TABLE 1.—Striped bass fingerling production in ponds fertilized with high or low levels of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

Treatment (N:P ratio)	N:P rates (µg/L)	Pond	Fish stocked	Fish harvested	Weight harvested (kg)	Harvest length (mm)	Survival (%)
Low P (10:1)	300 : 30	7	50,156	6,792	6.85	44.3	0.14
Low P (10:1)	300 : 30	15	50,184	1,392	0.68	36.0	0.03
Low P (10:1)	300 : 30	16	50,184	10,270	7.17	41.6	0.20
High P (5:1)	300 : 60	9	50,000	4,317	5.22	47.2	0.09
High P (5:1)	300 : 60	13	50,184	0	0.00	0.00	0.00
High P (5:1)	300 : 60	67	52,693	0	0.00	0.00	0.00
Control	0	10	50,184	0	0.00	0.00	0.00
Control	0	12	50,184	0	0.00	0.00	0.00
Control	0	14	50,184	167	0.00	39.2	0.00

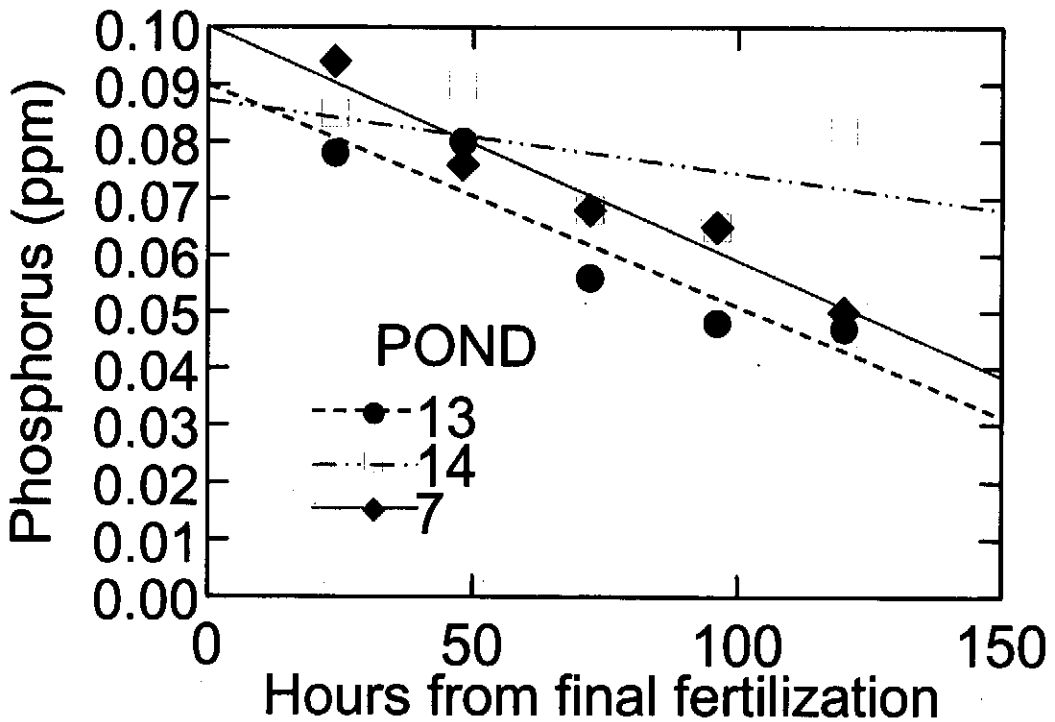

FIGURE 1.—Phosphorus decline rates in ponds fertilized with two 60-µg/L applications of phosphorus at the Dundee State Fish Hatchery in Spring 2002.

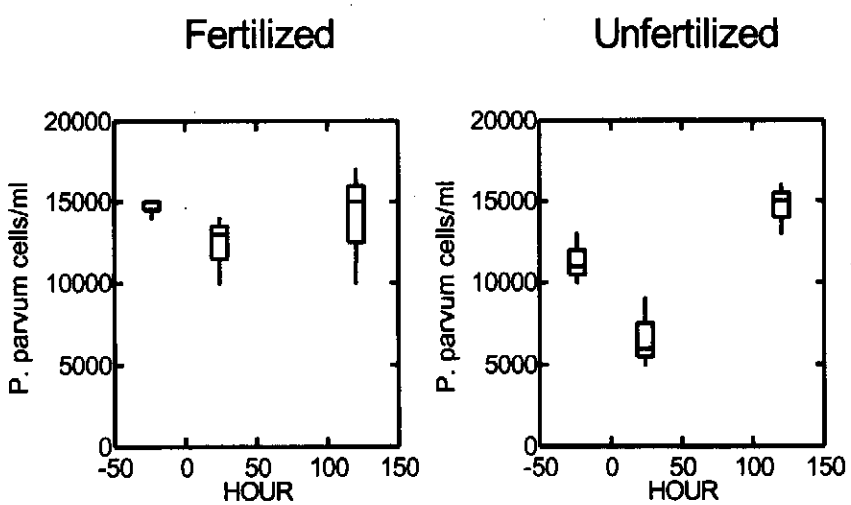

FIGURE 2.—*Prymnesium parvum* cell densities in ponds fertilized with 60 µg P/L or unfertilized with phosphorus at the Dundee State Fish Hatchery in Spring 2002.

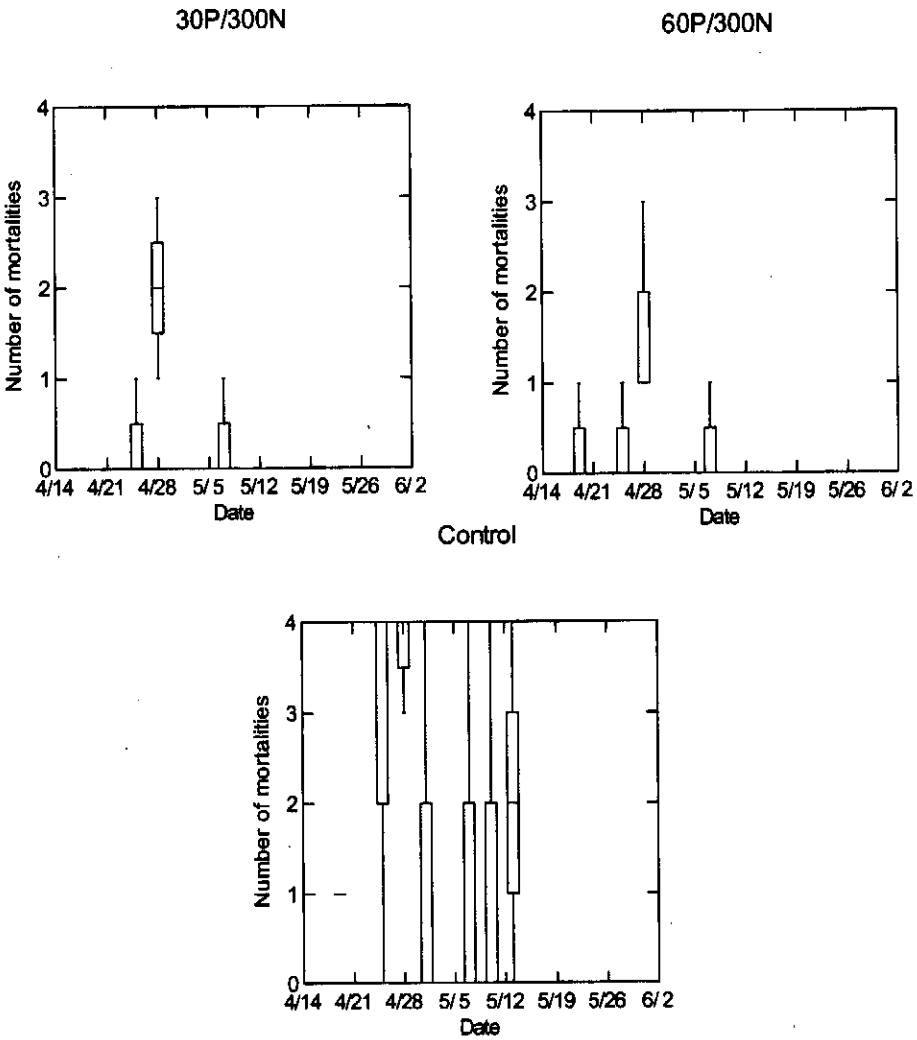

FIGURE 3.—Mortality among test fish in bioassays with undiluted water from ponds fertilized with high or low levels of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

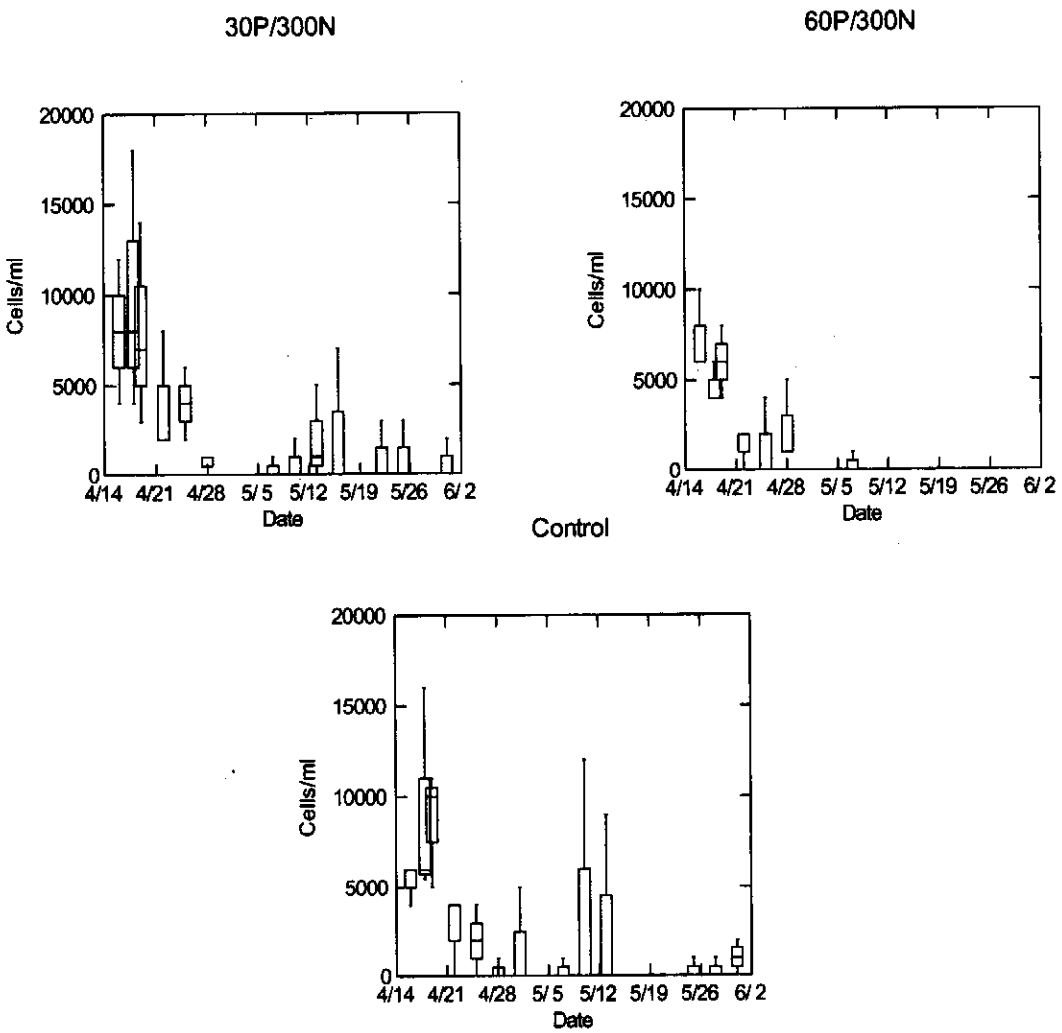

FIGURE 4.—*Prymnesium parvum* cell densities in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

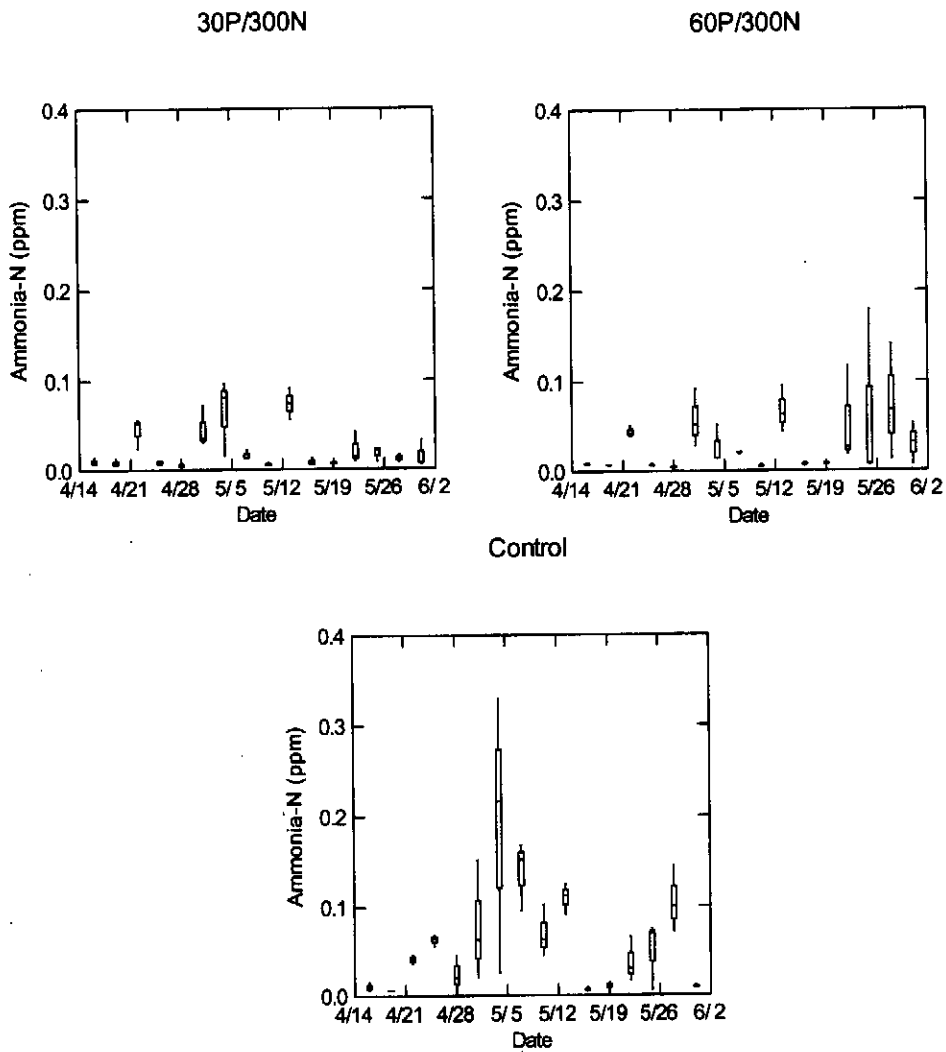

FIGURE 5.—Ammonia nitrogen concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

