Fish & Shellfish Immunology 35 (2013) 525–531

Contents lists available at SciVerse ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Combined effects of carbonate alkalinity and pH on survival, growth and haemocyte parameters of the Venus clam *Cyclina sinensis*

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ARTICLE INFO

Article history:
Received 25 March 2013
Received in revised form
3 May 2013
Accepted 6 May 2013
Available online 24 May 2013

Keywords:
Cyclina sinensis
Carbonate alkalinity
pH
Survival
Haemocyte parameters

ABSTRACT

Carbonate alkalinity (CA) and pH are considered to be two important stress factors that determine the response of aquatic animals to sudden transfers into saline-alkaline water. To evaluate the potential for aquaculture production of Venus clams (*Cyclina sinensis*) farmed in saline-alkaline water, the combined effects of CA (2.5 (control), 10.0, 20.0 and 40.0 meq/l) and pH (8.0 (control), 8.5, 9.0 and 9.5) on survival rate was monitored every day for 10 days. Length gain rate (LGR) and weight gain rate (WGR) were also monitored for two months, and total haemocyte count (THC), phagocytic rate (PR) and haemocyte mortality (HM) were measured for 3, 6, 12 and 24 days under the same water temperature (20 °C) and salinity (15‰) conditions. The results showed that survival rates in treatments of CA $\leq$ 20.0, combined with pH $\leq$ 9.0, were 100%. LGR and WGR in treatments of CA 2.5 & pH 8.0 (control), CA 2.5 & pH 8.5 and CA 10.0 & pH 8.0 exhibited the largest values ($P > 0.05$), while in other treatments, they showed a decreasing trend with an increase in either CA or pH or both ($P < 0.05$). Similarly, for THC, PR and HM, no significant differences were observed among the fast growth treatments during the entire experimental period ($P > 0.05$), however, in other treatments, they presented significant differences, especially on day 3 and 6 ($P < 0.05$), most notably with increases in CA or pH, but returned to control levels on day 12. In conclusion, in this study, a strong interaction between CA and pH was observed. Additionally, it was ascertained that the Venus clam *C. sinensis* can withstand the stress of CA 20.0 combined pH 9.0, although individuals grows slowly and may take approximately 12 days to recover to the unstressed condition.

1. Introduction

Large areas of saline-alkaline water are distributed widely throughout the world [1,2]. In China, saline-alkaline water covers approximately 45.87 million hectares, and brackish water lakes account for approximately 55% of total lake area [3]. Saline-alkaline water has poor buffering capacity, an unstable proportion of major ions, high pH values of approximately 8.8, and high carbonate alkalinity (CA) concentrations [1,4]. As a result of these characteristics most saline-alkaline regions still remain unexploited. With the increasing shrinkage of available water resources inland, countries around the world have begun to investigate reasonable uses of saline-alkaline water [2,5]. Previously, two major approaches have been recognised for saline-alkaline water utilisation: modification of saline-alkaline water suitable for crop irrigation or aquaculture and exploitation of plants or aquatic animals tolerant to saline-alkaline water. However, the former use requires more manpower, material and financial resources than the latter [1,6].

Our group has been engaged in investigating the use of saline-alkaline water for aquaculture for more than 20 years. In 1994, the shrimp *Penaeus chinensis* was first successfully farmed in saline-alkaline waters in northwest China [7]. Soon after that advance, successful farms of freshwater prawn (*Macrobrachium rosenbergii*) [8], silverfish (*Protosalanx hyalocranius*) [9], whiteleg shrimp (*Litopenaeus vannamei*) [10], medaka (*Oryzias latipes*) [3] and sturgeon (*Acipenser baerii*) [11] in saline-alkaline water throughout inland China were established, providing economic benefits to local farmers. However, in recent years, as saline-alkaline water aquaculture has become well-developed, a major problem has emerged. Namely, the pH of water in aquaculture farms can increase dramatically (potentially reaching values greater than pH 9.5) due to microalgal over-multiplication during the middle and late rearing periods, thereby causing damage to the reared organisms. Methods of pH reduction, in addition to running

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http://dx.doi.org/10.1016/j.fsi.2013.05.006

an aerator to increase the CO_2 content in the water, rearing bivalves with a high algae-filtering capacity to remove the excessive microalgae may be also a workable one [12–14].

The Venus clam *Cyclina sinensis* is an intertidal clam that is naturally distributed along the coastal muddy sands of China, Korea, Japan and Southeast Asia. This species tolerates a wide range of temperature (−2–33 °C with an optimum of 20–25 °C) and salinity (5–35‰, with an optimum of 15–25‰) [15]. Additionally, this clam is fast-growing [16] and has a high algae-filtering capability [17]. *C. sinensis* farms were first developed in the 1980s and have expanded greatly since the late 1990s. Recently, to improve the increasingly valuable *C. sinensis* industry, research has been conducted on large-scale artificial breeding technology [18], nutrition [19] and genetic diversity [20]. In this study, the effects of CA and pH, two of the most important stress factors for determining the survival, growth and haemocyte parameters of *C. sinensis* in saline-alkaline water, were investigated. The main objective was to determine the feasibility of farming *C. sinensis* in saline-alkaline water, thereby introducing a new species into saline-alkaline water aquaculture and also providing a potential tool for alleviating the sharp pH increase during the middle and late rearing periods.

