Proc. World Maricul. Soc. 10:392-402 (1979)

Macrobrachium rosenbergii CULTURE IN POLYNESIA: pH CONTROL IN EXPERIMENTAL POND WATERS BY PHYTOPLANKTON LIMITATION WITH AN ALGICIDE

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ABSTRACT

Macrobrachium rosenbergii ponds at the Centre Océanologique du Pacifique are subject to increases in pH exceeding 10.5 during growing experiments. The high levels of pH are responsible for mortality of prawns, especially when molting. The photosynthetic activity of dense phytoplankton blooms are primarily responsible for high pH. The algicide Clarosan (CIBA-Geigy) was used to thin phytoplankton blooms. Experiments showed that treatments with 0.02 mg/liter result in a rapid fall in the pH. At this dose Clarosan is not deleterious to the prawns.

INTRODUCTION

During growing experiments of *Macrobrachium rosenbergii*, conducted at the COP, the monitoring of water quality showed important increases in pH exceeding 10.5. Weakened animals were observed during molting and subsequent mortality was observed. When the pH rises, the fraction of toxic un-ionized ammonia (NH_3) which is cellular permeable (Milne et al., 1958) increases. The animals are most sensitive during molting because of water absorption.

The photosynthetic activity of unicellular algae is primarily responsible for the pH variations. An increase in water exchange can depress the phytoplankton bloom but this is not always possible. A second

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solution is a reduction of phytoplankton activity through the use of algicides.

The objective of this study was to describe the relationship between photosynthetic activity and pH and evaluate the algicide Clarosan for controlling phytoplankton blooms in ponds.

MATERIALS AND METHODS

PHOTOSYNTHESIS AND pH

To determine the daily variation in pH regulated by photosynthetic activity, it is necessary to know the CO_2 fixation rate by the phytoplankton. This rate is evaluated from the difference in the dissolved oxygen (DO) concentration measured in the afternoon (1700 hours) and in the morning (0800 hours) in a growing pond. The equation for the carbon metabolism by the phytoplankton is, after Stumm and Morgan (1970):

$$106\ CO_2 + 16\ NO_3^- + HOP_4^{2-} + 122\ H_2O + 18\ H^+ + \text{oligoelements} + \text{energy}$$

Photosynthesis $\downarrow\uparrow$ Respiration

$$C_{106}\ H_{263}\ O_{110}\ N_6\ P_1 + 138\ O_2 \qquad (1)$$

Thus, the evolution of one mg of dissolved oxygen corresponds to a fixation of 2.4×10^{-5} moles of CO_2. Likewise, the assimilation of CO_2 modifies the carbonate-bicarbonate equilibrium as follows:

$$H_2O + CO_2 \rightleftharpoons H_2\ CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

The $[H^+]$ concentration can be joined to the concentration in total carbon (CT).

By definition Alkalinity $= [HCO_3^-] + 2\ [CO_3^{2-}] + [OH^-] - [H^+]$ (2)

$$C_T = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \qquad (3)$$

where $[H_2CO_3] = C_T\alpha_0$

$[HCO_3^-] = C_T\alpha_1$

$[CO_3^{2-}] = C_T\alpha_2$

Therefore, Alkalinity $= C_T(\alpha_1 + 2\alpha_2) + [OH^-] - [H^+]$

and $$C_T = \frac{Alk - [OH^-] + [H^+]}{\alpha_1 + 2\alpha_2} \qquad (4)$$

where $$\alpha_1 = \left(\frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right)^{-1}$$

$$\alpha_2 = \left(\frac{[H^{+2}]}{K_1K_2} + \frac{[H^+]}{K_2} + 1\right)^{-1}$$

When replacing α_1 and α_2 in Eq (4) some terms can be ignored according to the level of pH.

