

Responses of Plankton to Different Chlorine Concentrations and Nutrient Enrichment in Low Salinity Shrimp Pond Water

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Abstract

An experiment was conducted to determine the effects of chlorine at concentrations of 0, 2, 4, 6 and 10 mg·l⁻¹ on plankton which were sampled from a low salinity shrimp pond and tested in glass containers under controlled temperature (27 to 28.5°C). The residual effect of chlorine on phytoplankton with nutrient enrichment (N and P) was also evaluated.

The results show that the chlorine concentration at 2 mg·l⁻¹, corresponding to total residual chlorine (TRC) concentration of 0.25 mg·l⁻¹, adversely affected phytoplankton growth as it reduced primary productivity by more than 50% and killed more than 40% of zooplankton populations. However, this chlorine dose did not completely inactivate bacteria in the pond water. The sensitivity of phytoplankton species varied with *Selenastrum* sp., being the most insensitive, even in the treatment with dose at 10 mg·l⁻¹, corresponding to TRC concentration of 1.2 mg·l⁻¹. Among the zooplankton, the species of *Hexarthra* and *Brachionus* were sensitive to TRC concentration of 0.25 mg·l⁻¹.

Enrichment of chlorinated pond water with nitrogen and phosphorus at a ratio of 4:1 and N concentration of 2.8 mg·l⁻¹ stimulated phytoplankton growth in pond water in all tested chlorine concentrations. Although phytoplankton production was reduced by 48% at 10 mg·l⁻¹ chlorine comparing to that in the control, the number of phytoplankton species increased in all treatments after 3 days of nutrient enrichment, and *Selenastrum* sp. regained its dominance in 6 days.

Introduction

Chlorination has been adopted by shrimp farmers as a common procedure to disinfect unwanted organisms during preparation of shrimp ponds in Thailand and elsewhere in the world (Lin and Nash 1996, Boyd and Massaut 1999). Chlorine is usually applied at concentrations ranging from 3 to 30 mg·l⁻¹ during pond to eliminate *Vibrio* spp., *Monodon baculovirus* (MBV) and other virus vectors particularly wild crustaceans (Chanratchakool

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1995, Kongkeo 1995, Hedge et al. 1996, Hedge et al. 1996, Lin and Nash 1996, Boyd and Massaut 1999). In some cases, it is also applied during the culture period to control phytoplankton bloom at a concentration of $0.1 \text{ mg}\cdot\text{l}^{-1}$ (Boyd and Massaut 1999).

Chlorination of shrimp pond during pond preparation or grow-out period would not only disinfect pathogenic bacteria but also affect the plankton abundance, species composition and diversity. The chlorine efficacy and required dosage depend on organic matter concentration in suspended solids in water column and bottom of the shrimp pond (Dierberg and Kiattisimkul 1996, Hopkins et al. 1993).

Many freshwater and estuarine phytoplankton species are sensitive to chlorine, with an adverse effect to TRC concentrations as low as $0.05 \text{ mg}\cdot\text{l}^{-1}$ (Ho and Robert 1986). The acute toxicity of chlorine concentration to phytoplankton was reported in a range from 2 to $5 \text{ mg}\cdot\text{l}^{-1}$ (Junli et al. 1997), and to Cladoceran species from 0.12 to $0.28 \text{ mg}\cdot\text{l}^{-1}$ (Manning et al. 1996). Most studies on chlorine effect on plankton were related to industrial effluents and domestic wastewater. Little information is available on chlorine effect on plankton of shrimp ponds, despite the fact that chlorine is applied routinely to most shrimp ponds in Thailand, where a considerable area is involved in low salinity shrimp farming. The objectives of this study were to determine: (1) the effect of different chlorine concentrations on bacteria and plankton in low salinity shrimp pond water, and (2) the response of phytoplankton to nutrient enrichment in chlorinated pond water.

Materials and Methods

For the experiment, a pond (200 m^2 and 1 m depth) with salinity of 5‰ was stocked with 15 day post larvae (PL 15) of *Penaeus monodon* at $30 \text{ shrimps}\cdot\text{m}^{-2}$. The shrimps were cultured under a closed system without water exchange for 5 months and fed with commercial feed containing 37% crude protein at a rate of $3.5\% \text{ shrimp body weight}\cdot\text{day}^{-1}$ (Chanratchakool et al. 1994). Pond was aerated with a 2 hp paddle wheel from one corner of the pond.