2. Materials and methods

2.1. Experimental muddy sand and animals

Muddy sand was collected from the shallows in Yueqing Bay, Zhejiang Province, China. After transportation to the laboratory, muddy sand was filtered through a screen mesh with a diameter of approximately 500 μm, then sterilised by boiling and sun-dried. Venus clams (20.42 ± 0.25 mm shell height, 2.18 ± 0.14 g body weight) were sampled from a bivalve hatchery in Yueqing city. After arrival at the laboratory, clams were acclimatised in fiberglass tanks containing pre-covering sterilised muddy sand with a thickness of 10 cm and aerated seawater with a salinity of 15‰, water temperature of 20 °C, pH of 8.0, and CA of 2.5 meq/l. Clams were fed a mixture of *Chlorella* sp. and *Platymonas* sp. at densities of $5.3 \sim 6.7 \times 10^5$ cells/ml and $2 \sim 2.5 \times 10^4$ cells/ml, respectively [18]. Rearing water was changed daily, and muddy sand was renewed weekly. After two weeks of acclimatisation, clams were used in a CA and pH stress experiment.

2.2. Experimental water

The CA and pH stress experiment was set up in a factorial design with four levels of CA (2.5 (control), 10.0, 20.0 and 40.0 meq/l) and four levels of pH (8.0 (control), 8.5, 9.0 and 9.5), for a total of 16 treatments. Experimental water in each treatment was prepared by adding different volumes of 0.1 mol/l NaOH, or different quality proportions of Na_2CO_3 and $NaHCO_3$ to seawater (Table 1). CA was determined by acid–base titration, while pH was measured with a pH-meter (Mettler-Toledo, Greifensee, Zürich); measured values of CA and pH are shown in Table 2. Experimental water was maintained in plastic tanks for 24 h before use.

Table 2
Target and measured values of CA and pH ($n = 5$).

Target value CA & pH	Measured value CA & pH
2.5 & 8.0	2.5 & 8.0
2.5 & 8.5	2.60 ± 0.100 & 8.47 ± 0.058
2.5 & 9.0	2.63 ± 0.058 & 9.03 ± 0.055
2.5 & 9.5	2.73 ± 0.115 & 9.47 ± 0.058
10.0 & 8.0	9.83 ± 0.153 & 8.06 ± 0.026
10.0 & 8.5	9.90 ± 0.200 & 8.54 ± 0.061
10.0 & 9.0	9.93 ± 0.252 & 9.06 ± 0.023
10.0 & 9.5	10.03 ± 0.208 & 9.47 ± 0.020
20.0 & 8.0	18.53 ± 0.404 & 9.00 ± 0.006
20.0 & 8.5	18.30 ± 0.656 & 9.03 ± 0.055
20.0 & 9.0	18.23.±0.153 & 9.06 ± 0.061
20.0 & 9.5	18.20 ± 0.361 & 9.06 ± 0.012
40.0 & 8.0	36.50 ± 1.418 & 9.47 ± 0.021
40.0 & 8.5	36.27 ± 1.270 & 9.48 ± 0.030
40.0 & 9.0	35.80 ± 1.153 & 9.50 ± 0.021
40.0& 9.5	35.33 ± 1.539 & 9.48 ± 0.030

2.3. Experimental protocol

2.3.1. Experiment 1. Effect of CA and pH on survival

Venus clams were placed in 100 cm × 70 cm × 25 cm (length × width × height) plastic tanks containing pre-prepared experimental water and muddy sand and reared for 10 days. Each treatment consisted of three replicates with 50 individuals per replicate. During the rearing period, no feeding, aeration or muddy sand renewals were conducted, although 90% of experimental water was changed daily by siphon. Clams were observed every half-day, and dead individuals were removed promptly and recorded for survival rate calculation. The criteria of death was based primarily on clams lying on the muddy sand, rather than diving into the sand, keeping shells open for a long time, and not responding to touch. According to the result of experiment 1, treatments with a 100% survival rate were used for the succeeding experiments in which growth and haemocyte parameters were measured.

2.3.2. Experiment 2. Effect of CA and pH on growth

Clams were reared in the plastic tanks containing aerated experimental water and sterilised muddy sand for two months. Each treatment consisted of three replicates with 50 individuals per replicate. During the rearing period, clams were fed a mixture of *Chlorella* sp. and *Platymonas* sp. daily. Ninety per cent of the experimental water was changed daily by siphon and muddy sand was replaced weekly. After two months, the length and weight of each clam was measured using a vernier caliper and electronic balance, respectively, and length gain rate (LGR) and weight gain rate (WGR) were calculated. $LGR = (L_t - L_0)/L_0$, $WGR = (W_t - W_0)/W_0$, where L_t and L_0 are the length at two months and the initial length, respectively, and W_t and W_0 are the weight at two months and the initial weight.

2.3.3. Experiment 3. Effect of CA and pH on haemocyte parameters

Clams were reared in the tanks containing experimental water and muddy sand for one month. Each treatment consisted of three replicates with 30 individuals per replicate. During the rearing

Table 1
Volumes of 0.1 mol/l NaOH, and detailed quality proportions of Na_2CO_3 and $NaHCO_3$ used in preparing the desired experimental water.

	CA = 2.5	CA = 10.0		CA = 20.0		CA = 40.0	
	0.1 mol/l NaOH (ml)	Na_2CO_3 (mg)	$NaHCO_3$ (mg)	Na_2CO_3 (mg)	$NaHCO_3$ (mg)	Na_2CO_3 (mg)	$NaHCO_3$ (mg)
pH = 8.0	0.0	14.8	606.5	26.5	1428.0	40.3	3086.2
pH = 8.5	1.8	58.3	573.6	169.6	1201.2	365.7	2570.4
pH = 9.0	5.5	196.1	319.2	381.6	865.2	858.6	1789.2
pH = 9.5	12.5	323.3	117.6	689.0	378.0	1378.0	966.0