For pH < 9

$$[H^+] = \frac{C_3 + K_1\ CT + \sqrt{(C_3 - K_1CT)^2 - 4\ Alk(C_2 - 2\ K_2K_1CT)}}{2\ Alk} \quad (5)$$

For pH ⩾ 9

$$[H^+] = -\ C_2 + 2\ K_2K_1CT + \sqrt{(C_2 - 2\ K_2K_1CT)^2 + 4\ C_4(Alk\ K_1 - CT\ K_1)} \quad (6)$$

where $C_2 = K_1K_2\ Alk - 10^{-14}\ K_1$

$C_3 = Alk\ K_1 - 10^{-14}$

$C_4 = 10^{-14}\ K_1K_2$

$pK_1 = 6.3$

$pK_2 = 10.2$

In our conditions Alk = 1.14×10^{-3} equivalents/liter. To model the pH system, it is assumed that alkalinity remains constant during daylight hours and there is no exchange of CO_2 with atmosphere and no deposition of $CaCO_3$ during time period considered. To compute the theoretical pH at sunset, the C_T corresponding to the initial pH measured in the morning is evaluated with Eq (4). The quantity of CO_2 fixed by the phytoplankton is then subtracted from the initial CT (the rate of CO_2 fixation is estimated from the DO produced during the day [see Eq (1)]). The final pH can then be computed from the new CT and Eq (5) or (6).

The mole fraction of un-ionized ammonia (NH_3) is calculated using the formulae described by Colt and Tchobanoglous (1976).

ALGICIDE STUDIES

Algicide trials were conducted with the compound terbutryn which is commercialized under the name Clarosan (CIBA-Geigy). Preliminary tests on the algicide action were conducted on Chlophyceae (*Chlorella* sp.), Prasinophyceae (*Tetraselmis tetrathele*), Haptophyceae (*Monochrysis lutheri*) and Bacillariophyceae (*Skeletonema costatum*). Two different methods were utilized:

(1) Polarographic measurement of oxygen production in a 350 ml flask lighted (4,000 lux) and containing suspension of *Tetraselmis* (1.5×10^6 cells/ml) submitted to varying doses of Clarosan (0.02 to 0.10 mg/liter).

(2) Counting phytoplankton cells in monospecific cultures (300 ml) incubated at a constant temperature (25°C) and light intensity (2,500 lux). Each flask contained 50 ml of inoculum (*Tetraselmis, Monochrysis* or *Skeletonema*), 200 ml of seawater enriched with Conway formula (Walne, 1974) (plus 1 mg/liter Na_2SiO_3 for *Skeletonema*) and 0.10 mg/liter of Clarosan. Cells were counted daily and controls were used with each species tested.

Further tests were conducted in four 100-liter cylindrical tanks aerated and submitted to solar light. Each tank contained pond water rich in *Chlorella*, diatoms and dinoflagellates. The four tanks were treated with 0.00, 0.02, 0.10 and 0.50 mg/liter Clarosan and phytoplankton counts made after two days.

Toxicity tests on *Macrobrachium* (70 mm total length) were conducted in 100-liter tanks with sand bottoms. Each tank contained 15 prawns and the tests were run for 10 days. Test water was exchanged daily and a new dose of Clarosan added. Water temperature ranged from 24 to 26°C during the trials and DO was maintained at saturation by bubbling. Two concentrations of terbutryn were tested in double: 2 and 20 mg/liter.

POND TREATMENTS

Further algicide tests were conducted in 0.07 ha earthen ponds (one meter deep). The daily water exchange rate was 8-15% and the temperature ranged from 26 to 31°C during the study. Phytoplankton density was measured by Secchi disk visibility. DO and pH were measured (pH meter Knick Portamess 902 ± 0.05 pH and DO meter YSI 51A ± 0.1 mg/liter). Recording of pH and DO were conducted using a system SEMA 2 Safare.