The chlorine toxicity experiment consisted of two parts. The first part was to determine the acute chlorine effect to bacteria, phytoplankton, and zooplankton in 24 h; and the second part was to determine the response of phytoplankton to nutrient enrichment in chlorinated pond water. Pond water samples containing plankton were taken with a submersed pump from an experimental shrimp pond. The unfiltered pond water was used to test for chlorine effect on both phytoplankton and zooplankton, as “unfiltered treatment”. The other test was on nannoplankton, filtered through $60 \mu\text{m}$ plankton net, as “filtered treatment” since the phytoplankton community was dominated by unicellular species with size ranging from 10 to $30 \mu\text{m}$.

The experiment on the acute effect of chlorine to bacteria, phytoplankton as chlorophyll *a* and zooplankton was carried out in thirty 10 l glass cylinders, each of which contained 9 l water sample. These culture vessels were

placed on wooden racks and illuminated with light intensity at 3250 to 4035 lux using fluorescent light bulbs in a room with a constant temperature ranging from 27.0 to 28.5°C. All jars were aerated to maintain dissolved oxygen (DO) concentration above 5 mg·l⁻¹. Five active chlorine concentrations (0, 2, 4, 6, and 10 mg·l⁻¹) were applied in each type of water sample. Each chlorine concentration was made up with diluted bleach solution, sodium hypochlorite (NaOCl) containing active chlorine concentration of 24,093 mg·l⁻¹. Total bacteria count was done by taking 20 ml samples from each treatment jar at 0 and 24 h of chlorine exposure. These samples were inoculated on Petri dishes containing agar media following the Plate Count Droplet method (APHA 1992). The inoculated bacteria were then put into an incubator. Total bacteria were counted at two time intervals at 15 and 24 h of incubation at temperature 28 to 30°C. Twenty-four hours after chlorine treatment, phytoplankton biomass expressed as chlorophyll *a* was determined by acetone extraction and spectrophotometric method (APHA 1992). Response of zooplankton to different chlorine concentrations was indicated by the abundance and percent mortality at 0 and 24 h of chlorine exposure. From each treatment jar containing unfiltered water, 1 ml sample was taken to determine zooplankton populations using a Sedgewick-rafter counting chamber under 100x microscope magnification. Dead zooplankton were indicated by no heart beat or movement (Reish and Oshida 1986). The total number of zooplankton was counted after adding a small drop of buffered formalin to the chamber and recounted. The difference between those two counts was regarded as the survived zooplankton. To reduce the time effect, zooplankton in each replication was sequentially counted.

TRC concentration was measured at 0 and 24 h of chlorine exposure by taking a 300 ml water sample from each jar using a plastic hose, and chlorine concentration was analyzed by diethyl-r-phenylene-diamine (DPD) titrimetric method (APHA 1992). Water quality parameters such as pH, total alkalinity, total suspended solids (TSS), total ammonia nitrogen (TAN), filtered and total organic carbon (TOC_f and TOC respectively), filtered and total nitrogen (TN_f and TN respectively), orthophosphate and total phosphorus (TP) were analyzed at initial and 24 h of chlorine application.

The effect of chlorine on photosynthetic activity of phytoplankton was determined by primary productivity to indicate the effect of chlorine on phytoplankton activity. For primary productivity, samples taken from filtered water stock were placed in 15 sets of BOD bottles (2 light and 1 dark bottles per set) and treated in triplicates of 5 chlorine concentrations, 0, 2, 4, 6, and 10 mg·l⁻¹ respectively. These bottles were incubated at the photic depth of 15 to 20 cm in shrimp pond for 3 h. Concentration of DO in each bottle was measured before and after incubation using a DO meter (YSI Model 58). Primary productivity was calculated following standard methods (APHA 1992).

