Do Herbicides Impair Phytoplankton Primary Production in the Sacramento-San Joaquin River Delta?

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ABSTRACT

The effect of herbicide concentration on the maximum rate of phytoplankton primary production (P_{max}) was examined for 53 water samples collected at 9 sites in the Sacramento-San Joaquin River Delta between May and November, 1997. Samples were analyzed for P_{max} and the concentrations of diuron, atrazine, cyanazine, simazine, thiobencarb, and hexazinone. The herbicide concentrations ranged between 0 µg/L and 2.1 µg/L, with 50% of the values (n=318) between 0 and 0.018 µg/L. Herbicide concentrations in 52 of the water samples were well below the lowest observable effect concentrations (LOECs) that have been reported in laboratory experiments to inhibit primary production. P_{max} ranged between 2 and 11 milligrams of carbon per milligrams of chlorophyll a per hour (mg C (mg chl<u>a</u>-h))$^{-1}$ for the 52 samples where the herbicide concentrations were less than any reported LOEC. However, for the one sample where the diuron concentration (2.1 µg/L) exceeded the reported LOEC of 2.0 µg/L, P_{max} was the lowest observed during the study, 0.9 mg C (mg chl<u>a</u>-h)$^{-1}$. Herbicide concentrations we observed throughout the system do not appear to limit production; however, localized occurrences of elevated herbicide concentrations exist and may affect primary production.

INTRODUCTION

The Sacramento-San Joaquin Delta is at the confluence of the Sacramento and San Joaquin rivers. In the early 1800's, this conjunction comprised approximately 700,000 acres of Delta freshwater marshes (Atwater and others, 1979). However by 1920, 95% of the marshes were leveed for farming. Today, the Delta is a maze of levees that create small sloughs, vast lakes, and meandering rivers surrounding over twenty islands dominated by agricultural use. Over 500,000 acres of Delta farms grow alfalfa, corn, grapes, safflower, sorghum, sugarbeets, tomatoes, winter wheat, and orchards.

Each year, 500,000 pounds of over 30 different herbicides are applied on the agricultural lands within the Delta (California Department of Pesticide Regulation, 1996). Unwanted plant species that decrease crop production and crowd rights-of-way are treated with herbicides that have specific mechanisms designed to inhibit plant growth and primary production. These compounds have been detected within nontarget areas such as Delta waterways, yet the effect of these compounds on natural aquatic plant and phytoplankton communities in unknown.

Phytoplankton are the base of the food web thus providing much of the food resource to grazers in lower trophic levels. If primary production is significantly depressed by herbicide exposure, the food resource to higher consumers such as zooplankton and fish may be reduced.

In this study, we focused on a particular class of herbicides that inhibit photosynthesis. This class includes the triazines (atrazine, simazine, cyanazine, and hexazinone), the substituted urea-based compound diuron, and the thiocarbamate, thiobencarb. Laboratory experiments with algal cultures have demonstrated that these compounds can be potent inhibitors of phytoplankton photosynthesis (Day, 1993; Tubbing and others, 1993; Brown and Lean, 1995; Schneider and others, 1995; Peterson and others, 1997).

We designed this study to explore the hypothesis that phytoplankton primary production in an agriculturally impacted freshwater ecosystem is inhibited by herbicides. Our objective was to answer three specific questions relevant to the Sacramento-San Joaquin River Delta: (1) What is the range of concentrations of the herbicides which inhibit photosynthesis? (2) Are there times/locations when these compounds are present at concentrations known to inhibit algal photosynthesis in the laboratory? (3) Are there negative correlations between measured rates of phytoplankton photosynthesis and measured concentrations of these herbicides in the Delta ecosystem? To answer these questions we used two different approaches. First, we measured herbicide concentrations from a field study and compared them to the lowest observable effect concentrations (LOEC) for primary production found in the literature. Second, we performed a bioassay of photosynthetic performance on natural phytoplankton communities while simultaneously measuring herbicide concentrations at the same location. Data were then analyzed to determine if there were significant (negative) correlations between herbicide concentrations and photosynthetic performance of the natural phytoplankton populations.

