

Effect of Salinity on Embryo and Larval Development of Oyster *Crassostrea iredalei*

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Abstract: The effects of salinity on the embryonic and larvae stage of *Crassostrea iredalei* were investigated. Fertilised eggs and one day old D-larvae were subjected to salinities ranging from 0 to 30 ppt at temperature of 30±2°C. At salinity lower than 10 ppt, 100% mortality was observed. For embryo development, the highest survival was observed at salinity 25 ppt with 80.9±2.2% survival with no significant difference compared to 15 and 30 ppt. Shell height and length were both greatest at salinity 30 ppt. Throughout the 11 days culture, the highest larval survival occurred at salinity 15 ppt with no significant difference compared to all other salinities except 10 ppt. Larval shell sizes showed no significant differences between salinities, except for 10 ppt. Optimum culture condition for larvae growth are salinities ranging from 15 to 30 ppt whereby the larval of this species can tolerate wider range of salinity compared to other oyster species and thus, making it a competitive species to be cultured.

Keywords: Salinity, Tropical Oyster, Development, Survival

INTRODUCTION

The early life stage is the most sensitive stage in the life cycle of a bivalve and its tolerance towards various environmental conditions increases as they develop into a benthic juvenile (Bayne *et al.* 1976; Chapporo *et al.* 2008). Carry-over effects from early life stage stresses as such from embryonic stages. It would influence subsequent metamorphosis stages that expense high energetic cost as they begin with energetic shortfall (Gimenez 2010; Hettinger *et al.* 2012). Hence, the sensitivity of the early larval stage determines the bottleneck for species

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persistence and ecological success via successful settlement (Byrne 2011). Significant roles played by low salinity still uncertain due to lack of research conducted as compared to other extensive environmental study in ocean acidification and warming (Albright *et al.* 2008).

It is vital to conduct environmental tolerance study particularly for the complex-life stages of a species, so that species stock can be replenished through optimum condition for hatchery produced high quality seeds. Known publication for the salinity tolerance for early larvae of tropical oyster was limited. Hence, this paper aims to investigate the effect of different salinity regimes on embryonic and early larval development and survival of *Crassostrea iredalei*, one of the commercially important species.

MATERIALS AND METHODS

The experiment was conducted at a research hatchery located at Pulau Betong, Pulau Pinang, Malaysia. Eight test salinities (0, 5, 10, 15, 20, 25, 30, and 35 ppt) were used in this experiment. Respective salinities were prepared and determined using refractometer (ATAGO Ltd.).

The oyster broodstock were stripped spawned. The gametes were sieved and pooled to fertilise in 25 ppt filtered seawater. Fertilised eggs were transferred to different test salinities in tanks containing 30 L seawater at stocking density of 10 embryo mL⁻¹ in triplicates. No aeration was supplied throughout the experiment. At the end of 24 hours, the samples were collected in 35 µm mesh sieve and concentrated to 1 L volume. Three 1 mL subsamples were collected and the number of normal D-hinged larvae was recorded and shell size measured (n = 30) using compound microscope. The normal D-hinged larvae were defined according to His *et al.* (1997).

For larval experiment, 24 hour old D-larvae harvested were added into 30 L culture tank with respective salinities in triplicates at density 10 embryos per mL⁻¹. Slow aeration was supplied throughout the experiment. Larvae were fed daily with phytoplankton *Isochrysis galbana* with initial feed concentration of 5000 cells mL⁻¹ and increased progressively as the larvae developed (Tan & Wong 1996). Mixture of *Chaetoceros calcitrans* and *I. galbana* were given starting Day 5. Culture seawater was replenished every two days during which samples were sieved and concentrated to 1 L for survival count and size measurements. The experiment were terminated when survival in all culture less than 50% at Day 11. Temperature during the experiment ranged within 30±2°C, dissolved oxygen 6.0±0.5 mg/L and pH 7.9±0.1. All data were test for normality using Shapiro-Wilk normality test and analysed with one way ANOVA and LSD significant test to examine the effects of salinity on development and survival of *C. iredalei*.