The phytoplankton response to nutrient enrichment in chlorinated water was tested 24 h after exposure to the above 5 chlorine concentrations and the residual chlorine was dechlorinated with 7 mg·l⁻¹ sodium thiosulphate (Na₂S₂O₃) for 1 mg·l⁻¹ residual chlorine. All glass jars were then enriched

with nitrogen (N) and phosphorus (P) at a ratio of 4:1 with 2.8 mg·l⁻¹N. Phytoplankton abundance and composition was measured prior to, and 3 and 6 days after nutrient enrichment by taking a 50 ml sample from each jar and preserved with Lugol solution (APHA 1992). About 10 to 15 ml sample was transferred to a 20 ml test tube and phytoplankton was allowed to settle for 24 h before removing the supernatant. The concentrated phytoplankton samples were counted using a Palmer-Malone counting chamber under a microscope at 100 and 200 magnifications (APHA 1992). Phytoplankton counts were expressed as colony ml⁻¹ for filamentous *Oscillatoria* sp. and *Spirulina* sp, and cell ml⁻¹ for other taxa. Identification of phytoplankton was done using taxonomical keys from Bellinger (1992), Whitford and Schumacher (1973), Mizuno (1970), and Scott and Prescott (1961). In nutrient enrichment experiment, samples were taken from each of the treatment jars at days 3 and 6 for analysis of primary productivity, chlorophyll *a*, phytoplankton composition, pH, orthophosphate, TP, and TN. Methods and procedures for analysis of these parameters were similar to water quality analysis for previous experiment.

Results on chlorophyll *a*, total bacteria and other water quality parameters obtained at different time intervals were analyzed by two-way analysis of variance (ANOVA), whereas primary productivity, zooplankton abundance and mortality were analyzed with one way ANOVA using a statistical software Statistica version 5.0.

Results

The initial water quality parameters were not significantly different ($P > 0.05$) between filtered and unfiltered water stocks (Table 1).

Effect of chlorine doses on plankton and bacteria in shrimp pond water

Since organic substance in intensive shrimp culture ponds was usually high, the predominant portion of applied chlorine was lost through chlorine

Table 1. Mean and standard error (SE) of initial water quality parameters in filtered and unfiltered stock water for plankton study

Parameters	Filtered Water		Unfiltered Water	
	Mean	SE	Mean	SE
pH	8.15	0.05	8.20	0.01
Total suspended solids (mg l ⁻¹)	59.17	0.83	58.00	1.53
Total ammonia nitrogen (mg l ⁻¹)	0.05	0.00	0.06	0.00
Total alkalinity (mg l ⁻¹ CaCO ₃)	142.0	0.12	141.3	0.23
Total filtered organic carbon (mg l ⁻¹)	6.28	0.00	6.23	0.03
Total organic carbon (mg l ⁻¹)	7.03	0.07	7.06	0.03
Orthophosphate (mg l ⁻¹)	0.08	0.03	0.01	0.00
Total phosphorus (mg l ⁻¹)	0.78	0.01	0.86	0.10
Chlorophyll- <i>a</i> (mg m ⁻³)	290.47	22.17	279.77	3.88
Total filtered nitrogen (mg l ⁻¹)	0.33	0.00	0.33	0.00
Total nitrogen (mg l ⁻¹)	0.44	0.02	0.44	0.00

demand in water column. The applied chlorine concentrations in all treatments were reduced by nearly 90% immediately (0 h) after chlorine application and were barely detectable after 24 h in both filtered and unfiltered water samples in all chlorine doses (Fig. 1). For example, the initial chlorine concentration decreased from 2 to 0.25 mg·l⁻¹ and 10 to 1.20 mg·l⁻¹ immediately after chlorine application. However, TRC concentration increased with initial chlorine concentration with insignificant difference in reduction between filtered and unfiltered water. The initial chlorine concentration of 2 to 10 mg·l⁻¹ (TRC concentration 0.40 to 1.20 mg·l⁻¹) inactivated bacteria progressively ($P < 0.01$), but did not completely disinfect the bacteria in both filtered and unfiltered water systems (Fig. 2). Surprisingly, the total bacteria counts in all chlorine concentrations drastically increased and the numbers were greater than that of the control and pre-chlorine treatments.

