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Na_2-EDTA Effects on the Development of Oyster, *Crassostrea belcheri* (Sowerby) Larvae

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ABSTRACT

The effect of different concentrations of a disodium salt of ethylenediamine tetraacetic acid (Na_2-EDTA) on the development of oyster *Crassostrea belcheri*, was studied and the highest percentage of fertilized eggs that developed into normal trochophore larvae was found to occur in seawater untreated with Na_2-EDTA. However, no significant differences in mean percentages of embryos that developed into normal D-larvae were found among untreated and treated units at Na_2-EDTA concentrations ranging from 5 to 20 ppm ($P > 0.05$). In the study on the effect of conditioning periods of Na_2-EDTA pre-treated seawater on the development of oyster larvae, non-significant differences were found among the mean percentages of fertilized eggs that developed into normal trochophore larvae in untreated seawater and seawater pre-treated with Na_2-EDTA and conditioned from 0 to 48 hr ($P > 0.05$). However, mean percentages of embryos that developed into normal D-larvae was highly significantly different among treatments ($P < 0.01$), with the highest percentage occurring in untreated Na_2-EDTA seawater.

Keywords: Na_2-EDTA, *Crassostrea belcheri*, trochophore, D-larvae.

INTRODUCTION

Heavy metals affect various developmental processes during the embryonic period, which reduces the quantity and quality of bivalve larvae. The effects of heavy metals on embryos and larvae of marine bivalves have been reported by several authors (for example, Calabrese *et al.*, 1977; MacInnes, 1981; Martin *et al.*, 1981; Yantain, 1989; Ruiz *et al.*, 1996). The most widely used techniques to remove heavy metals involve the process of neutralization and metal hydroxide precipitation (Hiemesh and Mahadevaswamy, 1994). In recent years, the remobilization of metals by synthetic chelating agents has received much attention. Synthetic compounds like ethylenediamine tetraacetic acid (EDTA) are known to be effective chelating agents for heavy metals (Licop, 1988). EDTA is the most commonly used chelator due to its strong chelating ability for different heavy metals (Norvell, 1991). EDTA has advantages due to its relatively low biodegradability and its strong capacity to complex with heavy metals (GESAMP, 1997; Kedziorek and Bourg, 2000). EDTA is used in the intensive culture of penaeid shrimp to increase both the hatching rate (Cook, 1969) and survival of larvae (Cook, 1969; Castille and Lawrence, 1981). To chelate and reduce the heavy metals in rearing water, a disodium salt of ethylene diamine tetraacetic acid (Na_2-EDTA) at doses of 3–10 ppm is generally applied (Licop, 1988; Boonyaratpalin,

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1990; Carpenter, 1992). The pre-treatment of seawater by adding EDTA helps render complex heavy metals non-toxic to the vulnerable early developmental stages of bivalve larvae (Helm *et al.*, 2004). Despite the widespread use of EDTA in bivalve larval culture, there is a paucity of information on the possible adverse effects on developing embryos and larvae. The present work was designed to study the effects of pre-treating seawater with Na_2-EDTA on the embryonic and larval development of oyster *Crassostrea belcheri* (Sowerby). The results will improve decision-making concerning the use of EDTA for treating seawater in bivalve hatcheries.

MATERIALS AND METHODS

Preparation of broodstock and larvae

Mature *Crassostrea belcheri* oysters were collected from the wild by people fishing in the Palian Rivar, Kantang district, Trang province. Mature oysters of a size bigger than 12 cm in length (antero-posterior measurement) were selected to use as broodstock. The selected oysters were cleaned and acclimated in sand-filtered seawater (30 ppt) in a hatchery for 2 d. During this period, they were fed an algal mixture containing *Chaetoceros calcitrans* and *Tetraselmis suecica*. Gametes were obtained from sacrificed oysters and the eggs fertilized using one male for four females by the method according to Charlermwath (2001). Fertilized eggs were gently aerated for 15–20 min until the first polar body was observed. The fertilized eggs were then transferred to aquaria to test the effect of EDTA on larval development.