RESULTS

Figure 1 depicts the relationship between the theoretical pH computed with Eq (5) and (6) and the pH measured at 1700 hours in the pond. The relation was highly significant ($P < 0.01$). Figure 2 shows the variations in pH at 1700 hours as a function of the initial pH (0800 hours) and different rates of CO_2 fixation. The increase of pH regulated by CO_2 fixation in photosynthesis is important. For an initial pH (0800 hours) of 8.00, a CO_2 fixation rate of 2.67×10^{-6} mole/liter/hour (corresponding to a ΔDO 1700 hours-0800 hours of 1.0 mg/liter) induces a pH of 8.40 at 1700 hours. Under identical conditions a CO_2 fixation rate corresponding to a ΔDO of 7 mg/liter results in a pH increase of 9.47 (1700 hours). The action of photosynthesis is the most sensitive between pH 7.50 and 8.50.

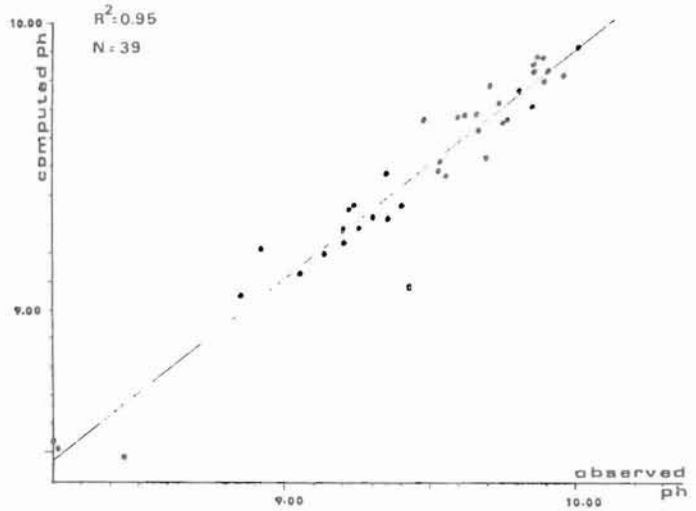

Figure 1. Relation between measured pH (1700 hours) and pH computed from Eq (5) or (6). pH computed = 0.81 pH observed + 1.82.