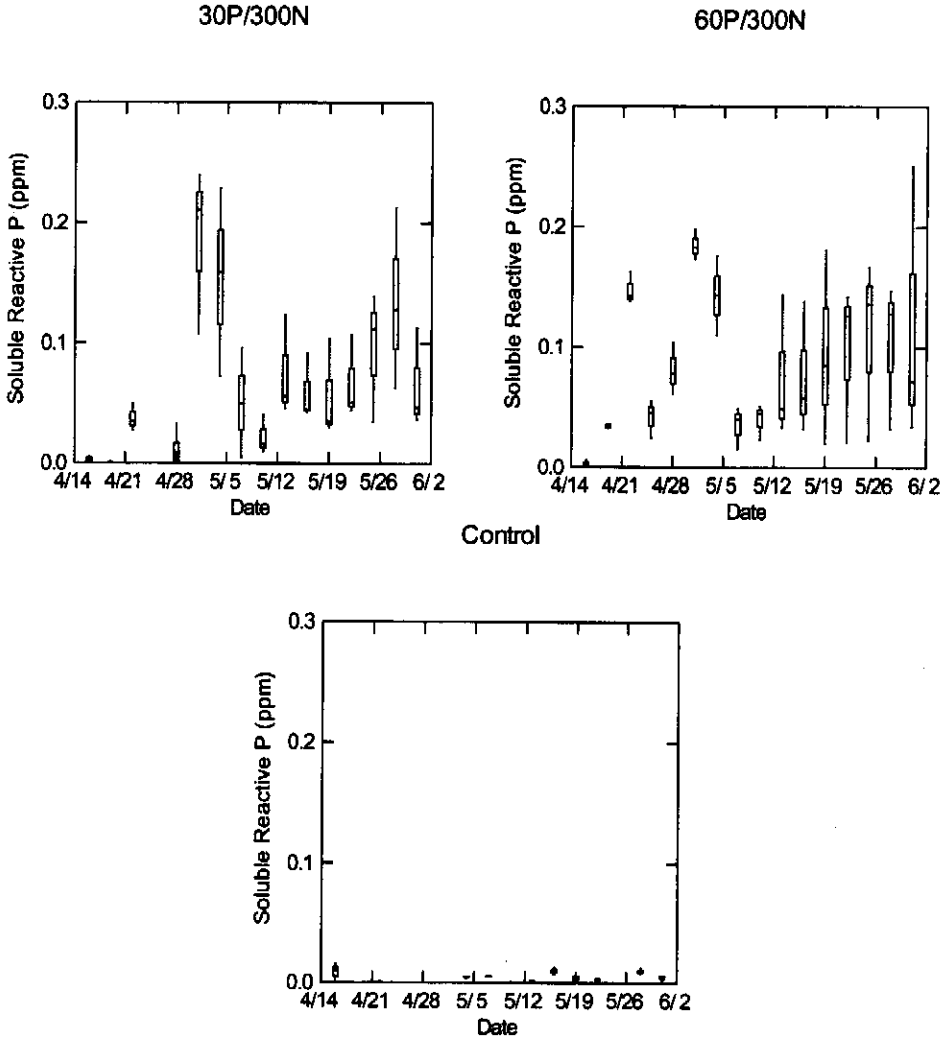

FIGURE 6.—Phosphorus concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

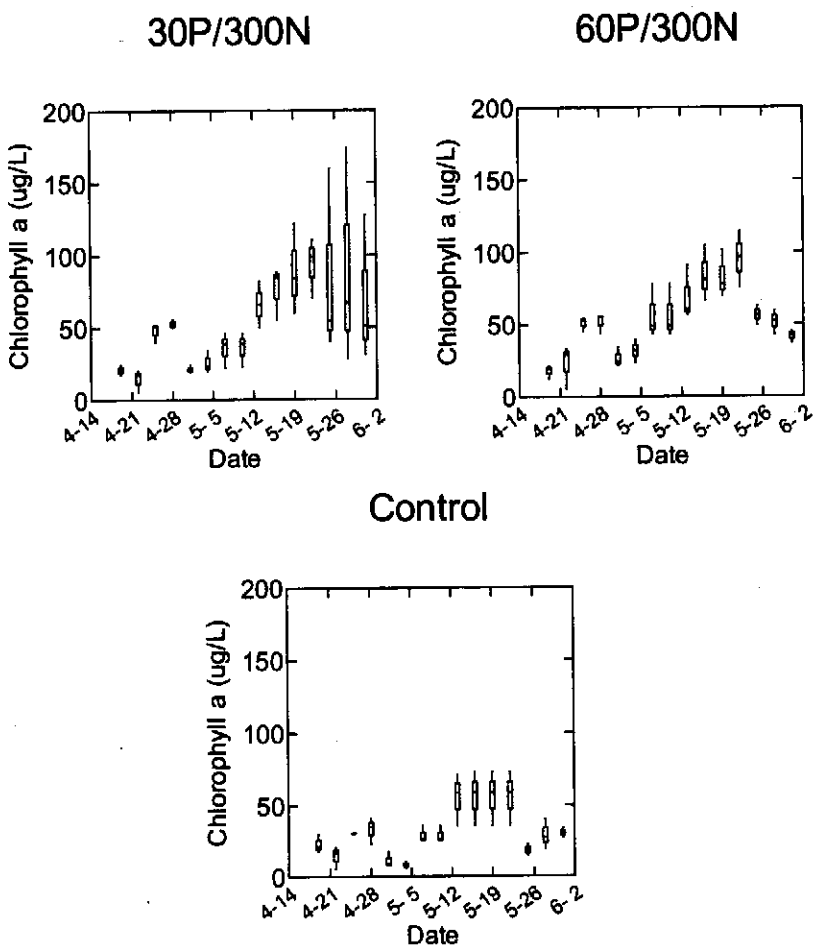

FIGURE 7.—Chlorophyll *a* concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

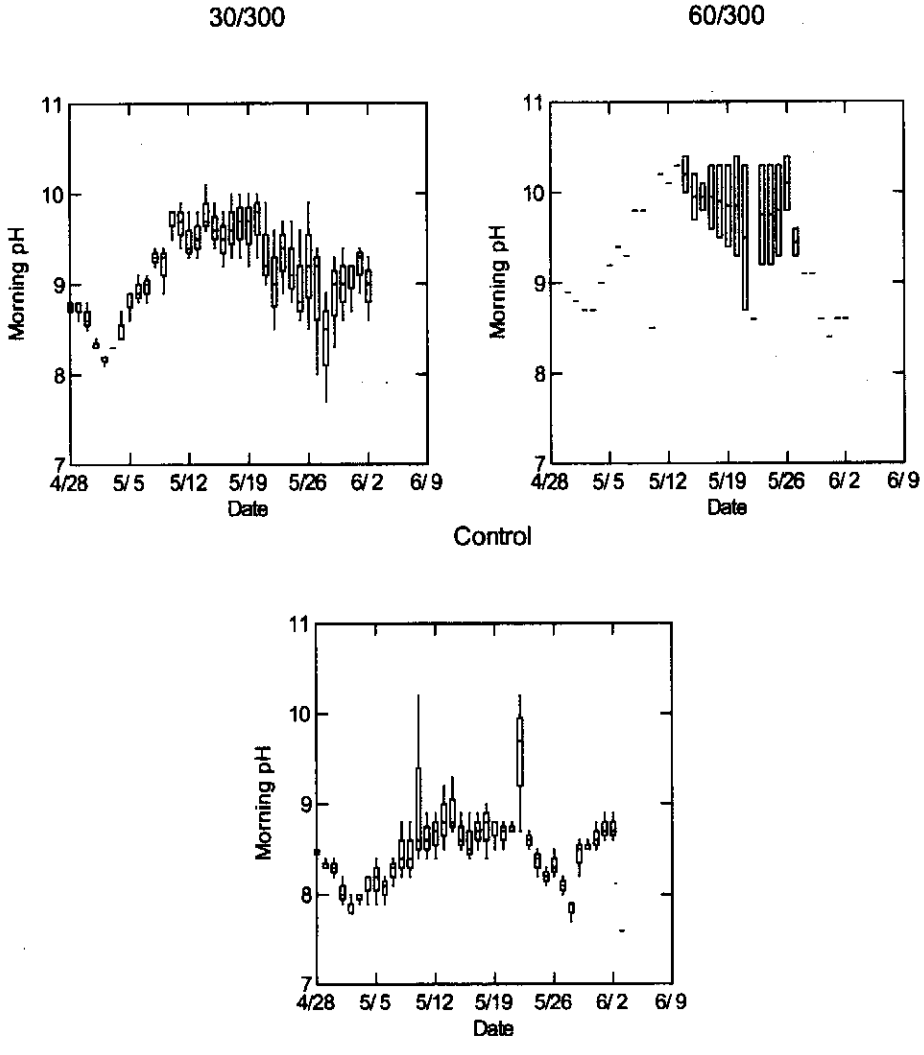

FIGURE 8.—Morning pH values in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

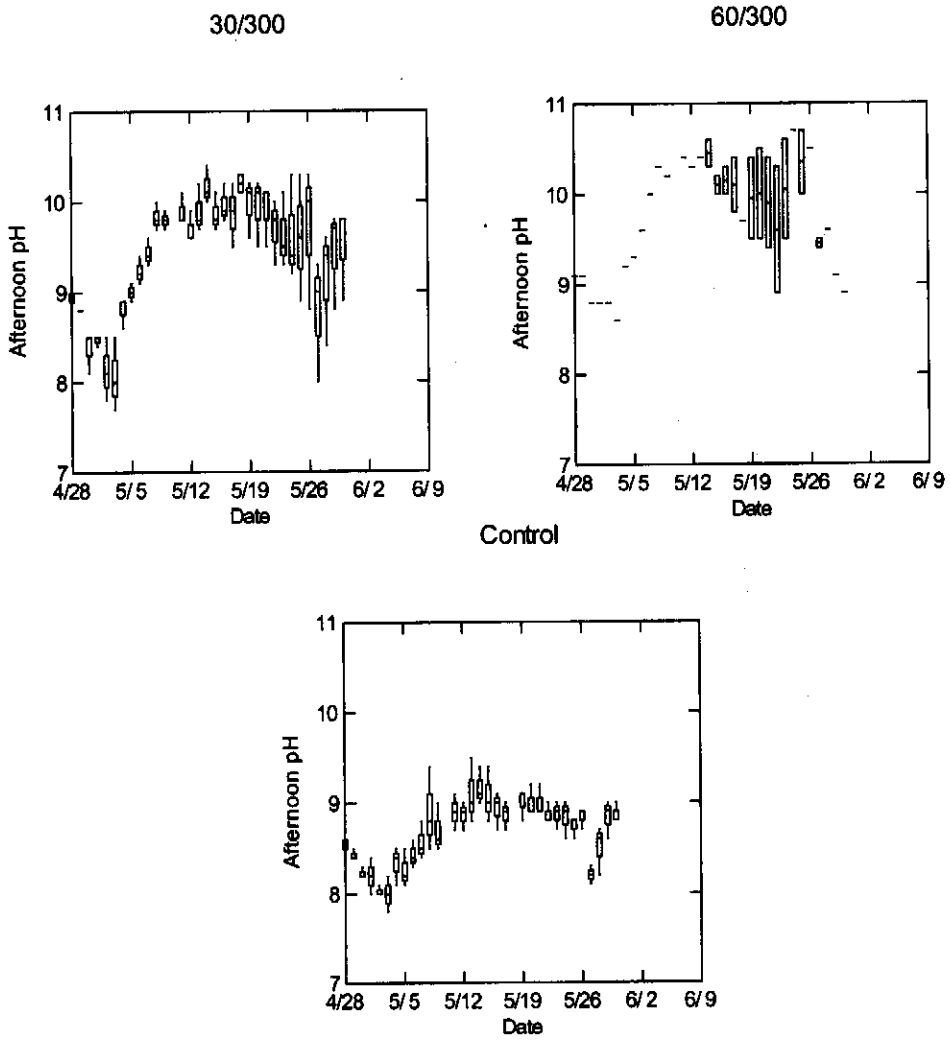

FIGURE 9.—Afternoon pH values in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

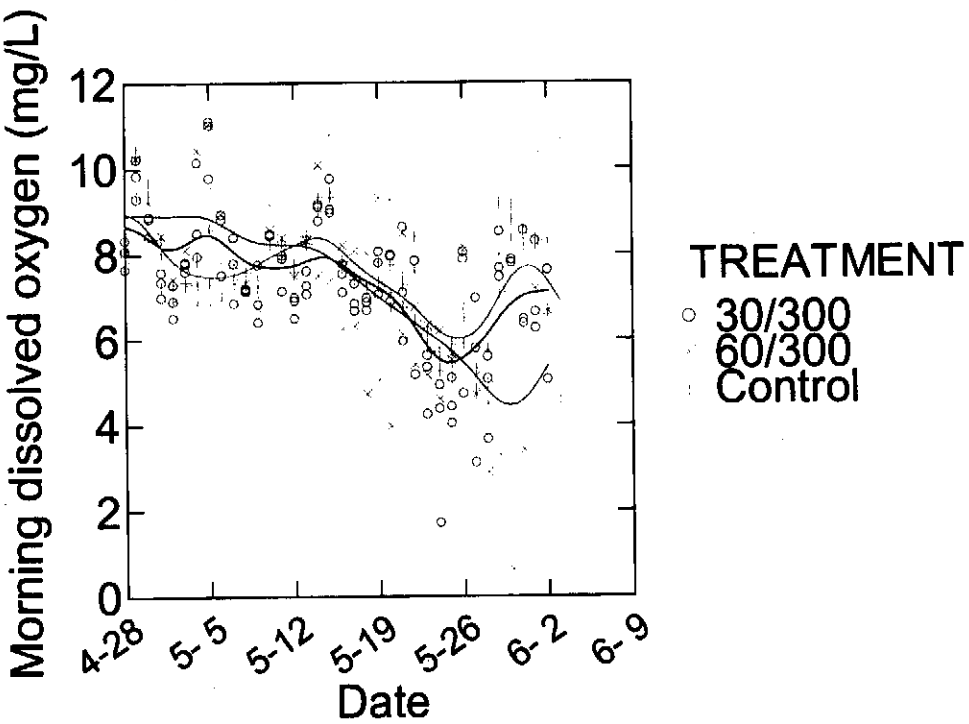

FIGURE 10.—Morning dissolved oxygen concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

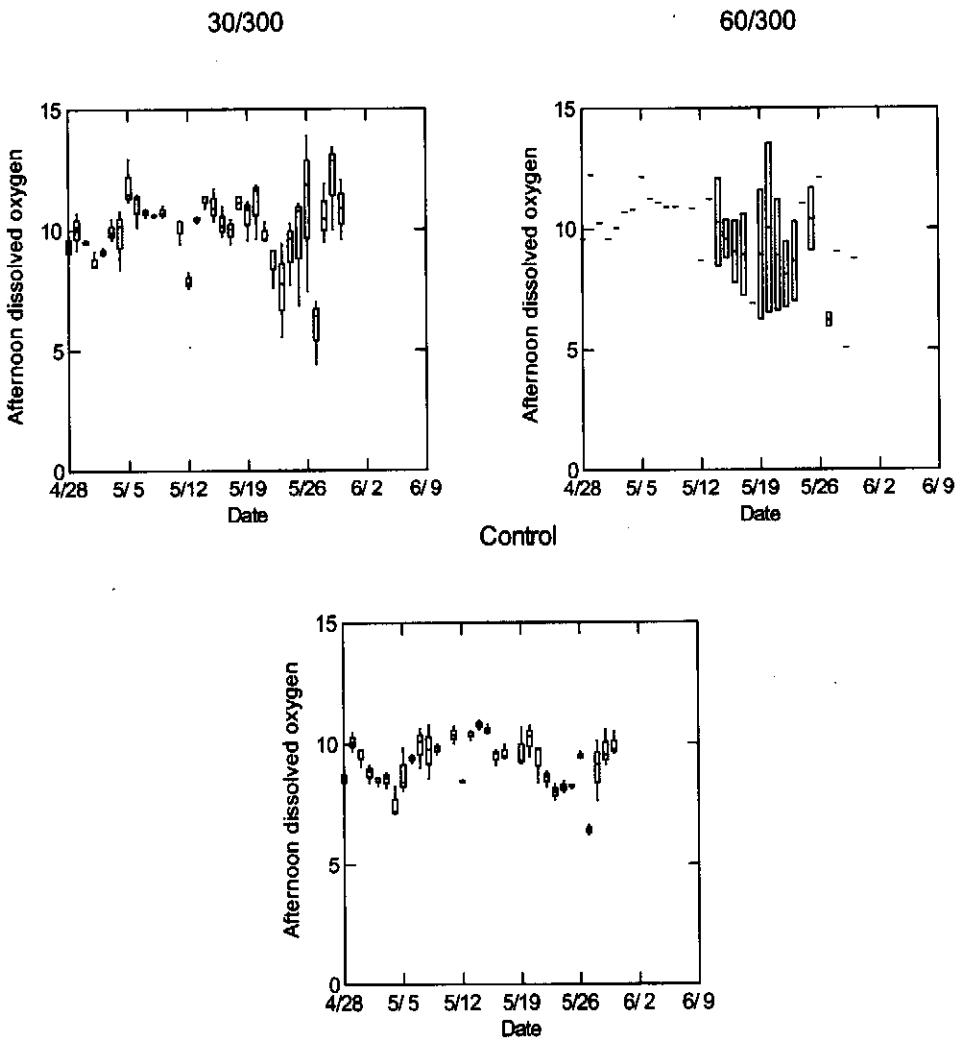

FIGURE 11.—Afternoon dissolved oxygen concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