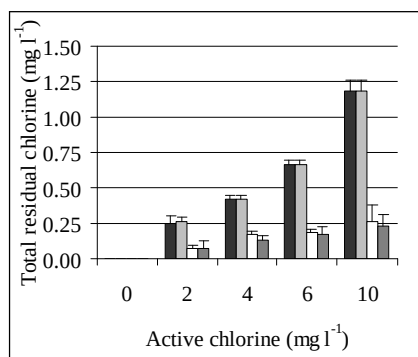


Fig. 1. Means and SE of TRC in filtered and unfiltered shrimp pond water after 0 h (black and light grey color) and 24 h (blank and dark grey color) treated with different chlorine concentrations

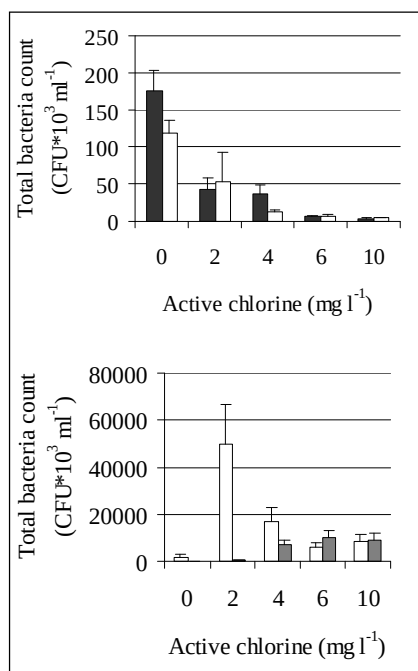


Fig. 2. Means and SE of total bacteria of filtered and unfiltered shrimp pond water after (a) 0 h (black and blank color) and (b) 24 h (blank and dark grey color) treated with different chlorine concentration. Bars represent SE

In phytoplankton experiment the chlorine application also significantly reduced net primary productivity (NPP) and chlorophyll *a* concentration ($P < 0.01$) in filtered and unfiltered shrimp pond water (Figs. 3 and 4). For example, the chlorophyll *a* concentration in high chlorine treatments was reduced from 153 to 6.23 $\text{mg}\cdot\text{m}^{-3}$ immediately after chlorine exposure and NPP at these concentrations could not be detected.

In the experimental pond, more than 75% of the phytoplankton community was dominated by *Selenastrum* sp. in both filtered and unfiltered water, except in 2 $\text{mg}\cdot\text{l}^{-1}$ chlorine treatment with corresponding TRC of 0.25 $\text{mg}\cdot\text{l}^{-1}$, where *Ankistrodesmus* sp. and *Cyclotella* sp. existed equally with *Selenastrum* sp. *Chlorella* sp. consisting 30 to 40% of initial phytoplankton community could not be detected in all treatments after 24 h exposure.

Acute chlorine toxicity to zooplankton immediately after chlorine exposure showed that chlorine concentration ranging from 4 to 10 $\text{mg}\cdot\text{l}^{-1}$ (TRC from 0.40 to 1.20 $\text{mg}\cdot\text{l}^{-1}$) killed 56 to 66% more zooplankton than that in 2 $\text{mg}\cdot\text{l}^{-1}$ (TRC 0.25 $\text{mg}\cdot\text{l}^{-1}$) (Fig. 5). Mortality of zooplankton at chlorine concentration of 6 $\text{mg}\cdot\text{l}^{-1}$ (TRC 0.70 $\text{mg}\cdot\text{l}^{-1}$) was not significantly different from that of 10 $\text{mg}\cdot\text{l}^{-1}$ ($P > 0.05$). Among five species of zooplankton recorded, three of them were rotifer dominated by *Brachionus* sp. and *Hexarthra* sp., which were sensitive to chlorine at TRC concentration as low as 0.25 $\text{mg}\cdot\text{l}^{-1}$. More than 60% of these animals were killed immediately after exposure to TRC concentration ranging from 0.40 to 0.70 $\text{mg}\cdot\text{l}^{-1}$ and 100% mortality at 1.2 $\text{mg}\cdot\text{l}^{-1}$ TRC concentration.

