures were not axenic.

Experimental batch cultures used a salinity of 4 psu, obtained by reducing the proportion of artificial seawater to ultrapure water. This salinity reflected a compromise between the low salinities characteristic of inland waters where *P. parvum* blooms have occurred (1–3 psu) and the optimal growth salinity for this strain, 22 psu (Baker et al., 2007). After dilution of artificial seawater, nutrients ($NaNO_3$, NaH_2PO_4, vitamins, trace metals) were added at f/2 concentrations and sodium bicarbonate was added at 93 μmol/L as a carbon source. The $NaNO_3$ and vitamins were added before autoclaving, but the other nutrients were filter sterilized (0.2 μm) and added aseptically after sterilization to minimize precipitation. One-liter flasks were filled to a working volume of 600 mL, and each flask was inoculated with 500 cells/mL of *P. parvum* from stock cultures in late exponential phase.

For experimental cultures, different incubators were used to achieve temperatures of 10, 20 and 30 °C. Flasks were distributed to combinations of temperature and dosing with ammonium or BSE in the experimental design described below. A photon flux of 150 μmol/m^2/s with a 12:12 photoperiod was used. The irradiance of experimental cultures was measured using a photon flux meter positioned at the sides, and above and below several culture vessels. Such measurements were taken in several areas of the incubator containing experimental cultures and then averaged to obtain the reported irradiance. This provided a daily light dose within the range characteristic of reservoirs in Texas during the cooler months (Grover and Chrzanowski, 2004) when *P. parvum* blooms have been observed. Flasks were mixed daily by gently swirling, and positions within incubators were rotated regularly.

2.2. Sampling and analysis

Samples of experimental batch cultures were taken periodically until cultures reached stationary phase (day 23). Aliquots of 5 mL were preserved with 0.15 mL Lugol's iodine and settled in sedimentation chambers for direct counts of cells of *P. parvum* with an inverted microscope (Margalef, 1969). Aliquots of 10 mL were preserved with 0.5 mL formalin for bacterial counts after staining with acridine orange