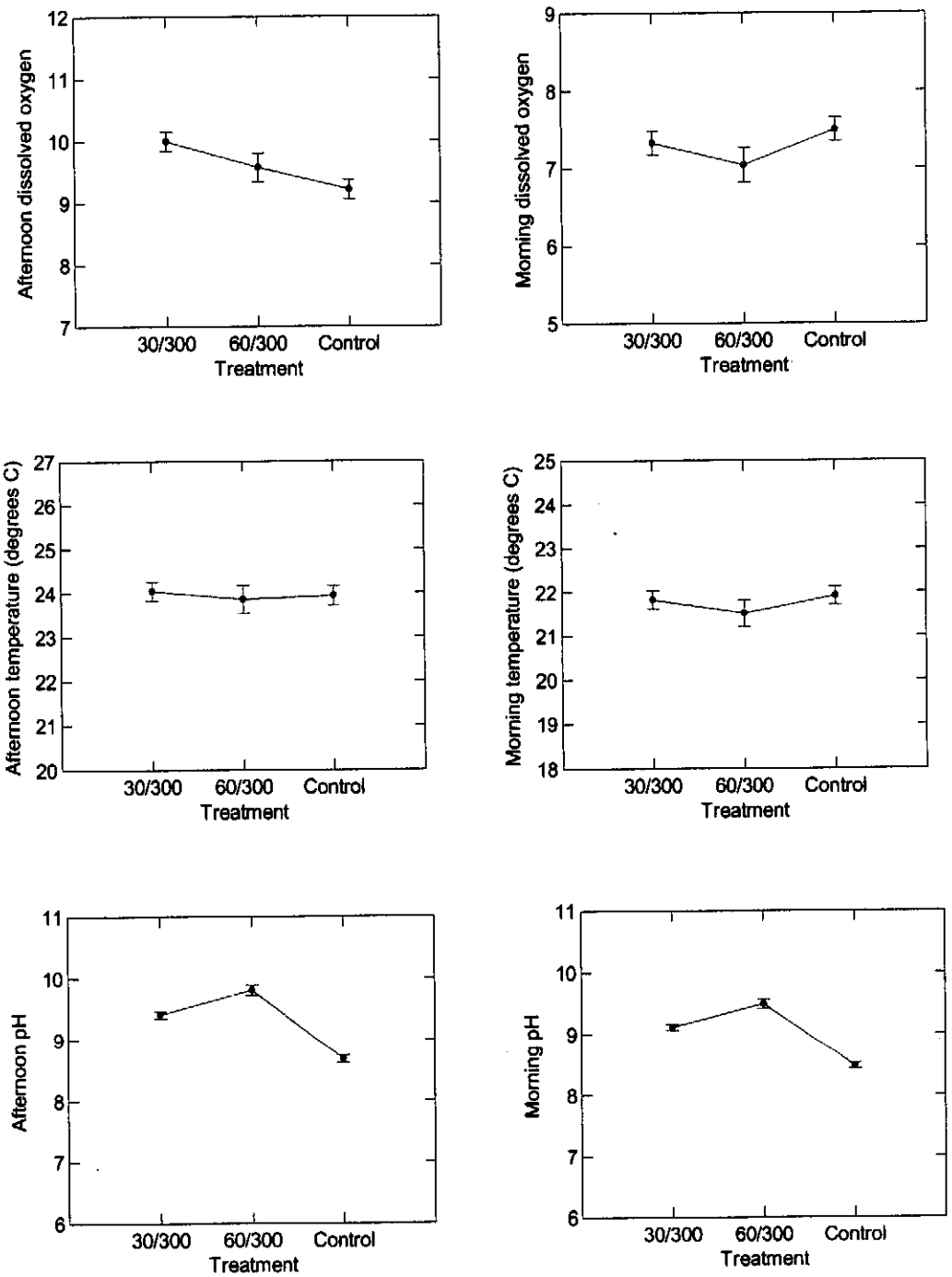

FIGURE 12.—Least squared means for water quality parameters in ponds fertilized with two concentrations of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

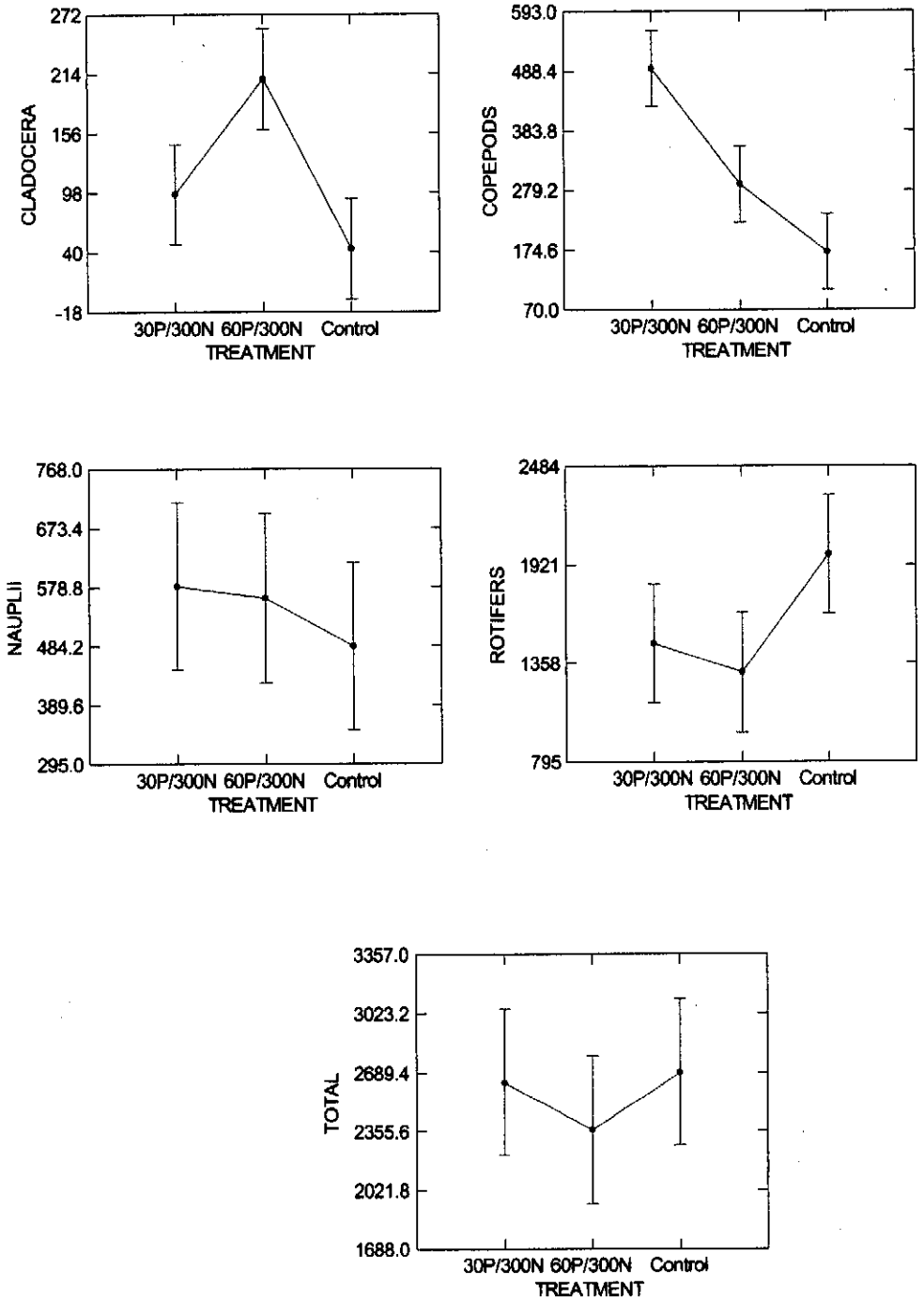

FIGURE 13.—Least squared means of zooplankton densities in ponds fertilized with two concentrations of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

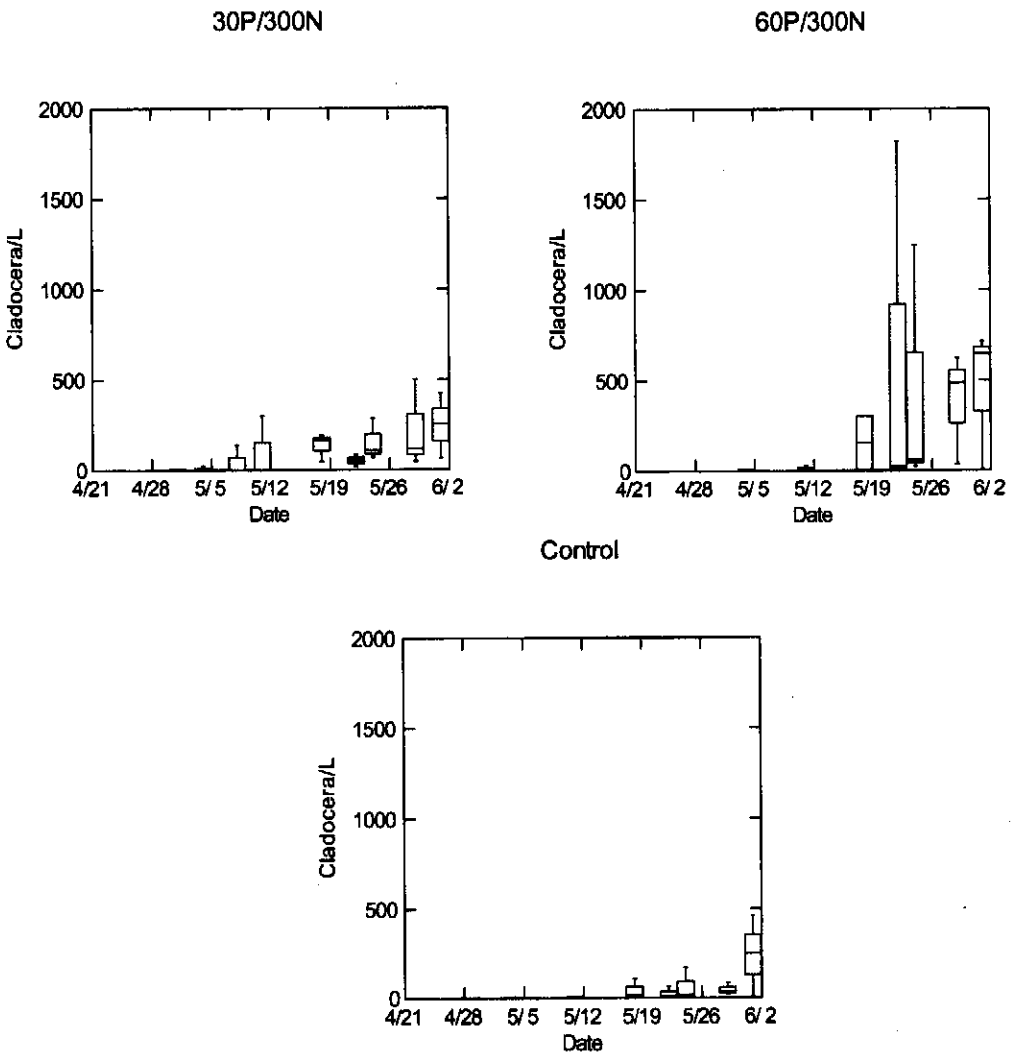

FIGURE 14.—Cladoceran densities in ponds fertilized with two concentrations of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

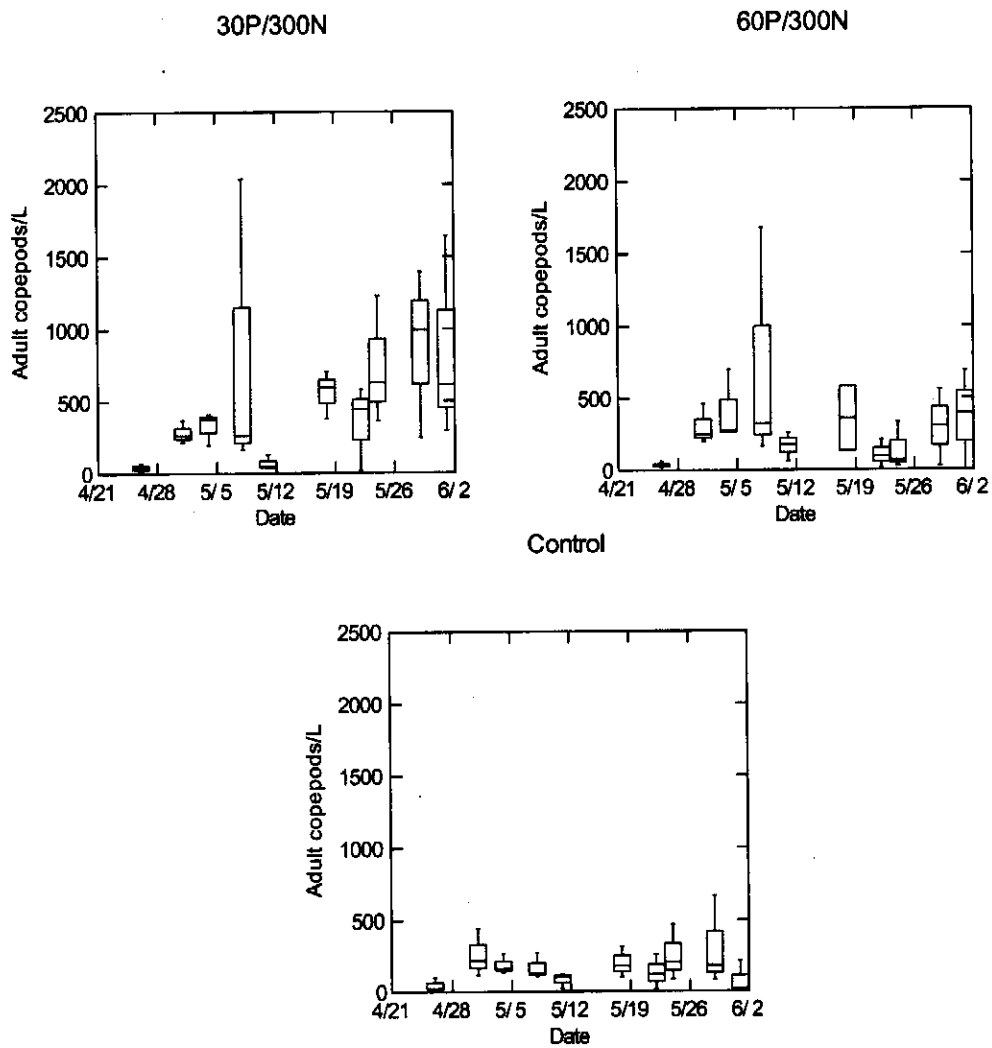

FIGURE 15.—Adult copepod densities in ponds fertilized with two concentrations of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in spring 2002.

CHAPTER 9

Efficacy of Ultraviolet Radiation to Control *Prymnesium parvum* Cells and Toxicity

DENNIS G. SMITH

Abstract

The effects of ultraviolet (UV) radiation on *Prymnesium parvum* cells and ichthyotoxicity were investigated using water from Lake Diversion, Texas. Reservoir water was toxic to fish on three of four testing days and sublethal on the remaining day. *P. parvum* cells were present in the water throughout the study. The water flowed through the UV unit at 11.5-11.6 CFM (i.e., cubic feet per min). The radiation was emitted at a mean dose of 210 (range = 193- 220) mJ/cm^2 and mean intensity of 91.5 (range = 84-96) mW/cm^2. The radiation completely destroyed all *P. parvum* cells and reduced toxicity from lethal to sublethal levels or from a sublethal to undetectable level.

Introduction

Prymnesium parvum, which produces toxins collectively known as prymnesins (Igarashi et al. 1996), has caused extensive fish mortality in rearing ponds at the Dundee State Fish Hatchery (DSFH) which necessitated a need for effective control of *P. parvum* cells and toxicity. This study was designed to determine whether ultraviolet (UV) radiation was a viable method to destroy *P. parvum* cells and ichthyotoxin. If UV radiation was determined to be effective, a treatment unit could be installed to sterilize incoming water to the hatchery. Water to the hatchery flows under gravity through a pipeline from Lake Diversion which is known to contain *P. parvum* and has experienced periodic fish kills attributable to the alga.