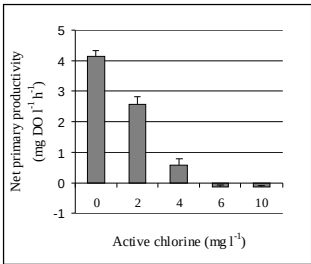


Fig. 3. NPP of filtered (black color) shrimp pond water after being treated with different chlorine concentrations. Bars represent SE.

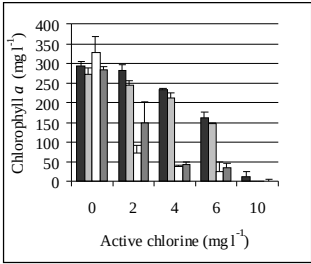


Fig. 4. Chlorophyll-*a* of filtered and unfiltered shrimp pond water after 0 h (black and light grey color) and 24 h (blank and dark grey color) treated with different chlorine concentrations. Bars represent SE.

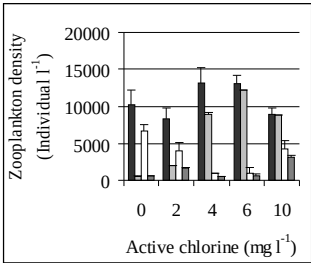


Fig. 5. Zooplankton abundance and mortality after 0 h (black and light grey color) and 24 h (blank and dark grey color) treated with different chlorine concentrations. Bars represent SE.

Water quality measurements showed that concentration of orthophosphate, TAN and filtrable total nitrogen (TNf) were significantly higher in water with chlorine treatments than those in the control ($P < 0.01$) (Table 2). The orthophosphate concentration was higher in TRC concentration of $0.40 \text{ mg}\cdot\text{l}^{-1}$ than in other chlorine treatments.

Response of phytoplankton to nutrient enrichment in chlorinated water

Enrichment of chlorinated pond water with nitrogen and phosphorus stimulated the growth of phytoplankton in control and chlorine treatments. Three days after nutrient enrichment, the NPP and chlorophyll *a* concentration in water treated with chlorine at TRC concentrations of 0.40 , 0.70 and $1.20 \text{ mg}\cdot\text{l}^{-1}$ (Figs. 6 and 7) were significantly lower ($P < 0.01$) than those in control and in TRC concentration of $0.25 \text{ mg}\cdot\text{l}^{-1}$. After 6 days of nutrient enrichment, NPP and chlorophyll *a* in the control were not different from those at TRC concentration of $1.20 \text{ mg}\cdot\text{l}^{-1}$ ($P > 0.01$). Phytoplankton growth was reduced in high TRC concentration compared to low TRC and the control (Figs. 6 and 7).

Table 2. Means and SE of water quality parameters of filtered and unfiltered water after 24 h treated with different chlorine concentrations

Water quality parameters	Active chlorine (mg l^{-1})	Filtered		Unfiltered	
		Mean	SE	Mean	SE
PH	0	8.84	0.01	8.79	0.01
	2	8.78	0.00	8.77	0.00
	4	8.82	0.00	8.80	0.02
	6	8.85	0.00	8.83	0.01
	10	8.91	0.01	8.91	0.01
Total alkalinity ($\text{mg l}^{-1} \text{ CaCO}_3$)	0	136.7	13.4	144.0	2.7
	2	142.0	1.2	141.7	1.5
	4	144.0	1.2	143.3	1.5
	6	144.0	0.6	143.0	1.5
	10	144.0	3.1	141.0	0.0
Total ammonia Nitrogen (mg l^{-1})	0	0.00	0.00	0.00	0.00
	2	0.33	0.07	0.29	0.02
	4	0.21	0.05	0.19	0.02
	6	0.11	0.02	0.34	0.07
	10	0.09	0.02	0.13	0.02
Total suspended solids (mg l^{-1})	0	43.18	1.47	53.17	3.92
	2	21.50	1.53	25.83	4.09
	4	43.50	1.53	45.83	1.45
	6	37.67	1.74	32.33	3.35
	10	33.83	2.13	33.17	0.33
Orthophosphate (mg l^{-1})	0	0.015	0.003	0.016	0.004
	2	0.144	0.016	0.162	0.007
	4	0.224	0.008	0.234	0.005
	6	0.147	0.010	0.134	0.006
	10	0.130	0.023	0.136	0.041
Total filtered Nitrogen (mg l^{-1})	0	1.34	0.10	1.38	0.12
	2	1.74	0.13	2.02	0.11
	4	1.82	0.12	1.74	0.08
	6	1.62	0.13	1.80	0.02