We used a modified primary productivity method (Lewis and Smith, 1983) to measure phytoplankton photosynthetic performance. We used this method as a bioassay to directly determine effects of herbicides on photosynthesis. The method uses radiocarbon ($NaH^{14}CO_3$) as a tracer for measuring the incorporation rate of CO_2 into algal cellular carbon. The rate of photosynthesis varies with light intensity, so the procedure includes measurement of $H^{14}CO_3$ assimilation rate across a range of light intensity. Photosynthesis-irradiance (P/I) functions are hyperbolic, and are described with empirical functions such as the one described by Platt and others (1980):

$$P^B = P_{max} [1 - \exp(-\alpha I / P_{max})] \qquad (1)$$

Here, P^B is the rate of carbon fixation normalized to chlorophyll a concentration; P_{max} is the maximum rate of carbon fixation at saturating irradiance levels; α is the slope of the P/I function at low irradiance, and I is the instantaneous irradiance (photon flux density, PFD, of photosynthetically active light (PAR)). Herbicides that inhibit photosynthesis directly affect the light reaction of photosynthesis by blocking the electron transport chain in photosystem II (Arsalane and others, 1993). The energy generated by the light reaction is lost as fluorescence and therefore not transferred to the dark reaction (Calvin cycle) for carbon uptake. We focused on the parameter P_{max}, which measures the efficiency and performance of both the light and dark reactions, including the photosystems and Calvin cycle.

Results of this study were not consistent with the hypothesis that system wide phytoplankton primary production is impaired by herbicides within the Delta. However, our results do indicate that there may be localized events when production is reduced.

METHODS

Experimental design

Sampling was designed to maximize the information content from each sample. Sampling occurred at nine sites to determine spatial variability and one site to determine temporal variability across the network of sloughs and primary channels throughout the Delta. Spatial sampling was designed to characterize the potential effects of herbicide compounds and concentrations in areas of varying water retention times and phytoplankton production. Stations D1 (Vernalis), and D2 (French Camp Slough) and D9 (Sutter Slough) represent inputs from the San Joaquin and French Camp slough, and Sacramento watersheds, respectively (for a map of the stations, please see Kuivila and others, 1999). Water flow through the central Delta was sampled at Stations D4 (Old River) and D5 (Middle River), representing a mixture of both external and internal inputs. Stagnant flow with a variety of high concentrations of pesticides was sampled at D3 (Paradise Cut), D6 (Werner), and D7 (Beaver Slough). Station D8 (Mokelumne River) was chosen as a control site because few herbicides in low concentrations have been detected previously at this site. The nine sites were sampled at five

times through the sampling period. High herbicide concentrations were expected during the first three sampling dates May 27-29, June10-12, and June 24-26. In contrast, low herbicide concentrations were expected during the later sampling dates, October 14-16 and November 11-13. In addition, Middle River (D5) in the central Delta was sampled bi-monthly to better characterize temporal variability of water quality, herbicide concentrations, and phytoplankton primary production in May through November.

Analytical methods

A 10 L water sample was collected from 1 meter depth at each site using a 2.5-L Teflon-lined Niskin bottle. The sample was collected in a stainless steel milk can and then split equally into eight 1-L bottles. Three kinds of measurements were made; chemical indicators of water quality and origin, herbicide concentrations, and the bioassay of phytoplankton photosynthetic performance.

Water quality

The water quality parameters included specific conductivity, temperature, suspended particulate matter (SPM), chlorophyll a, nutrients, dissolved inorganic carbon, and phytoplankton community composition. Ambient water temperature (°C) was measured using a glass mercury thermometer during the water collection at the sampling site. Suspended particulate matter was determined gravimetrically, as described by Hager (1993). Using 47-mm polycarbonate membrane filters 100-500 mL of sample was filtered onto preweighed 0.4-µm, and allowed to air dry for 48 to 72 hours before being re-weighed. Triplicate chlorophyll a samples of 500 mL were filtered through a Gelman A/E glass fiber filter and immediately frozen on dry ice. Filters were transferred to a freezer at the lab and allowed to dry for 72 hours. The dried filters were ground in 90% acetone and replaced in the freezer for 24 hours for chlorophyll extraction. Centrifuged samples were then analyzed on a Hewlett Packard 8452A diode array spectrophotometer. Chlorophyll a concentrations were calculated using Lorenzen's equations (1967). Nutrient samples were collected in an acetone-rinsed high-density polyethylene bottle, filtered through a 0.4-µm nuclepore filter, with the filtrate collected into a 30-mL high-density polyethylene bottle, and frozen until analyzed. Concentrations of ammonium (NH_4), nitrate plus nitrite (N+N), nitrite (NO_2), dissolved reactive phosphate (DRP), and dissolved silica were measured simultaneously on a Technicon AutoAnalyzer II system. Total dissolved inorganic carbon was measured by injecting 4 mL of sample into a sealed 20-mL serum bottle, acidified with 0.1-mL 6N HCl, and analyzed on a Perkin Elmer Sigma 2000 gas chromatograph. Samples of 100 mL were preserved with Lugol's solution and analyzed for phytoplankton species composition, density and cell volume.