Experiments

Effect of Na_2-EDTA concentrations

The experiment was conducted in 5 L aquaria filled with 3 L of treated seawater (filtered (1 μm) and UV-treated). The disodium salt of ethylene diamine tetraacetic acid (Na_2-EDTA) at concentrations of 5, 10, 15 and 20 ppm was added to treated seawater. A treatment without Na_2-EDTA was used as a control. The fertilized eggs were stocked at a density of 40 embryos. mL^{-1} in each experimental unit. Gentle aeration was supplied via air diffusers throughout the study. The temperature varied from 27 to 29 °C during the experimental period (24 hr). The designation of experimental units followed a completely randomized design (CRD) with three replicates. At the end of 2 and 24 hr, three samples from each experimental unit were collected and the numbers of embryos that had developed into normal trochophore or D-larvae were counted using a Sedgewick Rafter counting chamber under a binocular compound microscope (Olympus CH2, Japan). The numbers of trochophore and D-larvae were expressed as a percentage of development (100 × total number of trochophore or D-larvae / total number of fertilized eggs).

Effect of conditioning periods of Na_2-EDTA pre-treated seawater

At 10 ppm, Na_2-EDTA was added to the 5 L aquaria filled with 3 L of the treated seawater, which was then vigorously aerated until use. The seawater was conditioned to degrade Na_2-EDTA. Four conditioning periods were used in the experiment: 0, 12, 24 and 48 hr. The treated seawater without adding Na_2-EDTA was used as a control. The experiment was set up using a CRD with each treatment in triplicate. The experimental procedure was similar to that described for the experiment on the effects of Na_2-EDTA concentration.

Data analysis

Data were analyzed statistically using analysis of variance (one-way ANOVA) to determine differences among Na_2-EDTA concentrations and conditioning times. If significant effects were present, then data were subjected to Duncan's multiple range test to analyze differences among treatment means.

RESULTS

Effect of Na_2-EDTA concentration

The mean percentage of fertilized eggs that developed into normal trochophore larvae differed significantly in seawater among untreated (88.67 ± 7.86%) samples and samples treated with Na_2-EDTA at concentrations of 10, 15 and 20 ppm (67.33 ± 6.77, 64.00 ± 11.55 and 63.33 ± 12.17%, respectively). However, no significant differences ($P > 0.05$) were found between samples that were untreated (88.67 ± 7.86%) and treated with Na_2-EDTA at a concentration of 5 ppm (73.11 ± 14.79%). The mean percentages of embryos that developed into normal D-larvae were 34.44 ± 6.54, 28.44 ± 7.70, 24.22 ± 7.00, 22.00 ± 5.93 and 21.33 ± 7.06% in untreated seawater and in treated seawater at concentrations of 5, 10, 15 and 20 ppm respectively. No significant differences in mean percentages of embryos that developed into normal D-larvae were found among untreated and treated units at Na_2-EDTA concentrations ranging from 5 to 20 ppm ($P > 0.05$) (Figure 1).

Effect of conditioning period of Na_2-EDTA pre-treatment seawater

There were no significant differences in mean percentages of fertilized eggs that developed into normal trochophore larvae among untreated and pre-treated Na_2-EDTA seawater conditioned for different periods ($P > 0.05$). The mean percentage trochophore larvae were 60.00 ± 10.90, 45.50 ± 2.65, 47.75 ± 7.04, 49.67 ± 1.58 and 53.83 ± 2.02% for untreated seawater and pre-treated Na_2-EDTA seawater conditioned for 0, 12, 24 and 48 hr, respectively. However, Figure 2 shows that the mean percentages of embryos that developed into normal D-larvae differed highly significantly among treatments ($P < 0.01$), with the highest occurring in the untreated Na_2-EDTA seawater (37.17 ± 2.57%).

DISCUSSION

This study demonstrated that seawater pre-treated with Na_2-EDTA affects the development of oyster (*Crassostrea belcheri*) larvae compared with untreated seawater. Evidence exists of