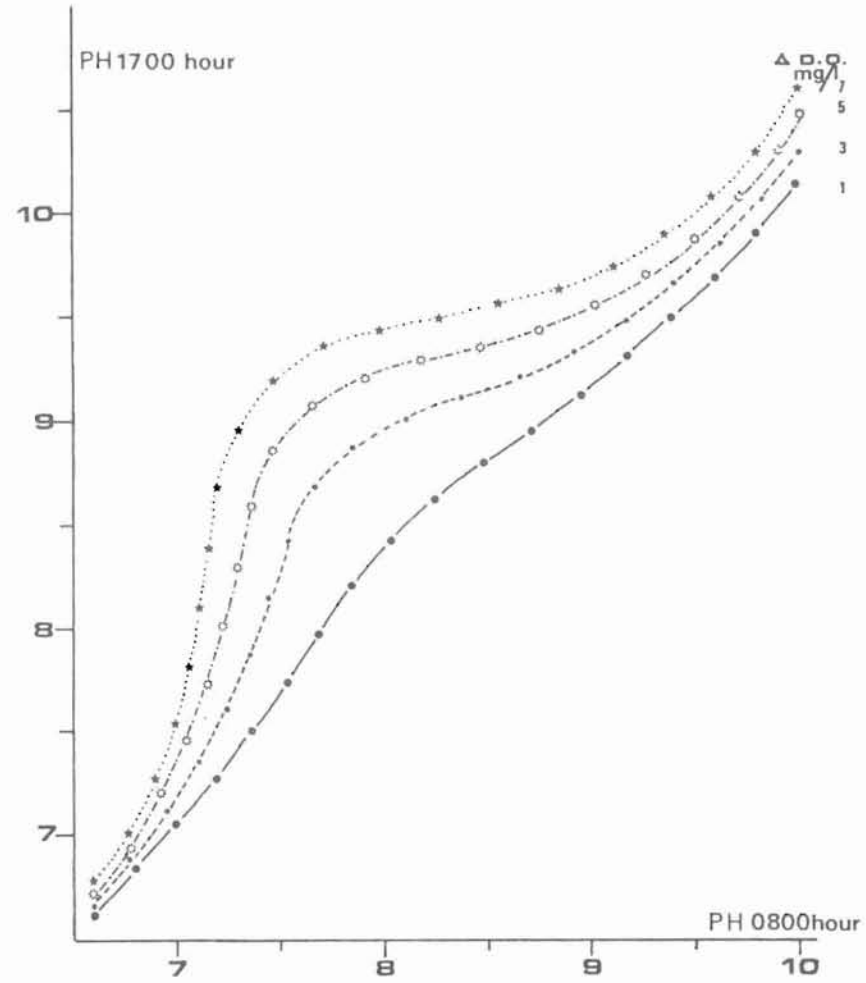

Figure 2. Effects of early morning pH (0800 hours) and dissolved oxygen change (ΔDO) on the theoretical afternoon pH (1700 hours) in *Macrobrachium* pond.

Figure 3 depicts the variation in oxygen production by a *Tetraselmis* culture submitted to varying doses of Clarosan. At a dose of 0.10 mg/liter, photosynthetic activity stopped 7 min after the introduction of the algicide. Subsequently the DO concentration decreased, indicating cellular respiration. At 0.04 mg/liter the photosynthetic inhibition appeared after 15 min. At 0.02 mg/liter the photosynthesis decreased 20 min after the addition of Clarosan. Thus it appears that the 0.02 mg/liter dose is sufficient to treat the phytoplankton community in *Macrobrachium* ponds.

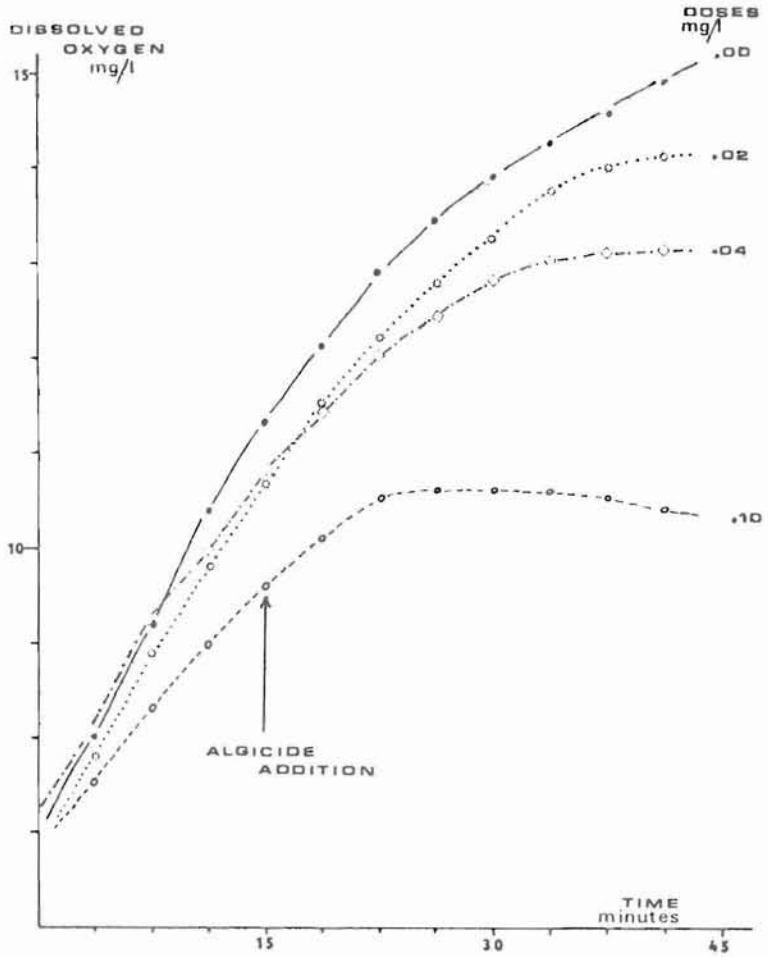

Figure 3. Action of varying doses of Clarosan on net photosynthesis of *Tetraselmis tetrathele*. Light intensity, 4,000 lux; temperature, 27°C.

