DOSAGE MINIMIZATION OF CHLORINE TO IMPROVE WATER QUALITY AND ITS APPLICABILITY FOR SHRIMP LARVAL REARING OPERATIONS IN HATCHERY

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ABSTRACT

Administration of higher dosage of chlorine leads to a concern about proper dosage determination for shrimp hatchery operations. Hence, the dosage application needs to be reworked at the present context. Accordingly a Completely randomized design experiment with 6 treatments (control, 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm of active chlorine content) with 3 replications was conducted. The water quality and the bacterial load were monitored once in 3 hours continuously. The salient observations of the study was that the exposure time for residual chlorine to be nil for the tank with chlorination of 10 ppm concentration was 6 hours, for 20 ppm and 30 ppm it was 18 hours and for 40 ppm and 50 ppm it was 21 hours. Also the results shows that bacterial load was nil in all the treatments viz. 10 to 50 ppm. The pH of the water gets increased and then stabilized. It could be concluded from the study that the chlorination is required in shrimp hatcheries. But the optimum dosage is 10 ppm for ensuring better water quality in shrimp hatchery which is very much less when compared to the general dose of upto 30 ppm for other purposes. Another experimental trial with three replications was conducted to ascertain the survival of post larvae of P. monodon from PL5 to PL 20 with the 10 ppm active chlorine. The study showed that survival was high in 10 ppm..

Key Words: Chlorine, Hatchery, Shrimp larvae, Water quality

INTRODUCTION

Incoming water used in shrimp hatcheries should be disinfected prior to use to minimize the chance of viral, bacterial, fungal and protozoan diseases entering and causing disease problems in the hatchery. Current methods for the disinfection of seawater, which reduce bacterial loading in water supplies and/or avoid blooms of potentially pathogenic microorganisms, include treatments with antibiotics, ozone, filtration, heat, and UV irradiation (Whipple and Rohovec, 1994; Pascho *et al.,* 1995; Chang *et al.,* 1998; Liltved and Cripps, 1999; Munro *et al.,* 1999; Frerichs *et al.,* 2000). However, each of these treatments has specific disadvantages such as high cost, need for sophisticated equipment, production of toxic residues for the cultured

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organisms, or the appearance of resistant microorganism strains (Liltved *et al.,* 1995; Bullock *et al.,* 1997; Reilly and Kaferstein, 1997).

The commonest and best chemical treatment for such disinfection is the use of chlorine in the hatchery. Chlorine has a high efficiency as a disinfectant, and is readily available at low cost and in its various forms has been widely used for microbiological control in seawater and for surface sanitation in some intensive aquaculture facilities (White, 1992; Pascho *et al.,* 1995). Laboratory and field studies have demonstrated the high biocidal efficiency of chlorine against viruses and bacterial pathogens (Sako *et al.,* 1988; Inouye *et al.,* 1990; Frerichs, 1990; Pascho *et al.,* 1995; Arimoto *et al.,* 1996; Chang *et al.,* 1998). At allowable levels, chlorine does not affect flora or fauna. If an excess amount of chlorine is released by accident into aquatic environments, it may harm aquatic plants and animals until it is diluted to a harmless level (Anonymous, 1999).

Chlorine can be bought either as a powder (calcium hypochlorite, usually 60–70 percent active ingredient), liquid (sodium hypochlorite, usually 7–10 percent active ingredient) or as tablets (sodium dichloroisocyanurate, usually > 90 percent active ingredient). Any of these forms of chlorine is effective and can be used depending upon price and availability. Normally a level of active chlorine in the water of 10–20 ppm for 12–24 h is sufficient to kill most viruses, bacteria and fungi (FAO, 2007).

The effect of chlorination at permissible levels, does not affect flora or fauna but excess amount of chlorine may harm aquatic plants and animals until it is diluted to a harmless level. Hence the knowledge of exact dosage to be applied needs to be ascertained and the effect of chlorination and the optimum dosage for maintaining the hatchery inlet water will depend on the water source and it is location specific. Hence this study was conducted with the main objective to determine optimum chlorine demand of hatchery water, complete residual time and effective chlorine dose required to disinfect the micro organisms and to investigate the effect of optimum dose of chlorine with the stocking density of shrimp larvae.