Materials and Methods

A UV-treatment unit was rented from Aquionics® and installed on a levee of a 0.1-ha rearing pond. Water flowed from the fill line of the pond through the UV unit and a flow meter into the rearing pond. A polyvinyl polycarbonate (PVC) pipe on either side of the treatment unit was fitted with a drain valve as close as possible to the unit. These drain valves allowed acquisition of untreated (inlet side of UV unit) and treated (outlet side of UV unit) water samples. The control and display panel of the UV unit was mounted on the safety railing of the pond. Water flow was adjusted with the pond fill valve to maintain a rate of approximately 327 L/min. The UV unit was in continuous operation for 11 days. The radiation dose and intensity and related data as well as water flow rates were recorded once or twice daily (Table 1). Water samples for cell counts were collected twice daily from the drain valves by flushing water through them for a few minutes and then simultaneously collecting some water from each drain valve in 19-L buckets. Three separate aliquots were

taken from each bucket for cell counts using a hemacytometer (Appendix A). Toxicity bioassays (Appendix B) also were performed on water collected in the same manner on the second, fourth, sixth, and eleventh day of operation. Bioassays were performed in duplicate for each sample.

Results and Discussion

Water was treated with a mean radiation dose of 210 (range = 193-220) mJ/cm^2 and mean intensity of 91.5 (range = 84-96) mW/cm^2 (Table 1). Mean cell density of the untreated water ranged from 0 to 14,000 cells/mL (Table 2), which is reflective of a patchy distribution of *P. parvum* in Lake Diversion. There were no viable cells in any of the treated water samples examined, indicating that UV radiation at the dose and intensity tested was effective at destroying *P. parvum* cells. This suggests that UV sterilization could prevent contamination of rearing ponds with *P. parvum* during pond filling. Furthermore, UV irradiation could be used to reduce or eliminate the number of viable *P. parvum* cells in water for fish transport and thereby lower the risk of unintentional introduction of *P. parvum* into lakes where the fish are stocked.

The bioassay results revealed that UV radiation did not always eliminate the toxin, but effectively reduced toxicity from lethal to sublethal levels or from sublethal to undetectable levels. The mortality data showed that toxicity was present in Lake Diversion water throughout the study occurring at lethal concentrations during the first three sampling days and at a sublethal concentration during the last sampling day. In the undiluted water with no co-factor (i.e., water that fish in rearing ponds would be exposed to), there were no mortalities in UV-treated water for the first three test dates (Table 3), although on those same days mortalities did occur in the other bioassay treatments (those with the cofactor). This observation indicates that toxicity was present at sublethal levels in the UV-treated water. On 8 October 2001, mortality occurred only in the untreated undiluted water without co-factor suggesting that the sublethal concentration of the toxin was further reduced to an undetectable level. These results suggest that, for a given radiation and water flow rate, detoxification is dependent upon toxin level such that complete removal is only accomplished at lower toxicity levels and at higher levels, toxicity is reduced but not eliminated.

TABLE 1.—Ultraviolet radiation characteristics and flow rate (cubic feet per min) of water subjected to radiation to destroy *Prymnesium parvum* and its ichthyotoxin.

Date	Time	Intensity (%)	Intensity (mW/cm^2)	Low UV Set point	Cumulative Run time (h)	Temperature (°C)	Dose (mJ/cm^2)	Wiper delay	Wiper cycles	Flow (CFM)
27 Sep 01	1430	98	84	70	47	20	193	2	90	11.5
28 Sep 01	1300	101	86	70	69	19	197	2	101	11.6
29 Sep 01	0901	105	90	70	89	19	207	2	111	11.5
29 Sep 01	1342	105	90	70	94	20	204	2	113	11.6
30 Sep 01	0853	108	92	70	113	19	211	2	122	11.6
30 Sep 01	1310	107	91	70	117	20	209	2	124	11.5
1 Oct 01	1100	108	92	70	140	19	211	2	135	11.5
1 Oct 01	1500	108	92	70	143	19	211	2	137	11.5
2 Oct 01	0900	110	94	70	161	19	216	2	145	11.6
2 Oct 01	1430	108	92	70	167	19	211	2	148	11.6
3 Oct 01	1030	108	92	70	187	19	211	2	158	11.5
3 Oct 01	1500	105	90	70	191	19	207	2	160	11.5
4 Oct 01	0900	109	93	70	209	19	213	2	168	11.5
4 Oct 01	1500	108	92	70	215	19	211	2	171	11.5
5 Oct 01	0800	109	93	70	232	18	213	2	180	11.5
5 Oct 01	1300	110	90	70	237	18	216	2	182	11.5
7 Oct 01	0900	112	96	70	256	18	220	2	203	11.6
7 Oct 01	1255	110	94	70	260	18	216	2	205	11.5
8 Oct 01	1100	111	95	70	282	18	218	2	216	11.5
Mean		107.4	91.5	70		18.9	210.3	2	151	11.5

TABLE 2.—Mean densities (range) of *Prymnesium parvum* in UV-treated and untreated water from Lake Diversion. Treated water passed through a UV unit at 11.5 CFM and subjected to radiation of 210.3 mJ/cm^2 at 91.5-mW/cm^2 intensity. Untreated water received no UV-radiation.

		Prymnesium parvum density (cells/mL)	
Date	*N*	Untreated water	Treated water
27 Sep 01	6	9,000 (2,000 – 30,000)	0
28 Sep 01	3	4,000 (2,000 – 6,000)	0
28 Sep 01	6	8,333 (4,000 – 16,000)	0
30 Sep 01	6	6,000 (2,000 – 10,000)	0
1 Oct 01	6	6,667 (2,000 – 16,000)	0
2 Oct 01	6	6,333 (2,000 – 10,000)	0
3 Oct 01	6	4,667 (0 – 10,000)	0
4 Oct 01	6	1,667 (0 – 4,000)	0
5 Oct 01	6	2,000 (0 – 4,000)	0
7 Oct 01	6	333 (0 – 2,000)	0
8 Oct 01	6	666 (0 – 2,000)	0

TABLE 3.—Bioassay (toxicity) results for untreated and treated water. Treated water passed through a UV unit at 11.5 ft^3/min and subjected to radiation of 210.3 mJ/cm^2 at 91.5-mW/cm^2 intensity. Untreated water received no UV radiation.

		Whole water with cofactor		1:5 Dilution with cofactor		Whole water with no cofactor	
Date	Water type	Test fish	Dead fish	Test fish	Dead fish	Test fish	Dead fish
28 Sep 01	Untreated	3	3	3	3	3	3
	Treated	3	3	3	2.5	3	0
1 Oct 01	Untreated	4	4	4	4	4	0.5
	Treated	4	4	4	0	4	0
3 Oct 01	Untreated	4	4	4	4	4	4
	Treated	4	4	4	0	4	0
8 Oct 01	Untreated	4	4	4	0	4	0
	Treated	4	0	4	0	4	0

CHAPTER 10

Evaluation of an Ultrasonic Device to Control Golden Alga *Prymnesium parvum* in Fish Hatchery Ponds

TOM DORZAB

Abstract

An ultrasonic device (i.e., Aquasonic Algae Controller) was evaluated to determine its efficacy at controlling *Prymnesium parvum* in hatchery ponds. This pilot study consisted of one pond with one Aquasonic Algae Controller (treatment) and two untreated ponds (control). Each 0.1-ha pond was stocked with five adult rainbow trout *Oncorhynchus mykiss*. Cell density *P. parvum* cell density was monitored in each pond for 21 days. The ultrasonic device appeared to be ineffective in reducing *P. parvum* cell density and had no discernable effect on survival of rainbow trout.

Introduction

The toxin producing, brackish-water phytoflagellate *Prymnesium parvum* was first identified at the Dundee State Fish Hatchery in March 2001. During the preceding month, about 7,000 rainbow trout *Oncorhynchus mykiss* died from exposure to the alga and its toxin. Since rainbow trout are known to be sensitive to copper sulfate (Hansen et al. 2002c) and ammonium sulfate is ineffective at controlling *P. parvum* at temperatures below 18°C (Sarig 1971), an alternative control method was needed for cold water conditions. This study was initiated to determine the effectiveness of the Aquasonic Algae Controller to control *P. parvum* in water with temperatures up to 18°C. This device is advertised for control of aquatic vegetation and reported to have successful application and wide acceptance (VoR Environmental, http://www.vor-env.com). The device works by emitting sound waves that cause cell death by breaking the cell vacuole (VoR Environmental)

Materials and Methods

Three 0.1-ha ponds were filled on 16 December 2001. Two of the ponds were used as control and received no treatment and the ultrasonic device was installed in the remaining pond on December 17. Each pond was stocked with five rainbow trout on December 17. *P. parvum* cell counts were conducted on December 17 and continued periodically through the end of the trial on January 7, 2002 using an established protocol (Appendix A). Morning temperatures, dissolved oxygen, and pH readings were recorded daily in each pond. Ponds were harvested on January 7, 2002 and the number of fish recovered from each pond was recorded.

Results and Discussion

At the beginning of the trial, cell counts showed *P. parvum* cells were present in one of the two control ponds as well as in the Aquasonic-treated pond (Table 1). Three days later, the same pattern was observed. Ten days after the start of the trial, *P. parvum* cells were present in the control pond that had previously had none while the two ponds that previously had cells now had none. Sixteen days after the trial began, cells were not observed in any of the ponds and after 21 days, *P. parvum* cells were found only in the pond with the ultrasonic unit. These observations suggest that the Aquasonic device was not effective in eliminating *P. parvum* from the pond after 21 days.

One fish (20%) died in the Aquasonic-treated pond while no fish died in either control pond, though the cause of the mortality is unclear. Although this study is not robust due to a lack of replication, the results presented herein suggest that further study of ultrasonic devices is unwarranted.

TABLE 1.—*Prymnesium parvum* cell densities and rainbow trout *Oncorhynchus mykiss* mortality in ponds with or without the Aquasonic Algae Controller.

Treatment	Pond	Fish mortality (%)	Cell density (number/mL)				
			17 Dec 01	20 Dec 01	27 Dec 01	2 Jan 02	7 Jan 02
Aquasonic	13	20	2,000	2,000	0	0	4,000
Control	9	0	0	0	2,000	0	0
Control	12	0	2,000	8,000	0	0	0

TABLE 2.—Water quality variables of trout ponds with or without the Aquasonic device for controlling *Prymnesium parvum*.

Treatment	Pond	Dissolved oxygen (mg/L)	Temperature (°C)	pH	Dissolved oxygen (mg/L)	Temperature (°C)	pH
Aquasonic	13	12.0 10.9 - 13.0	5.3 2.3 - 9.4	7.97 7.70 - 8.20	12.3 11.5 - 13.6	6.0 4.1 - 10.0	8.00 7.7 - 8.2
Control	9	12.0 10.8 - 12.8	5.1 1.7 - 9.5	7.91 7.80 - 8.10	12.0 11.4 - 12.9	6.4 3.8 - 10.3	7.98 7.80 - 8.10
Control	12	11.8 10.6 - 13.0	5.3 1.9 - 9.3	7.91 7.80 - 8.00	12.1 11.0 - 13.0	5.8 3.9 - 9.9	7.96 7.90 - 8.00

CHAPTER 11

Microscopy and *Prymnesium parvum*: Observations and Challenges

GREGORY M. SOUTHARD

Abstract

Prymnesium parvum is an algal species responsible for toxic fish kills in Texas reservoirs and two freshwater fish hatcheries. The current Texas Parks and Wildlife Department (TPWD) method for identifying and estimating densities of *P. parvum* in water samples uses a compound light microscope, hemacytometer, and trained personnel adept at identifying this particular species among mixed algal communities. Repeated observations of *P. parvum*-infested water samples using epifluorescence microscopy suggest that *P. parvum* may yield distinct fluorescence emission pattern(s) compared to the other types of algae in mixed samples. This observation lead to an investigative effort regarding the feasibility of specialized microscopic techniques, flow cytometry, and other fluorescence-based applications that might facilitate *P. parvum* cell enumeration. The following report is an overview of that investigation.

Introduction

Toxin-producing algae have been an area of health and economic concerns and study for marine biologists for decades and recently have become an issue for freshwater systems in the United States. *Prymnesium parvum*, also called "golden alga", is a chrysomonad haptophyte, which was first implicated in minor fish kills along the Pecos River in Texas during the 1980s (Linam et al. 1991). In recent years, this species has caused catastrophic fish mortalities in several Texas river systems and reservoirs as well as being problematic at two Texas Parks and Wildlife Department (TPWD) freshwater fish hatcheries.

An important aspect of toxic algal management plans at TPWD fish hatcheries involves monitoring cell densities to assess the need to implement control methods. Currently, trained personnel monitor *P. parvum* in reservoirs and hatchery ponds using light microscopy at magnification up to 1000X to identify the organism and a hemacytometer to calculate cell concentration.

Description of P. parvum

Microscopically, *P. parvum* has often been described as having variations in size and shape depending upon the phase of the life cycle as well as nutrient availability. Examination is facilitated by using unpreserved and unfiltered water samples since *P. parvum* can pass through or be damaged by most plankton nets and the cells are distorted by fixatives. Additionally, living cells have a characteristic swimming motion that aids in their identification. When samples must be preserved for long-term storage, buffered Lugol's

solution at 4°C is recommended although there may be some deleterious effects upon the cells, including loss of the haptonema.

The following description of *P. parvum* is adapted from Green et al. (1982). Cells glide smoothly in a forward direction while spinning on their longitudinal axis. Cells are 8-15 µm long and 4-10 µm wide, sub-spherical to elongate in shape with a rounded posterior end and tapered anterior end that bears two equal or sub-equal flagella of 12-20 µm long or approximately 1.5 times the length of the cell. A short haptonema (3-5 µm) is directed forward and arises from the same groove as the two flagella. There are two large yellow-green chloroplasts situated laterally and parietally, often deeply lobed and each containing an immersed pyrenoid. The nucleus lies centrally between the two chloroplasts. A golgi body is parabasal and a contractile vacuole is sometimes present. The flagellar action is heterodynamic in swimming cells with the anterior flagellum beating with a rapid, almost "flicking" movement (Green et al. 1982) as the posterior flagellum undulates freely. In resting cells, the flagellar action is slower and more regular with the amplitude of waves increasing towards the distal end of the flagellum. Cells are covered by organic scales of two types and in two layers: scales of the outer layer have narrow inflexed rims, a pattern of radiating fibrils on the proximal face and concentrically arranged fibrils, sometimes observed only in the central area, on the distal face; scales of the inner layer have surface patterning similar to the outer scales but with a rim strongly inflexed over the distal face. These scales require electron microscopy to be observed.

Light Microscopy

The oldest and simplest method for identifying algal species is by visual observation using a light microscope and a glass slide. While this approach is commonplace, it has many limitations. It requires trained personnel with an aptitude to distinguish subtle differences among taxa or variations associated with life history or nutrient availability (Culverhouse 1995). Cell counts can be very time-consuming, especially when phytoplankters are numerous. Additionally, density estimates using the hemacytometer likely are unreliable at low cell concentrations. In cases where the alga is present in low numbers or as a part of a complex mixture, it may be undetectable leading to the conclusion that the alga is absent when it is actually present. Despite these drawbacks, TPWD Inland Fisheries staff currently use light microscopy and hemacytometer counting chambers to identify and quantify *P. parvum* cells because faster, more accurate and inexpensive methods are unavailable.

Epifluorescent Microscopy

Epifluorescent microscopy was explored as an option to improve *P. parvum* cell identification and enumeration methodology. When illuminated using epifluorescence, *P. parvum* cells fluoresce a gold color that contrasts with the green or red color of many other algal species (Figure 1). This observation prompted further investigation into use of fluorescent microscopy to identify and quantify algae samples. Also of note, in some water samples we observed an organism that appeared to preferentially graze on *P. parvum* (Figures 2A, B, C, and D). The organism closely resembles the ciliate *Coleps* sp. (Fig. 2E and 2F), but it has not been positively identified. This protist could be useful as a biological

control agent if it is a significant predator of *P. parvum*, but more research is needed to demonstrate feasibility of using this organism as a control method. *Coleps* spp. have occasionally been associated with morbidity and mortality among fish (Szekely and Bereczky 1992; Wooster and Bowser 1994)

Pigment analysis via fluorescence excitation spectra has been studied and widely used for *in vivo* characterization of marine and freshwater phytoplankton communities. *In vivo* fluorometry (IVF) uses direct measurement of chlorophyll fluorescence in living cells and has been used by oceanographers and limnologists for locating and measuring algae for over 20 years. The IVF method is useful for gross estimates of algal growth patterns, but not very helpful to identify algal species.

Various attempts have been made to achieve taxonomic identification of phytoplankton based on *in vivo* bio-optical characteristics such as fluorescent excitation and absorption spectra (Hoepffner and Sathyendranath 1991). Oldham et al. (1985) were the first researchers to report identification of marine phytoplankton using total fluorescence "fingerprinting". Neveux and Panouse (1987) reported fluorescence characteristics of the chlorophylls and phaeophytins and the patterns associated with different algal taxa. Currently, fluorescence "signatures" of four taxonomic groups can be readily distinguished: chromophytes (golden-brown algae), chlorophytes (green algae), cryptophytes and rhodophytes (red algae), and cyanobacteria (blue-green algae). Specifically, the chlorophyll accessory pigment (CAP) ratio is used to differentiate these taxa in marine waters.