Response of different algae to several treatments of Clarosan appears in Tables 1 and 2. Chlorophyceae and Prasinophyceae were the most sensitive. Diatoms were most resistant both in monospecific culture (Table 1) and in pond waters (Table 2).

Clarosan was relatively non-toxic to *Macrobrachium rosenbergii*. A concentration of 20 mg/liter resulted in a 20% mortality after 10 days. Mortality was 6.7% at a dose of 2.0 mg/liter and did not differ from the control (Table 3). Consequently the algicide should be safe for pond use at a treatment rate of 0.02 mg/liter.

TABLE 1. Effects of Clarosan (0.10 mg/liter) on Three Species of Algae: Evolution of the Number of Cells (x 10^6/ml) in Monospecific Cultures. Tests conducted in 500 ml flasks.

Days	*Tetraselmis tetrathele*		*Monochrysis lutheri*		*Skeletonema costatum*	
	Control	0.10 mg/liter	Control	0.10 mg/liter	Control	0.10 mg/liter
0	0.2	0.1	0.5	0.5	1.5	1.5
1	0.5	0.0	2.5	2.5	4.0	2.0
2	1.2	0.0	4.5	4.5	3.0	1.5

TABLE 2. Effect of Different Doses of Clarosan on Cultures of Pluri-specific Phytoplankton Obtained from *Macrobrachium* Pond. Tests conducted in 100-liter tanks.

Dose (mg/liter)	No. of cells 2 days after treatment	
	Chlorellae (10^6/ml)	Dinoflagellates and diatoms (10^3/ml)
0.00	1.0	1.0
0.02	0.1	1.5
0.10	0.0	1.5
0.50	0.0	0.5

TABLE 3. Clarosan Toxicity to *Macrobrachium* after 10 Days (temperature 24-26°C, DO = 100% saturation)

Tank	Clarosan (mg/liter)	Initial no. of animals	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Mortality after 10 days (%)
1	0	15	M[a]	M	+[b]								6.7
2	2	15		M		M+			M				6.7
3	20	15			+			+	+				20.0
4	2	15	+										6.7
5	20	15			M+		++						20.0

[a]One prawn molting. [b]Dead prawn.

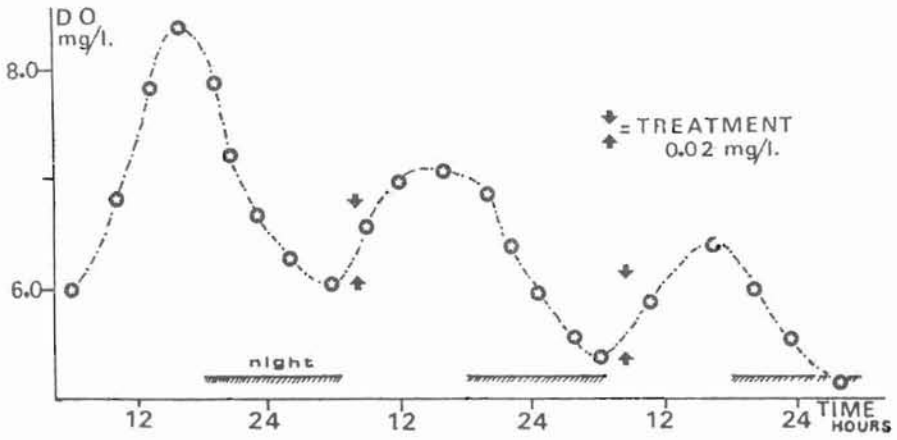

Figure 4. Effects of Clarosan on dissolved oxygen 2 days after algicide application in *Macrobrachium* ponds.