MATERIALS AND METHODS

Experimental design

A completely randomized design experiment was carried out with five active chlorine concentrations by adding calcium hypochlorite at 10, 20, 30, 40 and 50 ppm along with a control in triplicates (n = 3). Therefore, total 15 numbers of 50 lit FRP tanks with conical bottom have been used. Each chlorine solution of different concentrations was prepared by dissolving calcium hypochlorite and standardized with sodium thiosulphate (APHA, 1998). All tanks were aerated to maintain dissolved oxygen (DO) concentration above 5 ppm. The experiment was carried out outdoor of the shrimp hatchery under shaded condition for 24 h. Water quality parameters such as pH (pH-Scan-Eutech instruments, Singapore) and temperature (mercury thermometer) were analyzed at 3 h intervals from 0 h to 24 h of chlorine application.

Assessment of residual chlorine

When chlorine dissolves in pond water, it forms free residual chlorine. Part of this free chlorine which reacts with organic and inorganic substances and metals is referred as chlorine demand, and the residual parts oxidize and damage nucleic acid and/or protein of microorganisms and cause lethal damage (Acher *et al*., 1997; Chanratchakool, 1995). Concentrations of total residual chlorine (TRC) in treated water in each tank were analyzed in every 3h intervals after chlorine application. At each time interval, triplicate water samples were taken from each treatment. Total residual chlorine was analyzed by DPD titration method (APHA, 1998).

Assessment of microbial load

The total heterotrophic bacteria were determined in the shrimp rearing water by counting the colonies which grew on plates of Zobell Marine Agar (ZMA) (Hi-Media) with 1% of NaCl (Jorgensen *et al*., 1993). Before plating each sample onto agar medium, serial dilutions were made in physiological saline (0.9 % NaCl) solution (Sohier and Bianchi, 1985). The bacterial counts were expressed in colony forming units per ml of water (CFU ml^{-1}) (Smith, 1998).

Experimental trial on *P. monodon* survival at different stocking density

Based on the previous trial the optimum chlorine dosage was determined and in that another experiment was carried out to observe survival of *P. monodon* larvae from PL5 to PL 20 in different stocking densities viz. 100, 125, 150, 175 and 200 no./L. The water quality was monitored and survival % was calculated at the end of the experiment. Survival % was analysed using following formulae:

Survival (%) = Shrimp no. at the end of experiment / Shrimp no. at the beginning of experiment × 100

RESULTS AND DISCUSSION

pH and temperature

The mean and standard error of pH and temperature are presented in Table 1 and 2 respectively. From the results it was observed that the first four treatments, pH of water get increased slightly while in 50 ppm concentration it raised rapidly. This increment was observed up to 9 hrs then it decreased gradually whereas in control, the value increased as time increase. When a chlorination product is applied to water, it dissolves and chlorine speciation takes place based on pH. At pH below 2, chlorine gas is dominant. Between pH 2 and 6, HOCl is the only type. As pH increases beyond 6, OCl appears. At a pH of about 7.5, HOCl and OCl^- occur in equal proportions, but at higher pH, OCl^- is the most abundant source of chlorine. When chlorine is added to water in any of these forms, it creates hypochlorous acid. Hypochlorous acid (HOCl) is a weak acid that dissociates into hypochlorite ion ($OCl^–$) according to the following equation:

$$HOCl \leftrightarrow H^+ + OCl^-$$

Free chlorine refers to both hypochlorous acid (HOCl) and the hypochlorite (OCl^-) ion or bleach, and is commonly added to water systems for disinfection. When ammonia or organic nitrogen is also present, chloramines known as monochloramine, dichloramine, and trichloramine will quickly form. Chloramines are also known as combined chlorine. Total chlorine is the sum of free chlorine and combined chlorine. The level of total chlorine will always be higher than or equal to the level of free chlorine (Anon, 2011).

The two species exist in an equilibrium that is pH dependent. The equilibrium is also slightly affected by temperature. As the pH increases, the ratio of hypochlorous acid to hypochlorite ion decreases. Below a pH of 7.5, hypochlorous acid is the dominant species. Above a pH of 7.5, hypochlorite ion is the dominant species. The disassociation curve below illustrates the relationship between the chemical forms of chlorine and pH at 20° C. The graph indicates a significant change in the ratio of hypochlorous acid to hypochlorite between pH 6 and 9, within the typical pH range for drinking water treatment. The steepest portion of the curve is between pH 7 and 8. Even a 0.1 unit change in pH can cause a significant change in the ratio between HOCl and OCl^-. This is significant because HOCl is a stronger disinfectant than OCl^-. Therefore, the chlorination process is pH dependent. The germicidal effects of HOCl will be realized by chlorination at a lower pH.