Enrichment of chlorinated water with nitrogen and phosphorus changed the composition of phytoplankton. Phytoplankton composition initially dominated by *Selenastrum* sp was changed to *Ankistrodesmus* sp., *Cyclotella* sp., *Scenedesmus* sp. and *Coccus* sp. three days after nutrient enrichment. The community was again dominated by *Selenastrum* sp. six days after nutrient enrichment.

Discussion

Being an active compound in aquatic environment, the disinfectant efficacy of chlorine depends on various factors, such as radiation, pH, and concentration of particulate and dissolved organics in pond water. These factors affect the concentration of residual chlorine, which asserts toxicity to organisms. A significant loss of active chlorine that occurred immediately upon dissolving in pond water could be related to the relatively high pH value (> 8.15) and presence of total suspended solids ($59.17 \text{ mg}\cdot\text{l}^{-1}$). The TRC concentrations that ranged from 0.40 to $1.20 \text{ mg}\cdot\text{l}^{-1}$ did not completely inactivate bacteria but it reduced their growth. It was noted that the efficacy of chlorine to bacteria reduced with increasing TSS concentration in the pond water. Similarly, Harakeh (1986) reported that the efficacy of chlorine to inactivate virus was reduced by 50% in the presence of $50 \text{ mg}\cdot\text{l}^{-1}$ TSS. As chlorine efficacy to bacterial growth in open pond water reduced over time, the number of bacteria increased when the TRC decreased to $0.20 \text{ mg}\cdot\text{l}^{-1}$ with undetectable FRC concentration after 24 h of chlorine exposure.

Chlorine effect on NPP of phytoplankton was spontaneous impairment of metabolic function, while reduction in chlorophyll *a* concentration was a prolonged impact on growth. Compared to control, the NPP was reduced by 50 to 100% when the pond water contained TRC concentration increased

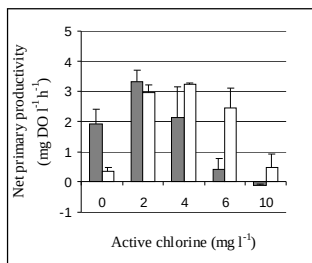


Fig. 6. NPP of chlorinated filtered shrimp pond water after 3 d (dark grey color) and 6 d days (blank color) after enriched with phosphorus and nitrogen. Bars represent SE.

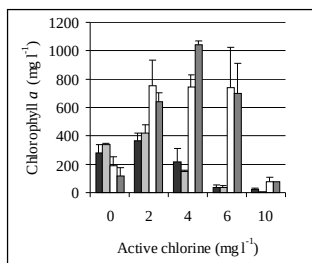


Fig. 7. Chlorophyll *a* of chlorinated filtered and unfiltered shrimp pond water after 3 d (black and light grey color) and 6 d (blank and dark grey color) enriched with phosphorus and nitrogen. Bars represent SE.

from 0.25 to 1.20 mg·l⁻¹. However, 50% reduction of chlorophyll *a* was only found at TRC of 0.7 mg·l⁻¹ treatment. These results indicate that the chlorine concentration that inhibits phytoplankton productivity inflicts lesser damage to its biomass. Ho and Robert (1986) also observed that with TRC concentration as small as 0.07 mg·l⁻¹ in chlorinated sewage on James River, the phytoplankton photosynthesis was reduced by 50%. They also found that phytoplankton primary productivity was lower in areas located 100 m from the chlorinated sewage source than in 4000 m, whereas chlorophyll *a* concentration showed an inverse result. For intensive shrimp culture, chlorine is applied for two purposes: one is to exterminate organisms in the pond water at preparation phase of shrimp culture, and the other is to control excessive plankton growth during the grow-out period. The chlorine concentration applied to meet these purposes is critical in pond management.