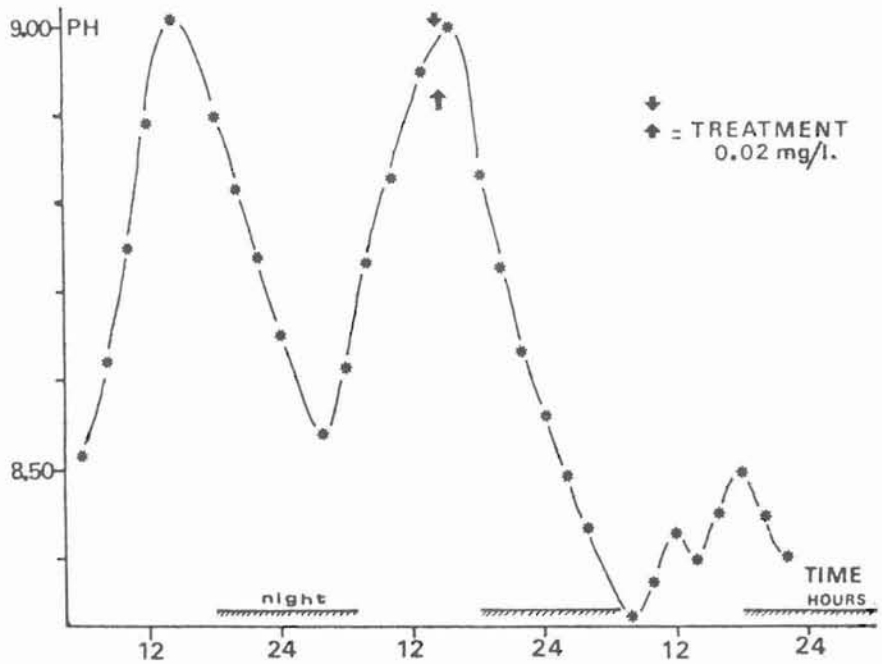

Figure 5. Effects of Clarosan on pH 2 days after algicide application in *Macrobrachium* pond.