Other techniques for monitoring algal communities include estimation of chlorophyll *a* concentration, algal biomass, and primary productivity (USEPA 446.0, 1997; Yentsch and Yentsch, 1979). Recently developed methods measure fluorescence based on fixed wavelength fluorometry using a filter system for excitation and detection, scanning spectro-fluorometry based upon the excitation or emission spectra of fluorescence, fast repetition rate fluorometry, and a pump and probe technique (Kolber and Falkowski 1993).

Although the new techniques are promising, there are some drawbacks of using fluorescent patterns to characterize algal species. A common feature among these techniques is pigment concentration is estimated through the correlation of *in vivo* fluorescence with the isolated photosynthetic pigment values. Attempts to quantify phytoplankton pigments *in situ* using fluorescence characteristics counter the fact that photosynthetic pigments are bound to protein in living cells (Cowles et al. 1993; Kolber and Falkowski 1993). Because of that, *in vivo* fluorescence of phytoplankton should also take into consideration these pigment-protein complexes, which have a variety of structures and different roles in photosynthesis. *In vivo* fluorescence excitation spectra of phytoplankton depend not only on the taxonomic position of algae, but also on the photoadaption state. The cellular pigment content, the ratio of total chlorophyll *a* to accessory pigments, and the efficacy of energy transfer to chlorophyll *a* are sensitive to the light conditions of culture growth (Wilhelm and Manns 1991). Vertical profiles of a species will vary in photoadaptative state due to the gradient of ambient light. The bulk of chlorophyll and carotenoid-protein complexes consists of various light harvesting pigment proteins that absorb light and then convert light energy into chemical energy. The characteristics of phytoplankton *in-vivo* fluorescence, including its fluorescence

efficiency, depend upon various factors other than only taxonomy. For *P. parvum*, quantitative pigmentation has been found to be nearly independent of growth phase (Wilhelm and Manns 1991) and the pigment pattern is sensitive to nutrient limitations. Also of note, for *P. parvum*, the predominant fraction of the light harvesting protein gave absorption spectra rich in chlorophyll *c* (c_1, c_2, and c_3) as well as xanthophylls (Wilhelm and Wiedemann 1991).

Flow Cytometry

Fluorescence becomes a powerful tool in cell identification when the cells can be separated and analyzed individually, as with flow cytometry (Børsheim et al. 1989). With the aid of laser-based flow cytometry and cell sorting, pigment autofluorescence, stain-induced fluorescence, and light scatter are used as probes to quantify and sort subpopulations of phytoplankton cultures and natural populations (Yentsch et al. 1983). Once fluorescence signature coordinates are established, flow cytometry is a convenient way to count individual species of phytoplankton in mixed samples. Chlorophyll *a* fluorescence and forward light scatter are two parameters measured using flow cytometry and that produce discriminatory signature ranges, but they are highly variable depending upon life history, distribution, concentration, and other factors (Yentsch et al. 1983). Thus, as with the visual method, there also are disadvantages with using flow cytometry to identify and quantify algal cells. Flow rates typically make selection of individual cells nearly impossible or investigation of organisms in low-concentrations time-consuming or impossible. Also, there is natural variation in the form or biochemistry of the algae themselves, ranging from morphological features to amount of chlorophyll or associated pigments produced (Culverhouse 1995).

Challenges

The TPWD Inland Fisheries Division has the need for a quicker and more reliable method to identify and quantify *P. parvum* in water samples, whether it occurs in the hatchery setting or as part of management plan for affected water bodies. Fluorescence characteristics may be useful to distinguish this alga from other species, but techniques in the field of microscopy or cytometry need to be developed and current TPWD personnel lack the expertise to develop these methods. Additionally, the specialized equipments are expensive and bulky. Thus, cost-effective alternatives to these technologies that are suitable for hatchery or field operations are needed

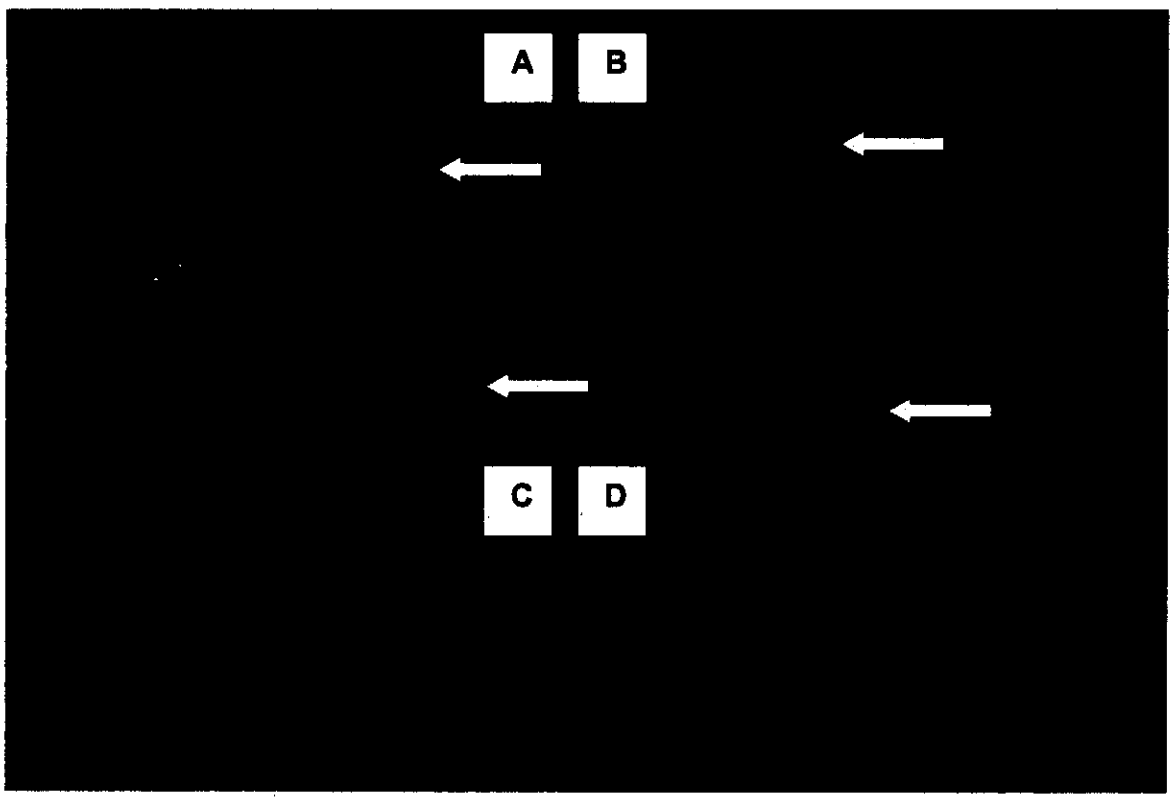

FIGURE 1.—Brightfield and epifluorescent microscopy images of mixed phytoplankton community containing *Prymnesium parvum* (arrows) cells: (**A**) brightfield (100X); (**B**) epifluorescence (100X); (**C**) brightfield (400X); (**D**) epifluorescence (400X).

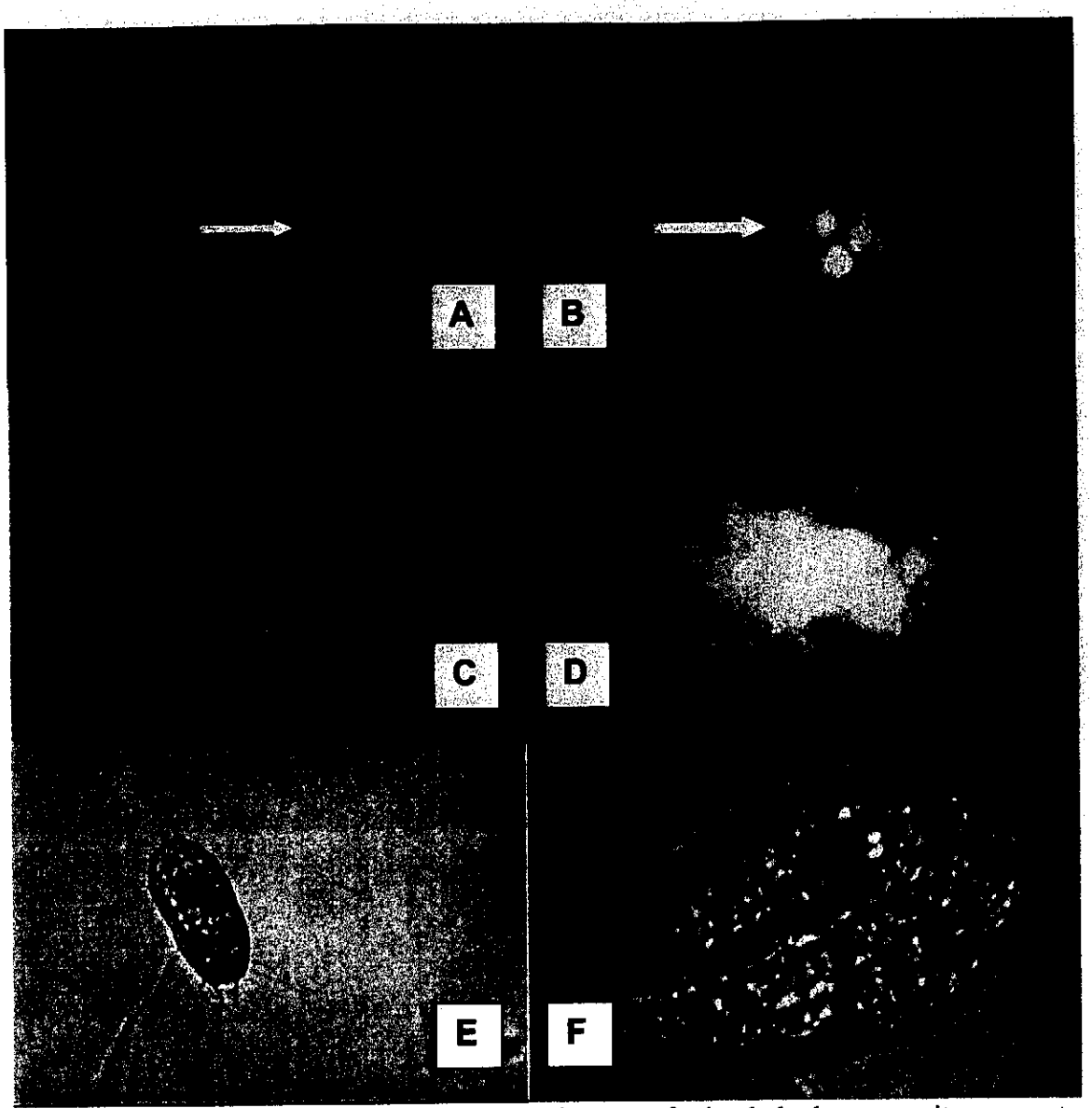

FIGURE 2.—Brightfield and epifluorescent images of mixed algal community, suspect *Prymnesium parvum* grazer (arrows), and the protist *Coleps* sp. **(A)** Mixed algal species (epifluorescence100X); **(B)** Mixed algal species (epifluorescence 400X); **(C)** Suspect *P. parvum* grazer (brightfield 400X); **(D)** Suspect *P. parvum* grazer (epifluorescence 1000X); **(E)** *Coleps* species (brightfield 400X) [http://www.funsci.com/fun3_en/protists/prot_14.jpg]; **(F)** *Coleps* species (brightfield 1000X) [http://www.ph-karlsruhe.de/NATUR/GARTEN/2001/kraeuterspirale/index.html].

CHAPTER 12

Dundee State Fish Hatchery *Prymnesium parvum* Management Plan

DENNIS G. SMITH

Abstract

This management plan was prepared as a guide to control the toxic alga *Prymnesium parvum* and its ichthyotoxin and eliminate, or at least minimized, its adverse impact on fish production. The plan includes monitoring presence and abundance of *P. parvum* and concentration of un-ionized ammonia nitrogen, and application of effective chemical treatments. Ammonium sulfate is applied at concentrations to raise the un-ionized ammonia nitrogen concentrations to 0.2-0.4 mg/L when water temperatures are 15°C or higher, and copper sulfate (or Cutrine-Plus) is applied at 0.2-0.4 mg Cu^{2+}/L when water temperatures are up to 15°C. The selected target concentrations of un-ionized ammonia nitrogen and copper depend on the tolerance of the fish that would be exposed to the treatments.

Introduction

The Dundee State Fish Hatchery is located in Archer County, Texas below Lake Diversion which supplies water to the hatchery. The hatchery has 97 ponds: 73 are plastic-lined totaling 24 ha (59.5 acres) and 24 are earthen ponds totaling 9.3 ha (23 acres) of surface water. Other culture units include four outdoor raceways and indoor 12, 1.8-m fiberglass round tanks, 90-jar egg incubation system and 4-trough (970-L) rearing system. All indoor culture systems can be operated as flow-through or closed systems. The spawning and rearing building which houses the indoor culture units also is equipped with an ozone generator and UV system for treating lake water containing *Prymnesium parvum* cells or toxins.

Fish species cultured at this facility include striped bass *Morone saxatilis*, palmetto bass (striped bass ♀ × *M. chrysops* ♂), channel catfish *Ictalurus punctatus*, black basses *Micropterus* spp., koi carp *Cyprinus carpio*, rainbow trout *Oncorhynchus mykiss*, walleye *Stizostedion vitreum* and saugeye (female walleye × male *S. canadense*).

In 2001 fishes on the hatchery suffered substantial mortality from *P. parvum* ichthyotoxicity. Losses included 5.1 million striped bass and palmetto bass, 1,500 black basses, and thousands of channel catfish, rainbow trout and koi carp. Through the efforts of hatchery staff and the Hatcheries Golden Alga Task Force, strategies have been developed to control *P. parvum*. These strategies form the basis of the *P. parvum* management plan described herein. This plan continues to evolve and modifications are made to it as more effective or efficient solutions to the *P. parvum* toxicity problem are discovered.

Prymnesium parvum Management Plan

Pond Management

- Fill ponds well in advance of fish stockings to allow water temperatures to rise so treatment with ammonium sulfate, if needed, can be effective.
- Avoid flushing ponds too rapidly and decreasing temperature if ponds must be flushed. If possible avoid pond flushing.
- Treat ponds at least two days prior to anticipated stockings to allow treatments to work and toxins to decompose.
- Perform bioassays and check for cells any time *P. parvum* toxicity is suspected and on the days before fish stockings.
- Maintain a minimum of 0.18 mg/L un-ionized ammonia nitrogen (UIA-N) or 2 mg Cu^{2+}/L in ponds depending on treatment option.

Prophylactic Treatments of P. parvum in Ponds

- Measure pond water temperature and pH
- If pond water temperatures are consistently above 28°C
    - *P. parvum* may be absent or present in very low numbers and ichthyotoxicity is unlikely. Treatment should be unnecessary.
    - Monitor ponds for presence of the alga and signs of toxicity at least once per week.
- If pond water temperature is 28°C
    - Check for presence of *P. parvum* cells twice per week.
    - If cells are present measure ammonia, temperature, and pH.
    - Calculate concentration of UIA-N.
    - Apply ammonium sulfate to raise UIA-N to 0.3 mg/L if UIA-N is less than 0.18 mg/L.
- If pond temperatures are below 28°C, consult an ammonia ionization table (Piper et al. 1992) or hatchery ammonia spreadsheet to determine proportion of total ammonia in the un-ionized form.
    - If the proportion of total ammonia in the un-ionized form is less than 5%
        - Apply Cutrine-Plus® or copper sulfate to raise copper concentration to 0.25 mg/L.
        - Measure copper concentration once per week.
        - Maintain copper concentration above 0.2 mg/L.
        - Check for presence of *P. parvum* cells once per week for monitoring purposes.
    - If the proportion of total ammonia in the un-ionized form exceeds 5%
        - Measure ammonia, temperature, and pH once per week (twice per week for sensitive species such as striped or palmetto bass).
        - Calculate concentration of UIA-N.
        - Apply ammonium sulfate to raise UIA-N to 0.3 mg/L if UIA-N is less than 0.18 mg/L.
        - Check for presence of *P. parvum* cells once per week for monitoring purposes.