The chlorine effect on phytoplankton was species specific among a dozen of phytoplankton present in the pond and *Chlorella* was more sensitive to the environmental change. Most *Chlorella* was found in both control and in all chlorine treatments. No significant difference on phytoplankton composition was found before and after chlorine application in all treatments, except at TRC 0.25 mg·l⁻¹, which may explain that counting the number of phytoplankton cells was not appropriate to see the short term toxic effect (acute toxicity) of chlorine. Even though chlorine with TRC ranging from 0.40 to 1.20 mg·l⁻¹ significantly affected phytoplankton through reducing NPP to undetectable level and chlorophyll *a* to less than 50 mg·m⁻³, enrichment with N and P triggered the phytoplankton growth of these chlorinated waters. This indicates that chlorine with TRC as high as 1.20 mg·l⁻¹ temporarily inhibited the growth of phytoplankton only if the residual chlorine is neutralized and the water is enriched with nutrients afterward. The growth response of survived phytoplankton in various chlorine treatments to nutrient enrichment resumed at different rates. At low TRC concentration of 0.25 mg·l⁻¹ phytoplankton growth was significantly greater than that in higher concentrations during the initial three days of fertilization. A strong and intermediate negative correlation was found between orthophosphate with chlorophyll *a* ($r = -0.83$) and NPP ($r = -0.62$) after 3 days of enrichment. Even though phytoplankton recovered in chlorine treatment with TRC 1.20 mg·l⁻¹, growth was very slow and smaller than chlorine with TRC ranging from 0.40 to 0.70 mg·l⁻¹. As a result of differential effect the dominant genera shifted from *Ankistrodesmus*, *Cyclotella* and *Coccus* to *Selenastrum* in chlorinated water with TRC ranging from 0.25 to 0.70 mg·l⁻¹.

The effect of chlorine on zooplankton was recorded within 24 h of exposure. Zooplankton abundance in high TRC (1.20 mg·l⁻¹) was higher than that in low TRC (0.40 and 0.70 mg·l⁻¹) and it was similar with TRC 0.25 mg·l⁻¹. However, results on direct counting of living and dead zooplankton gave a better picture of the negative effect of chlorine to zooplankton. Between the two dominant rotifers, *Brachionus* was less sensitive than *Hexarthra*. *Hexarthra* and *Brachionus* consisted of 80% of zooplankton community immediately after chlorine application. However, after 24 h almost 50% of the zooplankton was dominated by *Brachionus*. Junli et al. (1986) found that at

concentration of $5 \text{ mg}\cdot\text{l}^{-1}$, chlorine slowed down the swimming activity of *Philodina*, a member of Rotifer, and killed them after 20 min exposure to $6 \text{ mg}\cdot\text{l}^{-1}$ chlorine. Another study on a single cultured zooplankton recorded that 50% of the Cladoceran, *Ceriodaphnia dubia* was killed after 24 h exposure to chlorine concentration of $0.12 \text{ mg}\cdot\text{l}^{-1}$ (Manning et al. 1996). Ward and DeGraeve (1978) described the sensitivity of other Cladoceran, *Daphnia magna*, to chlorine relied on the age of this organism. TRC concentration of $0.07 \text{ mg}\cdot\text{l}^{-1}$ and $0.22 \text{ mg}\cdot\text{l}^{-1}$ killed 3 day old *D. magna* within 10.5 and 5.5 h respectively. At TRC concentration of $0.017 \text{ mg}\cdot\text{l}^{-1}$, it killed 50% of less than one day old *D. magna*.

Conclusion

Chlorine with TRC concentration as low as $0.25 \text{ mg}\cdot\text{l}^{-1}$ adversely affected phytoplankton as manifested in the reduction of primary productivity, chlorophyll *a* concentration and resulted to a mortality of more than 40% of zooplankton. For pond preparation, the purpose of chlorination is to exterminate pathogenic organisms and their hosts. The chlorine dose required depends on the chlorine demand of the water and sediment. Controlling excessive plankton growth during the grow-out period requires lower doses to avoid toxic effect on cultured shrimp.

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