The pH of chlorine is 11.7. It would seem logical that adding chlorine into water having a neutral pH would make the water more alkaline. When the pH of water is 7 or below, chlorine will act primarily as a sanitizer. At this level, it is very effective at killing bacteria. At 7.4, chlorine will act equally as a sanitizer and oxidizer. Above 7.8, the chlorine will act principally as an oxidizer. When chlorine is added to water it becomes hypochlorite ions (OCl) and hypochlorous acid (HOCl) in a quantity determined by the pH as indicated by the chart below:

pH	OCl	HOCI
6.0	3.50 %	96.50 %
6.5	10.00 %	90.00 %
7.0	27.50 %	72.50 %
7.5	50.00 %	50.00 %
8.0	78.50 %	21.50 %
8.5	90.00 %	10.00 %

The chart is helpful to work put the chlorine demand of hatchery water, complete residual time and effective chlorine dose required to disinfect the organisms, particularly pathogenic microorganisms and their carriers. The study indicated the percentage of OCl was higher compared to HOCl resulting the available chlorine in water as oxidizer. Besides, the higher the water temperature is, the lower the pH of water. Chunilall *et al.*, 2002 reported that addition of hypochlorite in seawater treatments can lower the BOD and COD of the water and also improve the water quality. Temperature was found gradually decreased in all treatments which helped to maintain the pH slightly alkaline during the experimental period. In general hypochlorite are widely used as domestic disinfectant and sanitizing agent in food processing industry (WHO 2000) as well as inactivation of viruses in shrimp ponds and hatcheries (Boyd, 1996; Cai, 1994; Hedge *et al.,* 1996).

Table 1. pH in 3 hrs interval with different chlorine concentrations

	Control	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
0 hrs	7.40 ± 0.014	7.53 ± 0.018	7.49 ± 0.012	7.47 ± 0.0	7.48 ± 0.003	7.48 ± 0.003
3 hrs	7.62 ± 0.024	7.70 ± 0.003	7.77 ± 0.009	7.77 ± 0.012	7.93 ± 0.017	8.04 ± 0.009
6 hrs	7.73 ± 0.021	7.78 ± 0.003	7.86 ± 0.009	7.92 ± 0.009	7.98 ± 0.009	8.05 ± 0.009
9 hrs	7.78 ± 0.023	7.78 ± 0.023	7.82 ± 0.003	7.91 ± 0.017	7.93 ± 0.006	7.97 ± 0.007
12 hrs	7.75 ± 0.035		7.87 ± 0.012	7.86 ± 0.02	7.95 ± 0.009	8.00 ± 0.012
15 hrs	7.82 ± 0.012		7.88 ± 0.009	7.94 ± 0.023	7.95 ± 0.027	7.97 ± 0.003
18 hrs	7.84 ± 0.009		7.90 ± 0.007	7.94± 0.007	7.95 ± 0.003	7.98 ± 0.006
21 hrs	7.82 ± 0.025		7.88 ± 0.009	7.91 ± 0.006	7.93 ± 0.006	7.95 ± 0.003
24 hrs	7.85 ± 0.015				7.83 ± 0.018	7.89 ± 0.037

Table2. Temperature in 3 hrs interval with different chlorine concentrations

	Control	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
0 hrs	32.07 ± 0.233	31.8 0± 0.200	31.67 ± 0.318	31.53 ± 0.348	31.90 ± 0.058	31.83 ± 0.033
3hrs	31.33± 0.145	31.57 ± 0.033	31.10 ± 0.058	31.13 ± 0.033	29.13 ± 0.186	29.03 ± 0.133
6hrs	30.57 ± 0.067	30.47 ± 0.033	30.17 ± 0.088	29.87 ± 0.067	29.90 ± 0.100	29.80± 0.000
9hrs	26.80± 0.058	26.8 ± 0.058	27.13 ± 0.133	26.87 ± 0.033	26.97 ± 0.120	27.23 ± 0.088
12hrs	26.83 ± 0.033		27.10 ± 0.100	26.40 ± 0.058	26.63 ± 0.186	26.87 ± 0.033
15hrs	26.37 ± 0.088		26.77 ± 0.033	26.20 ± 0.058	26.33 ± 0.088	26.43 ± 0.067
18hrs	27.07 ± 0.033		27.07 ± 0.033	27.07 ± 0.033	27.07 ± 0.067	27.13 ± 0.088
21hrs	30.23 ± 0.033		29.90 ± 0.058	29.87 ± 0.033	29.70 ± 0.058	29.87 ± 0.033
24hrs	32.60 ± 0.100				32.80 ± 0.058	32.67 ± 0.033