- If the proportion of total ammonia in the un-ionized form is low (5-15%) and pH is expected to increase above 8.5
  - Reduce target ammonium sulfate treatment to achieve UIA-N of 0.25 mg/L. This treatment level is high enough to control *P. parvum* but requires less ammonium sulfate and lower total ammonia. Thus, should pH rise the UIA-N generated may not be toxic to the fish. Treatments at this lower UIA-N rate may require more frequent applications.

Indoor Culture Units

- Use UV- and ozone-treated lake water (treated water) for all culture activities in the spawning and rearing building if lake water contains *P. parvum* or its toxin. High dosage UV (180 to 200 mJ/cm^2) and ozone treatment is required to eliminate *P. parvum* toxicity if toxins are present in the supply water.
  - Check treated water for presence of *P. parvum* or toxin to be sure the system is working.

Treatment of Ichthyotoxicity

- Treat ponds or other culture units with potassium permanganate at the demand rate or up to 2 mg/L above the demand rate for temporary relief if fish show signs of ichthyotoxicity.

Fish Harvest

- Check incoming lake water for toxicity and presence of *P. parvum* one day before fish harvest.
- If *P. parvum* or toxin is absent in lake water
  - Harvest fish using routine hatchery procedures.
- If *P. parvum* or toxin is present in lake water but water not toxic.
  - Do partial pond draining the day before harvest.
  - Harvest fish as scheduled within 2 hours using lake water.
  - Treat pond water with potassium permanganate if fish exhibit signs of ichthyotoxicity.
- If lake water is toxic
  - Suspend fish harvest until the condition improves.
  - If fish must be harvested, use non-toxic water from adjacent ponds or treated water and potassium permanganate treatment if fish show signs of ichthyotoxicity.

Fish Hauling Units

- Fill fish hauling units with treated water.
- Rinse fish to be transported off the hatchery with treated water before loading to avoid introducing *P. parvum* into hauling tanks and ultimately into stocked lakes.
- After fish loading check hauling unit water for *P. parvum*.
  - If *P. parvum* is absent deliver fish according to hatchery guidelines.
  - If *P. parvum* is present drain out some water, refill with treated water, and recheck for *P. parvum*. Repeat until no *P. parvum* is found.
- Upon return to the hatchery, disinfect hauling units with 10% chlorine bleach.

- Use lake water free of *P. parvum* cells or toxins, or treated water to transfer fish between hatchery culture units.

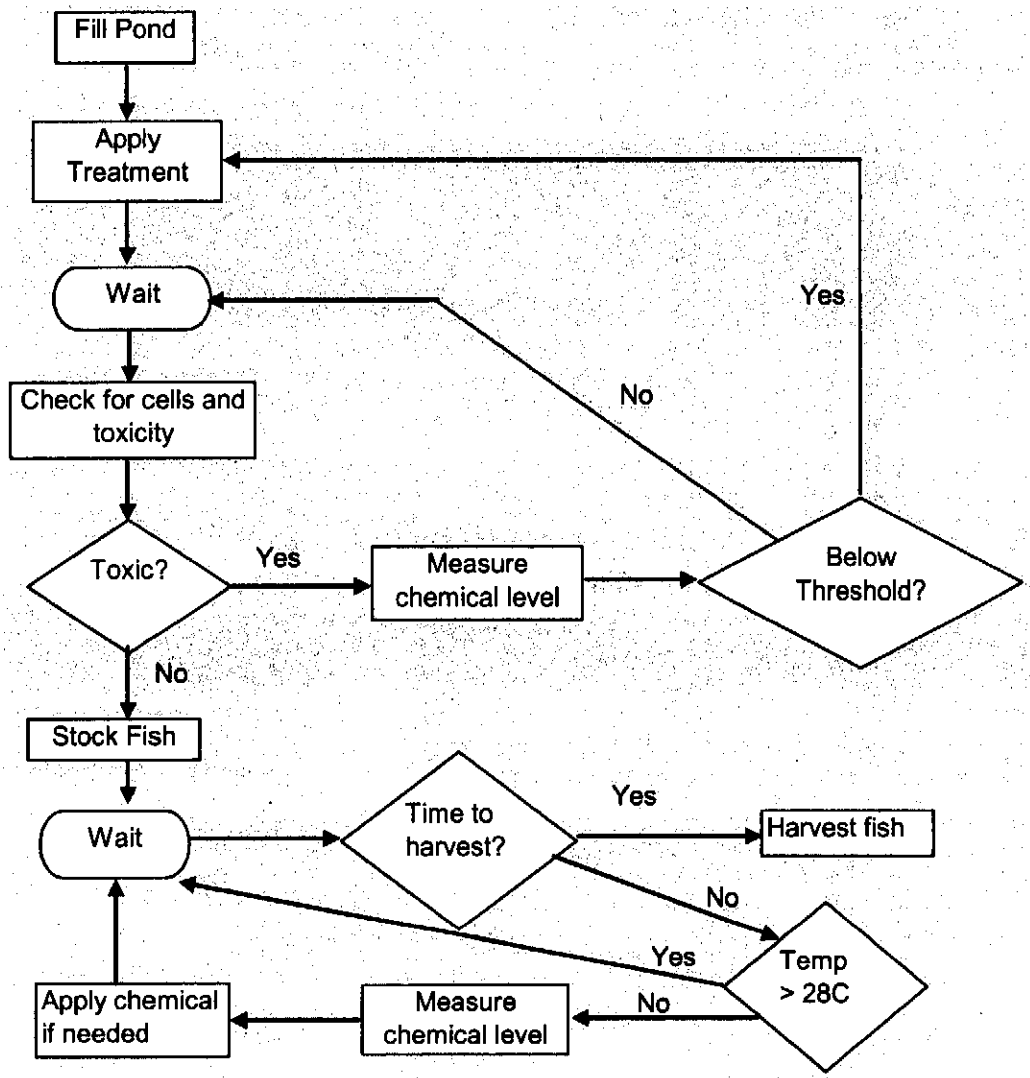

FIGURE 1.—A schematic diagram of the Dundee State Fish Hatchery pond management plan.

CHAPTER 13

Possum Kingdom State Fish Hatchery *Prymnesium parvum* Management Plan

DALE D. LYON, JAKE ISAAC, AND JOHN PARET

Abstract

This *Prymnesium parvum* management plan was prepared to provide a systematic approach to controlling this toxin-producing alga to make fish production possible at the Possum Kingdom State Fish Hatchery. The essential facets of the plan are monitoring presence and density of *P. parvum* and un-ionized ammonia levels, and application of chemical treatments. Ammonium sulfate is applied at 10 mg/L or concentrations to raise the un-ionized ammonia concentration to 0.2-0.4 mg/L when water temperatures are 15°C or higher and copper sulfate or Cutrine-Plus is applied at 0.75-1.0 mg Cu/L when water temperatures are up to 15°C. The target concentration of un-ionized ammonia or copper depends on the fish species being cultured.

Introduction

Possum Kingdom State Fish Hatchery is located in Palo Pinto County, Texas below Possum Kingdom Lake, the main source of water for the hatchery. The lake water comes to the hatchery through a 4.5-m (14.8 ft) deep intake valve (shallow water) or an 18-m (59-ft) deep intake valve (deep water). Additional water for the hatchery is provided by a well. Effluent water from ponds and indoor culture units can be reused in ponds after filtration by a re-circulation system. Culture units include 38 plastic-lined ponds (9.4 ha or 23.2 acres) and indoor raceway, 48-McDonald jar egg incubation system and six holding troughs. The incubation system can be operated as flow-through or closed system with filtration, heating and cooling capabilities. All holding troughs have flow-through capabilities but only four have re-circulation capabilities. The indoor re-circulation systems are equipped with an ultraviolet sterilizer for treating water infected with *P. parvum*.

Fish species cultured at this hatchery include striped bass *Morone saxatilis*, palmetto bass (female striped bass × male *M. chrysops*), channel catfish *Ictalurus punctatus*, smallmouth bass *Micropterus dolomieu*, koi carp *Cyprinus carpio*, bluegill *Lepomis macrochirus*, crappie *Pomoxis* spp., rainbow trout *Oncorhynchus mykiss*, and walleye *Stizostedion vitreum*.

P. parvum was first confirmed in Possum Kingdom Lake in 2001 following extensive toxin-related fish kills in the reservoir. This alga was found in our hatchery ponds in 2002 when ponds were filled with lake water following a renovation in 2001. This alga consistently appears to bloom during colder months (January-March), and blooms are usually associated with fish kills. During summer months, when temperatures exceed 28° C, the alga

usually disappears or occurs in very low density and toxin-related fish kills are rare. Spring and fall appear to be transitional periods when *P. parvum* densities fluctuate and fish kills are sporadic.

Since 2001 staffs at Possum Kingdom and Dundee hatcheries in cooperation with the Hatcheries Golden Alga Task Force have been developing strategies for controlling the alga. The strategies that seem to work best for this facility are formulated into the management plan described below. As more effective or efficient strategies are developed this management plan will be updated.

P. parvum Management Plan

This facility has adopted a prophylactic approach to managing *P. parvum* with the goal of elimination the alga from culture systems or keeping densities as low as possible. Therefore, if a single cell is detected in a water sample (i.e., 2,000 cells/mL), the infected pond is treated to control the alga. Before treatment, the un-ionized ammonia nitrogen (UIA-N) or Cu^{2+} concentration in the pond is determined and the difference needed to achieve the target treatment level is provided by applying ammonium sulfate or copper sulfate.

Brood fish Holding (striped bass or white bass)

- Fill indoor holding troughs with well water and operate as closed system
    - Check for the presence of *P. parvum* to be sure the system of free of the alga.
    - If no cells are present there should be no need for further monitoring.

Jar Rack Egg Incubation

- Fill egg incubation system with well water and operate as a closed system.
- Check system water for *P. parvum* cells.
    - If cells are present treat with UV radiation.
    - If no cells are present there should be no need for further monitoring.

Spring Fry Rearing (striped bass, smallmouth bass, koi carp, etc)

- Clean all pond bottom sediments 12-14 days before fry stocking.
- Begin filling ponds 11 days before fry stocking with deep lake water.
- Treat ponds with ammonium sulfate to achieve UIA-N level of 0.3 mg/L 6 days before stocking.
- Check ponds for presence of *P. parvum* 4 days and 1 day before fry stocking; treat if cells are present.
- For striped bass conduct 24-hour survival tests on all ponds before stocking.
- Check all ponds with fish for *P. parvum* once per week.
    - If *P. parvum* is present check affected ponds twice per week
    - Treat ponds containing *P. parvum* with ammonium sulfate to achieve UIA-N level of 0.3 mg/L if UIA-N is low and temperature is 15°C or higher.
    - Treat ponds containing *P. parvum* with copper sulfate (or Cutrine-Plus) to achieve 0.75 mg Cu^{2+}/L if temperature is less than 15 °C.

Spawning Ponds (smallmouth bass)

- Fill ponds with deep lake water
  - Check ponds for *P. parvum* once per week; when *P. parvum* is present check twice per week.
  - If *P. parvum* is present treat with ammonium sulfate to achieve 0.4 mg/L UIA-N.

Summer-Fall Fingerling Rearing (channel catfish and koi carp)

- Begin to fill ponds with lake water 7 days before stocking.
- Check ponds for *P. parvum* 2 days before stocking
  - If *P. parvum* is absent continue to fill ponds according to culture guidelines.
  - If *P. parvum* is present treat to raise UIA-N to 0.4 mg/L if temperature is 15 °C or higher, or treat to raise Cu^{2+} to 0.75 mg/L if temperature is below 15 °C.
- Check ponds for toxin 1 day before stocking and select ponds with no toxin for stocking with fish.
- After stocking fish monitor pond temperature and pH daily and *P. parvum* once per week.
  - If pond temperatures are consistently above 28°C.
    - No treatment should be necessary but monitor *P. parvum* twice per week.
  - If pond temperatures are 15-28°C.
    - Monitor UIA-N and treat to raise UIA-N to 0.4 mg/L if *P. parvum* present.
  - If pond temperatures are below 15°C.
    - Monitor Cu^{2+} and toxin, and treat with Cutrine-Plus to raise Cu^{2+} to 0.75 mg/L if toxicity is present.

Winter Holding Ponds

- Monitor ponds for *P. parvum* once per week or twice per week if *P. parvum* present.
  - If water temperatures are up to15°C treat to raise Cu^{2+} to 0.75 mg/L if *P. parvum* is present.

Raceway or Trough Culture (rainbow trout and channel catfish)

- 8 days before fish stocking fill with lake water and check for *P. parvum* cells.
  - If *P. parvum* is absent stock fish and operate raceway/trough as flow-through.
  - If *P. parvum* is present perform bioassay to test toxicity.
    - If lake water is not toxic stock fish and operate raceway/trough as flow-through.
    - If lake water is toxic do not use raceway/trough (Go to Trout Pond Production).

Trout Pond Production

Use ponds for trout production or holding, instead of indoor raceway or troughs, when lake water is toxic.

- 8 days before stocking fill ponds with lake water.
  - Treat with Cutrine-Plus to raise Cu^{2+} level to 1.0 mg/L if temperatures are less that 15°C.
  - Treat with ammonium sulfate to raise UIA-N to 1.0 mg/L if temperatures are 15°C and higher.

- 3 days before stocking check for *P. parvum* cells
    - If *P. parvum* is present treat as above.
- 1 day before stocking check for *P. parvum*.
    - If *P. parvum* is present test for toxicity.
- Stocking day
    - Stock only ponds with no toxicity.
- After stocking
    - Check for *P. parvum* twice per week and if present treat as described above.
- If lake conditions improve harvest fish (e.g., trout) and move to indoor raceway.

Fish Harvest

- At harvest check incoming lake water for *P. parvum*
    - If *P. parvum* is absent harvest fish using lake water
    - If *P. parvum* is present perform bioassay: if negative harvest fish using lake water; if positive use well water.
    - Fish leaving the hatchery must be rinsed in well water before loading into hauling unit.
    - Fish to be transferred between hatchery culture units need not be rinsed with well water.

Fish Transportation

- Fill hauling unit with well water and check all compartments for *P. parvum* after loading fish (Note: all fish leaving the hatchery must be rinsed in well water before loading).
    - If *P. parvum* is absent deliver fish according to hatchery guidelines.
    - If *P. parvum* is present drain out some water, refill and re-check for *P. parvum*. Repeat until no *P. parvum* is found.
    - Upon return to the hatchery, disinfect hauling unit with 10% chlorine bleach.
- Use *P. parvum*-free lake water or well water to transfer fish between culture units on the hatchery.

Monitoring Sites

- Monitor *P. parvum* in lake water at the dam, hatchery intake water, and ponds and indoor culture units in use.

REFERENCES

Anderson, R. O. 1993. Apparent problems and potential solutions for production of fingerling striped bass. Journal of Applied Aquaculture 2(3/4):101-118.

Anderson, D. P., and F. L. Mayer. 1993. Salmonid pharmacology and toxicology. Pages 432-439 *in* M. K. Stoskopf, editor, Fish Medicine. W. B. Saunders Company, Philadelphia, Pennsylvania.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Federation. 1995. Standard methods for the examination of water and wastewater, 19th edition. APHA, Washington, D.C.

Aure, J., and F. Rey. 1992. Oceanographic conditions in the Sandfjord system, western Norway, after a bloom of the toxic prymnesiophyte *Prymnesium parvum* Carter in August 1990. Sarsia 76:247-254.

Barkoh, A. 1996. Effects of three fertilization treatments on water quality, zooplankton, and striped bass fingerling production in plastic-lined ponds. Progressive Fish-Culturist 58:237-247.

Barkoh, A., D. G. Smith, and J. W. Schlechte. 2003. An effective minimum concentration of un-ionized ammonia nitrogen for controlling *Prymnesium parvum*. North American Journal of Aquaculture 65:220-225.

Bergerhouse, D. L. 1993. Lethal effects of elevated pH and ammonia on early life stages of hybrid striped bass. Journal of Applied Aquaculture 2(3/4):81-100.

Bold, H. C., and M. J. Wynne. 1983. Introduction to the algae, 2nd edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Børsheim, K.Y., Harboe, T., Johnson, T., Norland, S., and K. Nygaard. 1989. Flow cytometric characterization and enumeration of *Chrysochromulina polylepis* during a bloom along the Norwegian coast. Marine Ecology Press Series 54:307-309.

Boyd, C. E. 1979. Aluminum sulfate (Alum) for precipitating clay turbidity from fish ponds. Transactions of the American Fisheries Society 108:307-313.