Herbicide concentrations

Herbicide concentrations were determined by gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC) at the organic chemistry laboratory at the U.S. Geological Survey California District Office (see Kuivila and others,1999).

Bioassay

The water sample was split within 30 minutes after collection and phytoplankton production was measured immediately. Fifty milliliters of sample were spiked with ~5.0 µCi/mL $NaH^{14}CO_3$ and thoroughly stirred. Seventeen scintillation vials each were filled with 2-mL aliquots and incubated in the photosynthetron at ambient water temperature for 30 minutes over a light range of 0-1600 µEinst/m^2/s (Lewis and Smith, 1983). Three 2-mL aliquots were immediately acidified with 0.3 ml 0.2N HCl to account for any initial carbon uptake before incubation (time zero - T_0). Three 2-mL aliquots were fixed with 6N NaOH to determine total ^{14}C initially available. At the end of the incubation, the 17 samples were immediately acidified with 0.2N HCl under a ventilation hood and shaken for 1 hour to drive off the ^{14}C still in solution. Ten milliliters of OptiPhase 'HiSafe' scintillation cocktail were then added to each sample and analyzed in a 1209 Rackbeta scintillation counter. Irradiance measurements were made using a Biospherical QSL-100 4π light probe placed in a modified

scintillation vial inside the aluminum light tubes (Lewis and Smith, 1983).

Production and irradiance data were fit to the hyperbolic function (equation 1) to determine estimates of P_{max} . Negative correlation between P_{max} and herbicide concentration was determined using the software package Systat ($p = 0.05$)

RESULTS

Comparisons to published LOEC's

In 317 out of 318 samples, herbicide concentrations fell below reported LOECs. Atrazine, simazine, and cyanazine concentrations ranged from undetectable to very low throughout the sampling period (table 1). Atrazine concentrations ranged between 0 and 0.03 µg/L, well below the lowest observable effect concentration (LOEC), 26 µg/L (Caux and others, 1996). Simazine and cyanazine concentrations ranged between 0 – 0.07 µg/L and 0 – 0.13 µg/L, respectively, and also were well below the reported LOECs, 50 µg/L and 145 µg/L, respectively. All three herbicides were used in low quantities and were generally applied on rights-of-way throughout the Delta (Kuivila and others, this volume).

Table 1. Maximum herbicide concentrations measured in the Sacramento-San Joaquin Delta during May-November 1997, compared to lowest observable effect concentrations (LOEC) reported in the literature.

Herbicide	Maximum concentration measured in the Delta (µg/L)	LOEC (µg/L)	Reference
atrazine	0.03	26	Caux and others, 1996
cyanazine	0.13	145	Caux and others, 1996
diuron	2.14	2	Edmunds, U.S. Geological Survey, unpublished data, 1998
hexazinone	0.67	22.5	Schneider and others, 1995
simazine	0.07	50	Bryfogle and McDiffett, 1979
thiobencarb	0.31	17	Sabater and Carrasco, 1996

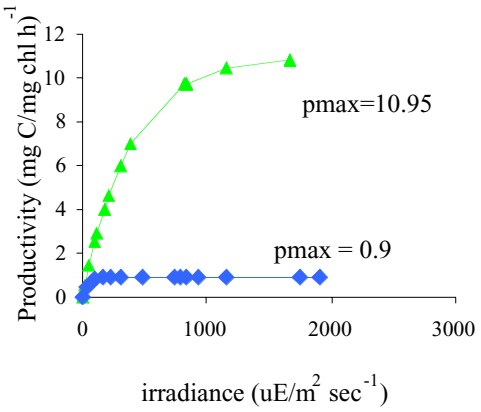
The highest thiobencarb concentration was detected (0.31 µg/L) on May 29 following the application to rice fields in the Sacramento River watershed. This concentration also was well below the LOEC for thiobencarb, 17 µg/L (Sabater and Carrasco, 1996).

The highest concentrations of herbicides detected were hexazinone (0.67 µg/L) and diuron (2.14 µg/L). Hexazinone and diuron are both applied to alfalfa, while diuron is also applied to rights-of-way and asparagus. The maximum hexazinone concentration was lower than the LOEC of 22.5 µg/L, however, the maximum diuron concentration slightly exceeded the diuron LOEC of 2.0 µg/L.