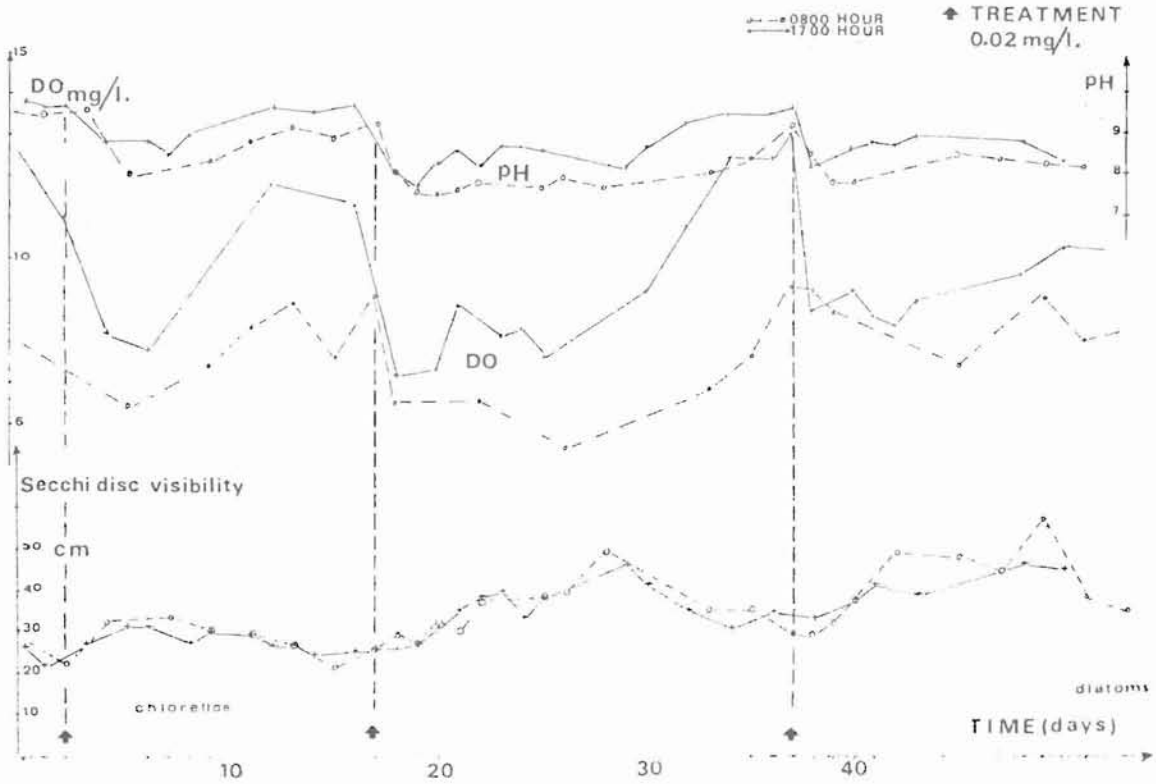

Figure 6. Changes in pH, dissolved oxygen (DO) and Secchi disk visibility over 50 days in a *Macrobrachium* pond treated with 3 applications of Clarosan (0.02 mg/liter).

Figures 4 and 5 show the immediate effects of Clarosan (0.02 mg/liter) on the pH and DO levels in a pond. A morning treatment results in a lower afternoon DO concentration (Fig. 4). If the pond is treated in the afternoon, a decrease in pH is observed the following morning with a fall from the afternoon value (8.50 instead of 9.01). Figure 6 shows the pH, DO and Secchi disk visibility in a growing pond during a 50-day period. The pond was treated three times with Clarosan. In spite of a daily water exchange of 10%, pH and DO continued to decrease 10-15 days after algicide treatment. The Secchi disk visibility increased also, with decreasing phytoplankton density. The nature of the phytoplankton bloom changed after three treatments: diatoms succeeded *Chlorella*.

DISCUSSION

The change in pH seems to be related to the density of phytoplankton as well as the species composition. In our ponds the blooms are dominated by *Chlorella* which tolerates high levels of pH and DO. This may be because of the carbonic anhydrase enzyme system (Pruder and Bolton, 1978). High pH and DO concentrations appear to favor photorespiration and inhibit photosynthesis in other algae, e.g., diatoms.

Maximum pH observed in the ponds exceeded 10.50 which results in a percentage of un-ionized ammonia greater than 96%. A NH_3-N level exceeding 0.1 mg/liter produces a growth reduction of 60% in *Macrobrachium* (Wickins, 1976). In our ponds this value is reached for a total ammonia concentration of 0.104 mg N/liter.

Mortality observed in tanks treated with 2 mg/liter of Clarosan (100 times the recommended application rate) was low. It would be important to know if Clarosan is accumulated by the prawns. The algicide also appears to be non-toxic to zooplankton and benthos. These results agree with those obtained by Sills (1964) with diuron (KARMEX).

It appears diatoms and *Monochrysis* are less sensitive to Clarosan than *Chlorella*. This might result in a dominance of diatoms in the ponds instead of *Chlorella*.

Clarosan applied at a rate of 0.02 mg/liter acts in a few hours on the phytoplankton and continues for 10 days. The decrease in activity of Clarosan is perhaps a result of algicide lost during the water exchange. After three treatments, diatoms become dominant in the pond. It is possible that the selectivity of the algicide, eliminating *Chlorella*, allows the installation of diatoms.

CONCLUSION

The utilization of the algicide Clarosan at low level (0.02 mg/liter) limits phytoplankton blooms and controls pH in *Macrobrachium* ponds. The treatment is simple and allows quick interventions on the ponds. Moreover, the dose utilized does not provoke the disparition of the totality of phytoplankton and is not deleterious to *Macrobrachium rosenbergii*.

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