Boyd, C. E. 1990. Water quality in ponds for aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama.

Boyd, C. E., and H. V. Daniels. 1993. Liming and fertilization of brackish water shrimp ponds. Journal of Applied Aquaculture 2:221-234.

Burleson, F. G., Chambers, T. M., and D. L. Wiedbrauk. 1992. Virology: A laboratory manual. Academic Press, Inc. San Diego, California.

Button, K. S., H. P. Hostetter, and D. M. Mair. 1977. Copper dispersal in a water supply reservoir. Water Resources 11:539-544.

Chang, F. H., and K. G. Ryan. 1985. *Prymnesium calathiferum* sp. Nov. (Prymnesiophyceae), a new species isolated from Northland, New Zealand. Phycologia 24:191-198.

Cowles, T. J., R. A. Desiderio, and S. Neuer. 1993. *In situ* characterization of phytoplankton from vertical profiles of fluorescence emission spectra. Marine Biology 115:217-222.

Cromar, N. J., and H. J. Fallowfield. 1997. Effect of nutrient loading and retention time on performance of high rate algal ponds. Journal of Applied Phycology 9:301-309.

Culver, D. A., S. P. Maden, and J. Qin. 1993. Percid pond production techniques: timing enrichment and stocking density manipulation. Journal of Applied Aquaculture 2(3/4):9-31.

Culverhouse, P. F. 1995. Automatic methods for the detection of harmful algae. Pages 695-706 *in* P. Lassus, E. Erard, P. Gentein, and C. Marcaillou, editors. Harmful Marine Algal Blooms. Lavoisier, Paris, France.

Dafni, Z, S. Ulitzur and M. Shilo. 1972. Influence of light and phosphate on toxin production and growth of *Prymnesium parvum*. Journal of General Microbiology. 70:199-207.

Droop, M. R. 1954. A note on the isolation of small marine algae and flagellates for pure cultures. Journal of the Marine Biology Association of the U. K. 33:511-514.

Edvardsen, B., W. Eikrem, J. C. Green, R. A. Andersen, S. Y. Moon-van der Staay, and L. K. Medlin. 2000. Phylogenetic reconstructions of the Haptophyta inferred from 18S ribosomal DNA sequences and available morphological data. Phycologia 39:19-35.

Emerson, K., R. C. Russo. R. E. Lung, and R. V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effects of pH and temperature. Journal of the Fisheries Research Board of Canada 32:2379-2383.

Fitzgerald, G. P. 1964. Laboratory evaluation of potassium permanganate as an algaecide for water reservoirs. Southwest Water Works Journal 45:16-17.

Gaikowski, M. P., J .J. Rach, and R. T. Ramsay. 1999. Acute toxicity of hydrogen peroxide treatments to selected life stages of cold-, cool-, and warmwater fish. Aquaculture 178:191-207.

Green, J. C., D. J. Hibberd, and R. N. Pienaar. 1982. The taxonomy of *Prymnesium* (Prymnesiophyceae) including a description of a new cosmopolitan species, *P.*

patellifera sp. Nov., and further observations on *P. parvum* N. Carter. British Phycological Journal 17:363-382.

Green, J. C., and B. S. C. Leadbeater, editors. 1994. The haptophyte algae. Systematics Association Special Volume No. 51. Clarendon, Oxford, England.

Groeger, A. W., P. F. Brown, T. E. Tietjen, and T. C. Kelsey. 1997. Water quality of the San Marcos River. Texas Journal of Science 49:279-294.

Guo, M., P. J. Harrison, and F. J. R. Taylor. 1996. Fish kills related to *Prymnesium parvum* N. Carter (Haptophyta) in the People's Republic of China. Journal of Applied Phycology 8:111-117.

Hansen, J. A., P. G. Welsh, J. Lipton, and D. Cacela. 2002a. Effects of copper exposure on growth and survival of juvenile bull trout. Transactions of the American Fisheries Society 131:690-697.

Hansen, J. A., P. G. Welsh, and J. Lipton. 2002b. Relative sensitivity of bull trout *Salvelinus confluentus* and rainbow trout *Oncorhynchus mykiss* to acute copper toxicity. Environmental Toxicology and Chemistry 21:633-639.

Hansen, J. A., J. Lipton, P. G. Welsh, J. Morris, D. Cacela, and M. J. Suedkamp. 2002c. Relationship between exposure duration, tissue residues, growth, and mortality in rainbow trout (*Oncorhynchus mykiss*) juveniles sub-chronically exposed to copper. Aquatic Toxicology 58:175-188.

Hipkins, P. L. 2002. Weed Control in Ponds and Lakes (Aquatic Weeds). Horticultural & Forest Crops. Virginia Department of Agriculture and Consumer Services Publication.

Hoepffner, N., and S. Sathyendranath. 1991. Effect of pigment composition on absorption properties of phytoplankton. Marine Ecology Progress Series 73:11-23.

Holdway, P. A., R. A. Watson, and B. Moss. 1978. Aspects of the ecology of *Prymnesium parvum* (Haptophyta) and water chemistry in the Norfolk Broads, England. Freshwater Biology 8:295-311.

Igarashi, T., M. Satake, and T. Yasumoto. 1996. Prymnesin-2: a potent ichthyotoxic and hemolytic glycoside isolated from red tide alga *Prymnesium parvum*. Journal of the American Chemical Society 118:479-480.

Igarashi, T., M. Satake, and T. Yasumoto. 1999. Structures and partial sterochemical assignments for prymnesin-1 and prymnesin-2: potent hemolytic and ichthytoxic glycosides isolated from the red tide alga *Prymnesium parvum*. Journal of the American Chemical Society 121:8499-8511.

Irwin, R. J. 1997. Environmental Contaminants Encyclopedia, 1-7. Copper Entry. National Park Service, Water Resources Divisions, Water Operations Branch. Fort Collins, Colorado.

James, T. L., and A. De La Cruz. 1989. *Prymnesium parvum* Carter (Chrysophyceae) as a suspect of mass mortalities of fish and shellfish communities in western Texas. The Texas Journal of Science 41:429-430.

Jeffery, G. H., J. Bassett, J. Mendham, and R. C. Denney. 1989. Titrimetric analysis. Pages 372-373 *in* Vogel's Textbook of Qualitative Chemical Analysis, 5th edition, Longman Scientific and Technical, Essex, England.

Johansson., N. 2000. Ecological implications of the production of toxic substances by fish killing phytoplankton species grown under variable N:P ratios. Ph. D. Dissertation, University of Lund, Lund, Sweden.

Kaartvedt, S., T. M. Johnsen, D. L. Aksnes, U. Lie, and H. Svedsen. 1991. Occurrence of the toxic phytoflagellate *Prymnesium parvum* and associated fish mortality in a Norwegian Fjord system. Canadian Journal of Fisheries and Aquatic Science 48: 2316-2323.

Kemp, H. T., R. G. Fuller, and R. S. Davidson. 1966. Potassium permanganate as an algicide. Journal of the American Water Works Association 58:255-263.

Kim, Y. S., and G. M. Padilla. 1977. Hemolytically active components from *P. parvum* and *G. breve* toxins. Life Sciences 21:1287-1292.

Kolber, Z., and P. G. Falkowski. 1993. Use of active fluorescence to estimate phytoplankton photosynthesis *in situ*. Limnology and Oceanography 38(8):1646-1665.

Kozakai, H., Y. Oshima, and T. Yasumoto. 1982. Isolation and structural elucidation of hemolysin from the phytoflagellate *Prymnesium parvum*. Agricultural and Biological Chemistry 46:233-236.

Larsen, A., and S. Bryant. 1998. Growth rate and toxicity of *Prymnesium parvum* and *Prymnesium patelliferum* (Haptophyta) in response to changes in salinity, light, and temperature. Sarsia 83:409-418.

Lawrence, J. M. 1956. Preliminary results on the use of potassium permanganate to counteract the effects of rotenone on fish. Progressive Fish-Culturist 18:15-21.

Linam, G., J. Ralph, and J.Glass. 1991. Toxic blooms, an unusual algae threatens aquatic resources. Chihuahuan Desert Discovery. No. 28(winter):6-7.

Lindholm, T., P. Öhman, K. Kurki-Helasmo, B. Kincaid, and J. Meriluoto. 1999. Toxic algae and fish mortality in a brackish-water lake in Åland, SW Finland. Hydrobiologia 397:109-120.

Lumsden, J. S., V. E. Ostland, and H. W. Ferguson. 1998. Use of hydrogen peroxide to treat experimentally induced bacterial gill disease in rainbow trout. Journal of Aquatic Animal Health 10:230-240.

Marking, L. L., and T. D. Bills. 1975. Toxicity of potassium permanganate to fish and its effectiveness for detoxifying antimycin. Transactions of the American Fisheries Society 104:579-583.

Meldahl, A. S., B. Edvardsen, and F. Fonnum. 1994. Toxicity of four potentially ichthyotoxic marine phytoflagellates determined by four different test methods. Journal of Toxicity and Environmental Health 42:289-301.

Meldahl, A. S., J. Kvernstuen, G. J. Grasbakken, B. Edvardsen, and F. Fonnum. 1995. Toxic activity of *Prymnesium* spp. and *Chrysochromulina* spp. tested by different test methods. Pages 315-320 *in* P. Lassus, E. Erard, P. Gentein, and C. Marcaillou, editors. Harmful Marine Algal Blooms. Lavoisier, Paris, France.

McIntosh, A. W. 1975. Fate of copper in ponds. Pesticide Monitoring Journal 8:225-231.

McLaughlin, J. L. A. 1958. Euryhaline chrysomonads; nutrition and toxigonesis of *Prymnesium parvum* with notes on *Isochrysis galbana* and *Monochrysis luteri*. Journal of Protozoology 5(1):75-81.

Neveux, J., and M. Panouse. 1987. Spectrofluorometric determination of chlorophylls and phaeophytins. Archives of Hydrobiology: Advances in Limnology 4:567-581

Nicholls, K. H. 2003. Haptophyte Algae. Pages 511-521 *in* J. D. Wehr and R. G. Smith, editors. Freshwater Algae of North America. Academic Press, Boston, Massachusetts.

Oldham, P., E. Zillioux, and I. Warner. 1985. Spectral "finger printing" of phytoplankton population by two dimensional fluorescence and Fourier-transform-based pattern recognition. Journal of Marine Research 43: 893-906.

Otterstrom, C. V., and E. Steelmann-Nielson. 1940. Two cases of extensive mortality in fishes caused by flagellate *Prymnesium parvum* Carter. Reports of the Danish Biological Station 44:4-24.

Padan, E., Ginzburg, D., and M. Shilo. 1967. Growth and colony formation of the phytoflaggelate *Prymnesium parvum* Carter on solid media. Journal of Protozool.ogy 14(3):477-480.

Padilla, G. M., and D. F. Martin. 1973. Interactions of prymnesin with erythrocyte membranes. Pages 265-295 *in* D. F. Martin and G. M. Padilla, editors. Marine Pharmacognosy: action of marine biotoxins at the cellular level. Academic Press, New York.

Paster, Z. 1973. Pharmacology and mode of action of *Prymnesium*. Pages 241-263 *in* G. M. Padilla and D. F. Martin, editors. Marine pharmacognosy: action of marine biotoxins at the cellular level. Academic Press, New York.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. U. S. Fish and Wildlife Service, Washington, D.C.

Rach, J. J., T. M. Schreier, G. E. Howe, and S. D. Redman. 1997. Effect of species, life stage, and water temperature on the toxicity of hydrogen peroxide to fish. Progressive Fish-Culturist 59:41-46.

Sarig, S. 1971. Toxin-producing algae: *Prymnesium parvum* Carter. Pages 17-43 *in* S.F. Snieszko and H.R. Axelrod, editors. Disease of fishes – Book 3: The prevention and treatment of disease of warmwater fishes under subtropical conditions, with special emphasis on intensive fish farming. T. F. H. Publications, Inc. Ltd., Neptune, New Jersey.

Shilo, M. 1967. Formation and mode of action of algal toxins. Bacteriological Reviews 31: 180-193.

Shilo, M. 1971. Toxins of Chrsophyceae. Pages 67-103 *in* S. Kadis, A. Ciegler, and S. J. Ajl, editors. Microbial toxins, volume 7. Academic Press, New York.

Shilo, M. 1972. Toxigenic algae. Pages 233-265 *in* O. J. D. Hockenhill II, editor. Progress in industrial microbiology, volume 2. Churchhill Livingstone Press, Edinburgh, England.

Shilo, M. 1981. The toxic principles of *Prymnesium parvum*. Pages 37-47 *in* W. W. Carmichael, editor. The water environment: algal toxins and health, Volume 20. Plenum Press, New York.

Shilo, M., and M. Aschner. 1953. Factors governing the toxicity of cultures containing the phytoflagellate *Prymnesium parvum* Carter. Journal of General Microbiology 8:333-343.

Shilo, M., and M. Shilo. 1953. Conditions which determine the efficiency of ammonium sulfate in the control of *Prymnesium parvum* in fish breeding ponds. Applied Microbiology 1:330-333.

Shilo, M. and M. Shilo. 1962. The mechanism of lysis of *Prymnesium parvum* by weak electrolytes. Journal of General Microbiology 29:645-658.

Skovgaard, A., C. Legrand, P.J. Hansen, and E. Graneli. 2003. Effects of nutrient limitation on food uptake in the toxic haptophyte *Prymnesium parvum*. Aquatic Microbial Ecology 31:259-265.

Sommer, U. 1985. Comparison between steady state and non-steady state competition experiments with natural phytoplankton. Limnology and Oceanography 30:346-353.

Straus, D. L., and C. S. Tucker. 1993. Acute toxicity of copper sulfate and chelated copper to channel catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society 24:390-395.

Szekely, C., and M. C. Bereczky. 1992. An unusual case of disease in pet fish stocks caused by *Coleps* sp. Diseases of Aquatic Organisms 13(2):143-145.

Thurston, R. V., and seven authors. 1984. Chronic toxicity of ammonia to rainbow trout. Transactions of the American Fisheries Society 113:56-73.

Tucker, C. S. 1989. Method for estimating potassium permanganate disease treatment rates for channel catfish in ponds. Progressive Fish-Culturist 51:24-26.

Tucker, C. S., and C. E. Boyd. 1977. Relationship between potassium permanganate treatment and water quality. Transactions of the American Fisheries Society 106:481-488.

Ulitzer, S. 1973. The amphiphatic nature of *Prymnesium parvum* hemolysin. Biochemica et Biophysica Acta 298:673-679.

Ulitzer, S., and M. Shilo. 1964. A sensitive assay system for determination of the ichthyotoxicity of *Prymnesium parvum*. General Microbiology 36:161-169.

Ulitzer, S., and M. Shilo. 1966. Mode of action of *Prymnesium parvum* ichthyotoxin. Journal of Protozoology 13:332-336.

Ulitzer, S., and M. Shilo. 1970a. Effect of *Prymnesium parvum* toxin, cetyltrimethylammonium bromide and sodium dodecyl sulphate on bacteria. Journal of General Microbiology 62:363-370.

Ulitzer, S., and M. Shilo. 1970b. Procedure for purification and separation of *Prymnesium parvum* toxins. Biochemica et Biophysica Acta 201:350-363.

USACE (US Army Corps of Engineers). 1985. Monoecious *Hydrilla* in the Potomac River. Miscellaneous paper A-85-5. Department of the Army, US Corps of Engineers, Washington, D.C.

USEPA. 1997. *In vitro* determination of chlorophylls a, b, c1 + c2 and pheopigments in marine and freshwater algae by visible spectrophotometry. National Exposure Research Laboratory, EPA/446.0/R 2/September 1997.

Warren, H. J. 2001. Striped bass production plan 2001. Management Data Series No. 185. Texas Parks and Wildlife Department. Austin, Texas.

Welch, E. B. 1980. Ecological effects of wastewater. Cambridge University Press, New York.

Wetzel, R.G. 1983. Limnology. CBS College publishing, Philadelphia, Pennsylvania.

Wilhelm, C., and L. Manns. 1991. Changes in pigmentation of phytoplankton species during growth and stationary phase-consequences for reliability of pigment-based methods of biomass determination. Journal of Applied Phycology 3:305-310.

Wilhelm, C., and I. Wiedemann. 1991. Evidence of protein-bound chlorophyll c_3 in a light-harvesting protein isolated form the flagellate alga *Prymnesium parvum* (*Prymnesiophyceae*)*. Photosynthetica 25(2):249-255.