Bioassay

P_{max} estimates for the 53 water samples ranged between 0.9 and 11 mg C (mg chl a-h)$^{-1}$ (Figure 1).

Figure 1. Highest and lowest P_{max} estimates depicted by productivity versus irradiance curves.



There was no negative correlation between herbicide concentrations and P_{max} for any of the six herbicides (Table 2). However, the lowest P_{max} was measured when the diuron concentration exceeded the reported LOEC, 2.0 µg/L (Peres and others, 1996).

Table 2. Correlation coefficients between measured P_{max} (bioassay of photosynthetic performance of natural phytoplankton communities) and concentrations of six herbicides that inhibit photosynthesis. In all cases, sample size n = 53

Herbicide	r
atrazine	0.19
cyanazine	0.25
diuron	0.32
hexazinone	0.31
simazine	0.08
thiobencarb	0.06

DISCUSSION

In our first approach, we measured concentrations, of atrazine, diuron, cyanazine, simazine, thiobencarb, and hexazinone, that typically were orders of magnitude lower what has been determined in laboratory experiments to inhibit algal photosynthesis. Only once was the herbicide concentration above the reported LOEC, indicating possible inhibition during localized or transient events.

High herbicide concentrations were expected during the spring application period, while low concentrations were expected during late fall. However, the highest herbicide concentration was measured after a small rain event in mid-November. Based on these initial results, we concluded that herbicide concentrations found within the Delta should not impair total ecosystem primary production, but it may be impaired on a localized episodic basis.

Our second approach consisted of a bioassay to determine P_{max} rates with simultaneous measurements of herbicide concentrations at the nine sites. This approach reaffirmed the initial conclusion that production was not significantly affected by herbicide concentrations in the Delta. A negative correlation between P_{max} and herbicide concentrations was not detected. The range of P_{max} measured varied by a factor of ten, but this variability is apparently due to factors other than herbicide concentration, such as available inorganic carbon. However, the one sample with a measured herbicide concentration that exceeded the LOEC, also had the lowest P_{max}. This illustrates that the bioassay technique we used was effective at demonstrating the inhibitory effects of herbicides when a LOEC was exceeded.

The temporal and spatial variability of herbicides and the generally low concentrations of herbicides seen in the Delta waterways may be due to a number of factors. Although more than 400,000 pounds of herbicides are applied annually to crops grown in the Delta, there are more than 30 different herbicides applied to over a dozen crops. Consequently, the concentration of individual herbicides is generally lower in the Delta even though the total use of herbicides is high. This situation differs from the dynamics of other agricultural regions found within the United States. For example, in the Midwest, one or two crops (usually corn and sorghum) dominate large agricultural regions. In the Delaware River Basin, located in southeastern Nebraska and northeastern Kansas, it is estimated that 240,000 pounds of atrazine alone are applied to corn and sorghum yearly (Stamer and others, 1994). Atrazine concentrations are found regularly within the Delaware River at levels exceeding the LOEC indicating that system-wide production in the Delaware River may be impaired.

The scarcity of observed effects also may be due to the peat rich soil common in the Delta. The Delta farmlands are valued in part because of their high organic carbon content. However, herbicide compounds sorb more readily to soil composed of high organic matter (Celis and others, 1998; Nkedi-Kizza and Brown, 1998). Herbicides also degrade due to light and bacterial metabolism (Schneider and others, 1995). The occurrence of a sample with a high concentration of diuron in the fall indicates that herbicides may be applied throughout the year and flushed from the fields during rain events. Our study design may have missed potentially high herbicide concentrations found during the rainy season.

Episodic high concentrations and persistent low-level concentrations of herbicides found in the Delta do not appear to impair the photosynthetic rate of the resident phytoplankton, but may alter the species composition. Other studies have shown large differences among phytoplankton species in their sensitivity to herbicide toxicity (for example, Day and Hodge, 1996). If persistent low levels of a wide variety of herbicides in the Delta alter the phytoplankton community composition, then the quantity and

quality of food available to higher trophic levels may be impaired even though ambient herbicide concentrations do not exceed LOECs.

CONCLUSIONS

We did not observe system wide reduction in phytoplankton primary production due to herbicide concentrations measured throughout the Sacramento-San Joaquin River Delta. However, this data is unique to this system. Herbicides have been detected in other systems at concentrations that exceed reported LOECs, and may impair production at the system level. Our results have introduced a useful assay to detect herbicide effects on natural phytoplankton populations and may be applied to these other systems where system-wide production may be reduced.

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