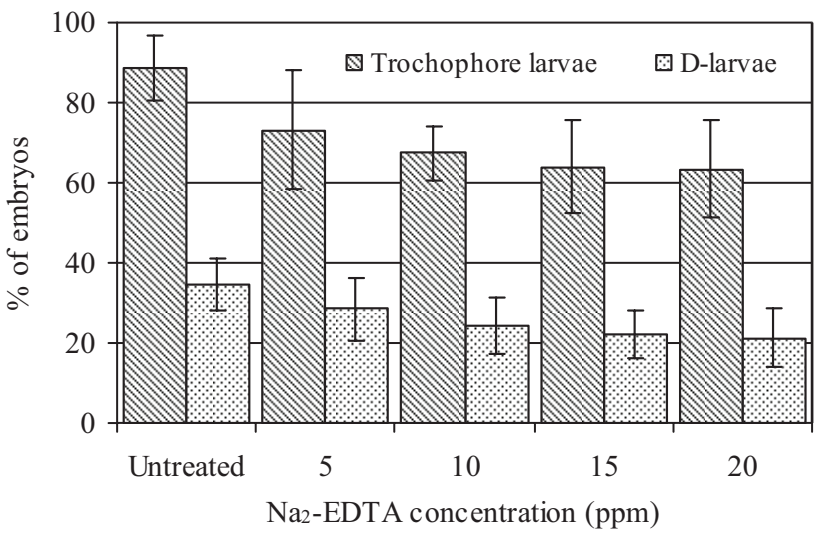

Figure 1 Mean (with bars showing ± SD) percentage of embryos that developed into normal trochophore and D-larvae of *Crassostrea belcheri* (Sowerby) in seawater treated with disodium salt of ethylenediamine tetraacetic acid (Na_2-EDTA) at different concentrations.

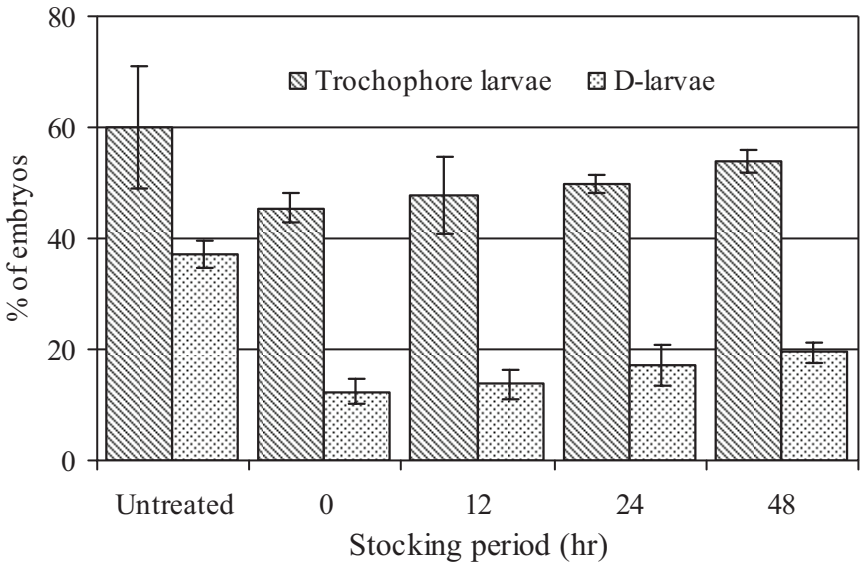

Figure 2 Mean with bars showing ± SD) percentage of embryos developed into normal trochophore and D-larvae of *Crassostrea belcheri* (Sowerby) in untreated and pre-treated seawater with disodium salt of ethylenediamine tetraacetic acid (Na_2-EDTA) concentration of 10 ppm and stocked for different periods.

deformational changes in animal chromosomes related to alterations in the colloidal properties of structural nucleoproteins by EDTA (Kaufmann and McDonald, 1953). That effect has been attributed to the removal by chelation of divalent cations essential for the maintenance of chromosomal integrity. Although increasing the conditioning period of pre-treated Na_2-EDTA seawater improved the percentage of surviving larvae, untreated seawater produced a better survival rate. However, where there is potential for trace metal toxicity, the use of EDTA in the culture of bivalve larvae would be beneficial. Knezovich *et al.* (1981) demonstrated that the presence of chelators (either naturally occurring dissolved organic matter (DOM) or added EDTA) reduced the toxicity of copper to embryos of the oyster *C. gigas*. Several studies have shown that the application of Na_2-EDTA to water polluted by metals enhances the survival chances of marine organisms (Lewis *et al*., 1972; Lawrence *et al*., 1981; Licop, 1988). In shrimp hatcheries, EDTA is normally added to larval rearing water to encourage larval development and survival (Simon, 1981; Mock, 1982; Fox, 1983; Liao *et al*., 1983; GESAMP, 1997). However, an overdose of chelating agents has deleterious effects on the development, survival and growth in crustacean larvae (Davey *et al.*, 1973). The present study demonstrated that untreated Na_2-EDTA seawater produced a better survival rate of *C. belcheri* larvae.

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