Wooster, G. A., and P. R. Bowser. 1994. Unusual infection of rainbow trout Oncorhynchus mykiss by the protozoan *Coleps* sp. (Ciliophora: Prostomatea) in a closed recirculation aquaculture system. Journal of the World Aquaculture Society 25(4):566-570.

Yariv, J., and S. Hestrin. 1961. Toxicity of the extracellular phase of *Prymnesium parvum* cultures. Journal of General Microbiology 24:165-175.

Yentsch, C. M, and 13 coauthors. 1983. Flow cytometry and cell sorting: a technique for analysis and sorting of aquatic particles. Limnology and Oceanography 28(6):1275-1280.

Yentsch, C. S., and C. M. Yentsch. 1979. Fluorescence spectral signatures: the characterization of phytoplankton populations by the use of excitation and emission spectra. Journal of Marine Science 37:471-483.

APPENDIX A

Identification and Enumeration of *Prymnesium parvum* cells, Version AEW-IDE 1.1

GREGORY M. SOUTHARD

Purpose and Scope:
This protocol outlines the accepted procedure for identification and enumeration of *P. parvum* in water samples.

Materials required:
Standard hemacytometer
Cover slip
Light microscope with scanning and high dry objectives
Pasteur pipette

Reagents/consumables:
Immersion oil
Lugol's solution
- 200 mL DI water
- 20 mL glacial acetic acid
- 20 g KI
- 10 g I

Procedure:

1. Carefully load the hemacytometer chamber using a Pasteur pipette at the loading groove, allowing capillary action to pull the water sample under the cover slip until chamber is full.
2. Using the scanning objective, observe for small, fast moving organisms. This is a tentative indication that *P. parvum* is present.
3. Switch to the 40X objective and look for *P. parvum* at 400X magnification. *P. parvum* will be identified by its small size (9-12 μm), two large chloroplasts (which may appear C-shape or saddle-shape), two long flagella, and one haptonema. All of the structures can be seen using 400X magnification, except the haptonema that can be visualized using the oil immersion lens (i.e., 1000X magnification). However, the oil immersion lens cannot be used with the hemacytometer.
4. Count the number of *P. parvum* cells in each of the five large squares of the hemacytometer grid, in each of the chambers (a total of 10 counts). The average number of cells per chamber (5 large squares) is the total cell count. For statistical accuracy, the total cell counts for each chamber should be within 10% of each other. If there is greater than 50 cells per large square, dilute the water sample to minimize time.
5. Total cells per mL is determined as follows:

$$\text{Total cells/mL} = (\text{total cell count}/5) \times (1/\text{dilution}) \times 10^4$$

Counting cell graphic (from Burleson, F. G., T. M. Chambers, and D. L. Wiedbrauk. 1992). Virology – a laboratory manual. Academic Press, Inc. New York. 250 pp.

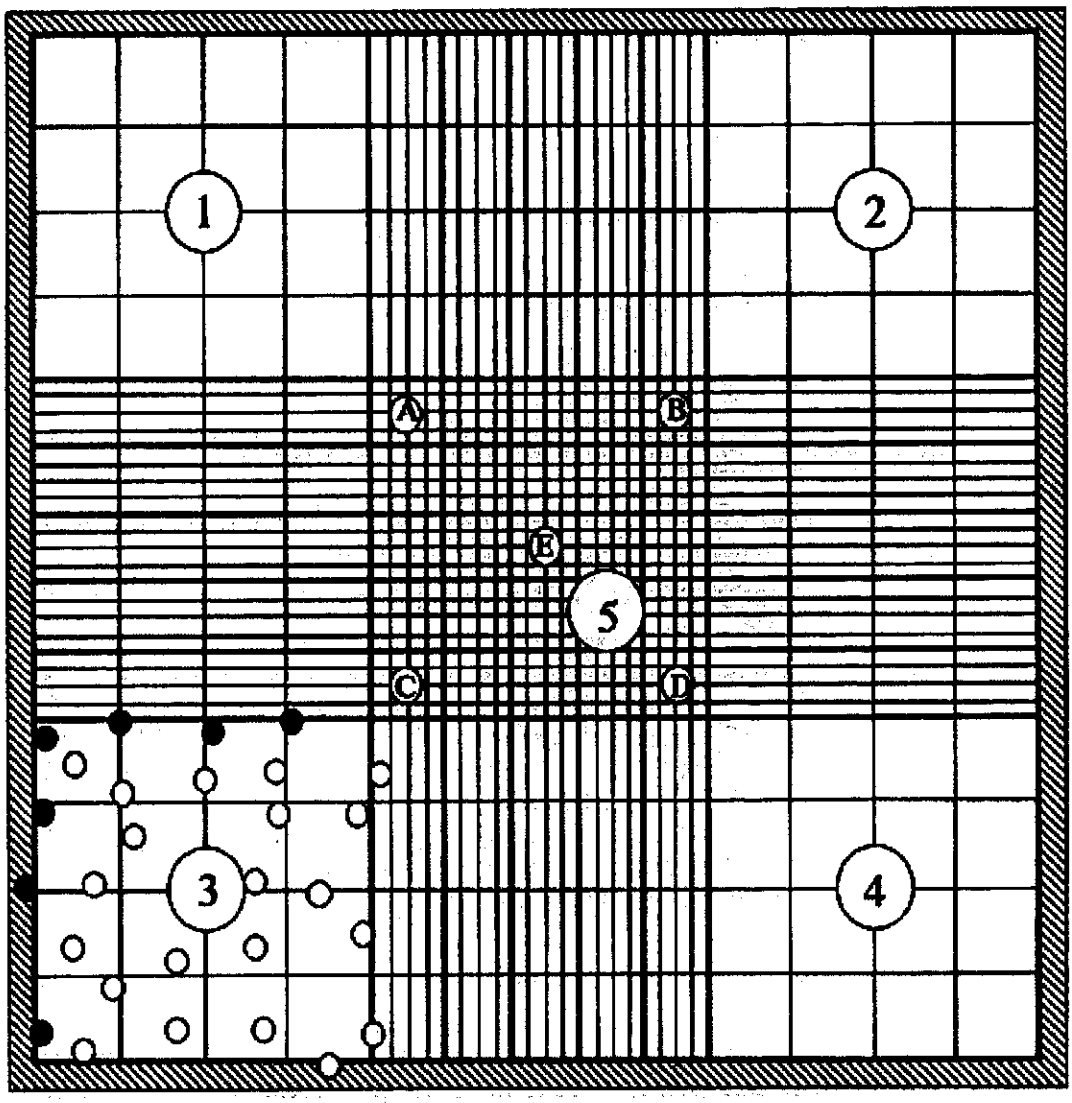

Figure 5.2 Enlarged view of the hemacytometer counting chamber, as it would appear through the low power of the microscope. The cells in the five large squares (1-5) are counted and this number is divided by 5 to obtain the average number of cells per large square. This value is multiplied by 10^4 and by the reciprocal of the dilution factor to obtain the number of cells/ml of the original cell suspension. Square #3 has a sample cell count. The open circles would be counted, whereas the dark circles indicate cells that would not be counted since they touch the top and left lines of that square.

Red blood cell counts are performed using the high dry objective of the microscope and counting the cells in 5 of the small squares (A,B,C,D, and E) of square #5. The cells/ml in the original suspension is calculated by multiplying the total number of cells in the 5 small squares by 5×10^4, and the reciprocal of the dilution factor.

APPENDIX B

Standard Bioassay of *Prymnesium parvum* Toxin, Version AEW-ITU 1.2*[1]

GREGORY M. SOUTHARD

Purpose and Scope: This test should be used when *Prymnesium parvum* is detected in hatchery water. The purpose is to evaluate the concentration of prymnesin toxin in ichthyotoxic units (ITU*) in hatchery water to determine the need for algaecide treatment.

*ITU (ichthyotoxic unit) = $1/25^{th}$ the lethal dose of prymnesin ichthyotoxin/mL for fish.

Equipment required:
Glass/metal aquarium (ca. 10 L)
with aquarium heater (a water bath may be substituted if available)
Thermometer
5 beakers (100 mL-1L)
pH meter
Graduated cylinder
Pipettes (mL and µL)

Reagents/consumables:
Distilled water
Pond water (test water)
Cofactor solution (see below)
12 test fish

Cofactor Solution Preparation:

1. Cofactor: 0.003 M DADPA /0.02 M tris buffer in distilled water, pH = 9.0.
   To 491.15 mL of distilled water in a dark storage container, add 9.85 mL of DADPA (= 0.15 M) and 60.5 g tris (= 1.0 M)
2. Store in the dark at 4°C.

(Note: 1 mL cofactor solution per 50 mL water is needed for each of four test beakers. DADPA = 3,3'-iminobispropylamine, Sigma cat # I 7006, supplied as 1.772-M solution.)

Procedure:

1) Fill the aquarium or water bath with enough water to incubate but not float the beakers.
2) Maintain temperature at 28°C ± 1.
3) Five beakers are used for each test. Size beakers according to size of test fish. (Note: Fish lengths and beaker sizes are guidelines and may be adjusted as needed.)
4) Add 1 mL of prepared cofactor per 50-mL total volume in each of the 4 beakers. 'Clean' water used for dilutions and controls is from *P. parvum*-free source (See Table 1 below).
5) Add 4 test fish to each beaker.
6) Observe for mortality and maintain constant temperature in aquarium.
7) Results are noted after 2 hours.

[1] Adapted from the Central Fish Health Lab, Nir David, Israel.

Interpretation:

1) Record mortality in each beaker (see Table 1) after 2 hours.

a) Mortality in beaker #2 (undiluted pond water + cofactor) indicates the presence of at least 1 ITU/mL, which is $1/25^{th}$ the lethal dose to fish in ponds. Toxicity is considered to be low. Treatment is not required, but continued monitoring is advised.

b) Mortality in beakers #2 and #3 (1/5 dilution + cofactor) indicates 5 ITU/mL, one-fifth the dangerous level in the ponds, which indicates the need for immediate treatment of the ponds. Toxicity is considered to be moderate.

c) Mortality in beakers # 2, 3 and #1 (undiluted pond water) indicates a high level of toxicity (25 ITU/mL or greater). The pond is toxic and fish should be displaying signs of prymnesin toxicity.

d) Partial mortality can occur in a beaker, which would indicate a level of ichthyotoxicity between those parameters confined by the dilutions and cofactor used in the bioassay. For example, there can be complete mortality in beaker #2, but only partial (e.g. 1 of 3 test fish) in beaker #3, which would indicate an ITU level between 1 and 5 ITU/mL. The same phenomenon can occur at higher toxin levels, with complete mortality occurring in beakers #2 and #3, or with partial mortality in beaker #1, indicating an ITU level between 5 and 25 ITU/mL. Partial mortality cannot occur in two of the beakers and if does, some other lethal mechanism is likely the cause.

e) Mortality in either control beaker suggests some other lethal mechanism. If fish die in one of the control beakers, the test should be repeated.

TABLE 1.—Suggested beaker size and experimental conditions to test for the presence of ichthyotoxin (from *Prymnesium parvum*) in hatchery water. If more than one pond is tested at a time, beakers 1 and 2 must be repeated for each pond, but only one set of controls (beakers #4 and 5) is required.

Fish length (mm)	Beaker size (mL)	Pond Water (mL)	Dilution water (mL)*	Cofactor (mL)	Dilution
≤ 50	100	50	0	0	1:1
		49	0	1	1:1
		10	39	1	1:5
		control	50	0	
		control + cofactor	49	1	
50 < length ≤ 75	400	200	0	0	1:1
		196	0	4	1:1
		40	156	4	1:5
		control	400	0	
		control + cofactor	396	4	
> 75	800	600	0	0	1:1
		588	0	12	1:1
		120	468	12	1:5
		control	600	0	
		control + cofactor	588	12	

* Dilution water must be free of *Prymnesium parvum* cells and toxin (e.g., conditioned tap water).

APPENDIX C

Recommended treatments for *Prymnesium parvum* blooms using liquid ammonia, ammonium sulfate, or copper sulfate as related to temperature and pH (after the Central Fish Health Lab (Israel) recommendations).

Temperature (°C)	pH	Liquid ammonia (mg/L)	Ammonium sulfate (mg/L)	Copper sulfate (mg/L)
> 20	> 9.0		10-12	
	8.6-9.0	10-12	15	
	< 8.6	12-13	15-17	
18-20	> 9.0	10-12	15	2
	8.6-9.0	12-13	20	2
	< 8.6	13	25	
12-18	> 9.0	13	25	2-3
	8.6-9.0	13		2-3
	< 8.6			2-3

Note: The toxicity of copper sulfate increases as water alkalinity decreases and a more appropriate treatment concentration may be determined as follows:

Treatment rate (mg/L) = alkalinity as $CaCO_3$ ÷ 100.

APPENDIX D

Copper – Bathocuproine Method, Version AEW-COP 1.1

STEVEN HAMBY

Purpose and Scope: This protocol describes methods used for measuring copper at concentrations of 0.02 to 0.5 mg/L. Color and turbidity of the sample can affect the accuracy of the method.

Materials Required:

Equipment

Class A 100-mL or 50-mL glassware (volumetric or graduated cylinder)
125-mL flask
Spectrophotometer, 1-cm path length
Hot plate

Reagents/consumables:

Deionized (D.I.) water that has been distilled in a glass still
1 +1 Hydrochloric acid (Conc. HCl diluted 1:1 v:v with water)
Hydroxylamine hydrochloride solution.
Sodium citrate solution
Disodium bathocuproine disulfonate solution
Stock copper solution (20 mg/L) for Cu standards:
- 10 mg/L intermediate
- 0.05 mg/L
- 0.1 mg/L
- 0.5 mg/L
- 0.2 mg/L

Procedure:

A. Prior to use, wash all glassware with concentrated HCl then rinse with copper-free water. Note: Many domestic waters have copper in detectable concentrations due to leaching from copper pipes.
B. Turn spectrophotometer on, set absorbance to 484 nm, and allow the unit to warm up.
C. Pour 50 mL of the blank (reagent water), standard, or sample into a separate 125-mL flask.
D. To each flask add, with mixing after each addition, the following: 1 mL (1 + 1) HCl, 5 mL $NH_2OH \cdot HCL$ solution, 5 mL sodium citrate solution, 5 mL disodium bathocuproine disulfonate solution.
E. After all flasks have been mixed, pour an aliquot into a cuvet, place it in the spectrophotometer, and record the absorbance. The following order is suggested: blank, 0.05 mg/L, 0.1 mg/L, 1 mg/L, samples, and the 0.5-mg/L continuing calibration verification standard.

Calculations:

Use the absorbencies from each standard to plot readings on a calibration curve to determine the amount of copper present.

Chemical recipes:

A. Hydroxylamine hydrochloride solution: 50g $NH_2OH \cdot HCL$ in 450 mL water.
B. Sodium citrate solution: dissolve 300 g $Na_3C_6H_5O_7 \cdot 2H_2O$ and make up to 1 L with water.
C. Disodium bathocuproine disulfonate solution: dissolve 1 g $C_{12}H_4N_2(CH_3)_2(C_6H_4)_2(SO_3Na)_2$ in water and make up 1 L.
D. Stock copper solution (20 mg/L): into a 250 mL flask, add 10 mL reagent water, 20 mg copper wire, and 5 mL conc. HNO_3. As reaction slows, warm to completely dissolve the copper, then

boil to expel nitrogen oxides. Cool and add 50 mL reagent water, carefully transfer to a 1-L volumetric flask, and fill to 1 L.

E. Cu standards:

10 mg/L intermediate (50 mL of 20 mg/L to 100 mL) or (25 mL of 20 mg/L to 50 mL)
0.5 mg/L (5 mL of 10 mg/L to 100 mL)
0.1 mg/L (1 mL of 10 mg/L to 100 mL)
0.05 mg/L (5 mL of 1 mg/L to 100 mL)
0.2-mg/L continuing calibration verification standard (1 mL of 20 mg/L to 100 mL)

Quality Control:

A. A laboratory duplicate sample should be tested along with the original sample. An analytical precision control chart should be kept to monitor these results.

B. Percent recovery of the spike standard can be calculate as follows:

$$\% \text{ Recovery} = \frac{(Cs - Csx) \times 100}{Ca}$$

Where Cs = result of spiked sample
Csx = average of the original and duplicate sample
Ca = value of the spike added

Reference:

Standard Methods for the Examination of Water and Wastewater 3500-Cu E, 18th edition.

NOTE: This method measures the free copper. To measure the chelated form perform the preliminary digestion described in Standard Methods 3500-Cu Neocuproine Method 4b then adjust pH to 4-5.