Table3. Residual chlorine in different treatments

Stocking Density (nos/lit)		100	125	150	175	200
PL 5	Control	100.00	100.00	100.00	100.00	100.00
	Treatment	100.00	100.00	100.00	100.00	100.00
PL 10	Control	87.00	92.00	84.67	84.00	72.00
	Treatment	86.00	84.00	85.33	89.71	92.50
PL 15	Control	79.31	84.35	72.44	85.03	75.69
	Treatment	88.37	94.29	84.38	92.36	86.49
PL 20	Control	55.07	74.23	72.83	76.00	79.82
	Treatment	86.89	84.85	78.70	86.21	91.88

Table 4.Finalsurvival (%) of *P. monodon* larvae from PL 5 to PL 20

Hrs	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
3	0.10	0.2	0.3	0.4	0.5
6	0.05	0.1	0.2	0.3	0.4
9	0.00	0.1	0.2	0.3	0.4
12		0.1	0.2	0.3	0.4
15		0.1	0.2	0.3	0.4
18		0.1	0.2	0.3	0.4
21		0.0	0.0	0.0	0.2
24					0.0

Residual chlorine

Residual chlorine from 0 to 24h in all treatments is presented in Table 3. In 10 ppm, Residual chlorine has been found zero after 9 hours while in 50 ppm residual time was about 24 hrs. Although chlorination treatment of seawater is known to produce significant toxic effects on microalgae such as *Nitszchia closterium*, *Dunaliella tertiolecta*, and *Microcystsis* sp. (Stauber, 1998; Tsuzuki *et al.,* 1999), neutralization of hypochlorite ion using sodium thiosulfate allowed the growth of *Isochrysis galbana* with no negative effects. This species of microalga is one of the main food sources used in the culture of larval marine invertebrates (Reitan *et al.,* 1998; Fidalgo *et al.,* 1998). Since food cultures are a serious source of contamination in culture systems (Elston, 1989), control of bacterial numbers and types in these systems is of prime importance. Therefore, the dosage of chlorine must be in proper range to avoid such kind of problems. Our studies showed that at 10 ppm treatments was quickly eliminated from the water compared to other higher treatments. Besides, it was quite effective to disinfect the water which is discussed in the later section.

Bacterial load

Bacterial load was found nil in all treatments right from 10 ppm to 50 ppm except in control. Therefore, the minimum dosage i.e. 10 ppm was effective to control the bacterial load in water. Laboratory and field studies have demonstrated the biocidal effects of chlorine on certain viral (Frerichs, 1990; Inouye *et al.,* 1990), protozoan (Bedell, 1971; Sanders *et al.,* 1972) and bacterial (Sako *et al.,* 1988) pathogens of fish. Calcium hypochlorite at 200 ppm is commonly recommended for disinfection of hatchery equipment and ponds (Brown and Gratzek, 1980; Piper *et al.,* 1982) but in our studies a significantly lower dosage (10 ppm) of calcium hypochlorite found to be effective to disinfect the inlet water for hatchery at lesser retention time. Baticados and Pitogo (1990) also reported that chlorine dosage of 5 to 30 ppm in water for shrimp culture could minimize the bacterial load within 6 hours.

Larval survival at different stocking density

Final survival % of *P. monodon* larvae from PL 5 to PL 20 and overall survival in different stocking densities is illustrated in Table 4. Survival % of *P. monodon* larvae in all stages i.e. from PL 5 to PL 20 was higher in 10 ppm treated water compared to control. Besides, it was observed that overall survival % was more than 84 % in all stocking densities and also found > 90 % survival at 200 no/L stocking density for PL 5 to PL 20. Therefore, an intensive stocking density of P. monodon larvae can be reared after water treatment with 10 ppm calcium hypochlorite. Douillet and Pickering (1999) reported that survival of fish larvae (*Sciaenops ocellatus*) improved significantly by filtering the seawater through 1 μm filter, followed by chlorination at 5 ppm Cl^- for 2 h, and dechlorination with sodium thiosulfate. Survival of different larval stages of *P. monodon* was reported as more than 70 % in seawater treated with 20 ppm chlorine for 24 hours (Soundarapandian and Babu, 2010).

CONCLUSION

It could be concluded that chlorination is required at the optimum dosage of 10 ppm for ensuring better water quality in shrimp hatchery which is very much less when compared to the general dose which is followed to the range up to 30 ppm.Shrimp larvae can be stocked @ 100 no/L or even higher density in 10 ppm chlorinated water which offer higher survival rate in shrimp hatchery.

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