RESULTS AND DISCUSSION

Percentage of *C. iredalei* eggs that developed into normal D-hinged larvae at salinity 5 to 30 ppt are shown in Figure 1. More than 70% normal D-hinged larvae were developed at salinity 15 to 30 ppt. The highest survival yield was recorded at 25 ppt (80.9%) and significantly different ($p>0.05$) compared to other salinities except 15 and 30 ppt. Total mortality was observed at salinities 0 and 5 ppt. Figure 2 shows the highest growth was recorded at salinity 30 ppt with mean shell height of $58.96\pm 3.48\ \mu\text{m}$ and shell length of $68.36\pm 2.12\ \mu\text{m}$. No significant differences ($p< 0.05$) were observed in shell height between embryo developed at salinity 30 ppt, with 20 and 25 ppt. As for shell length, there were no significant differences with all other salinities except for 10 ppt. Most embryos of tropical oyster species including *C. iredalei*, as shown in Table 1, thrive well at salinity around 25 ppt. An important aspect in determining the tolerance towards salinity variation for embryo is the broodstock condition and spawning environment (Chin & Lim 1975). The oyster broodstock used in this study were collected from oyster farm cultured at salinity ranging from 25 to 30 ppt, therefore justify the optimal salinity for this species.

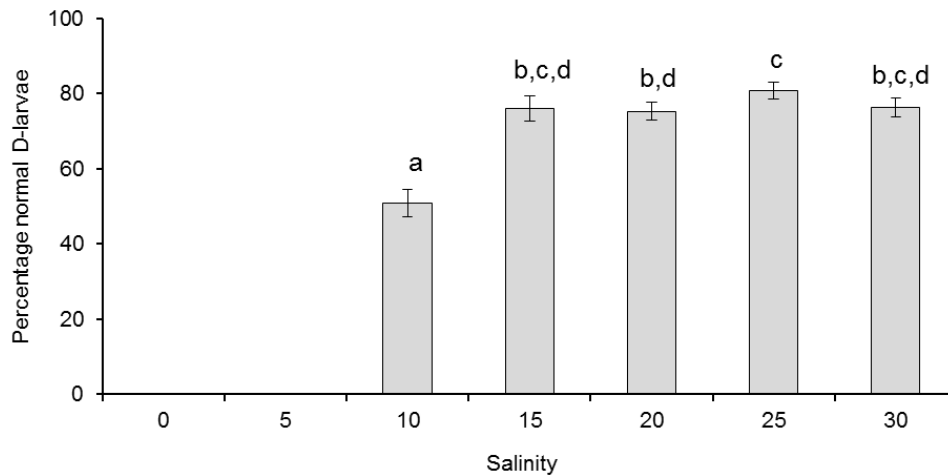


Figure 1: Percentage of *C. iredalei* eggs that developed into normal D-hinged larvae at different salinities.

Note: Bars with same alphabet(s) were not significantly different ($p>0.05$).

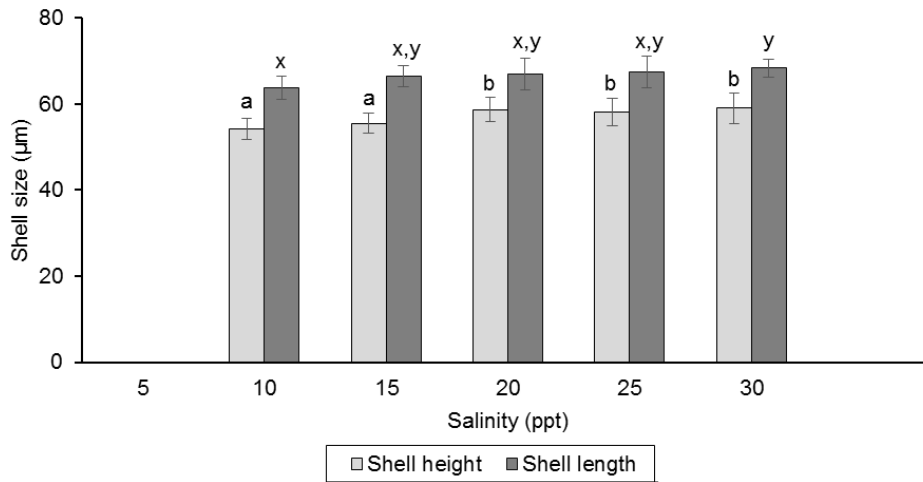


Figure 2: Mean shell size of *C. iredalei* normal D-hinged larvae at different salinities
 Note: Bars with same alphabet(s) were not significantly different ($p>0.05$).

Table 1: Optimum salinities and tolerance range for development of eggs to D-larvae of various commercial oysters.

Species	Salinity (ppt)		References
	Tolerance range	Optimum	
<i>Crassostrea iredalei</i>	10–30	15–30	This study
	10–40	25	Sudrajat (1990)
<i>Crassostrea belcheri</i>	12–30	24–30	Tan and Wong (1996)
<i>Crassostrea rhizophorae</i>	16–40	25–37	Dos Santhos and Nascimento (1985)
<i>Saccostrea cucullata</i>	15–40	25	Sudrajat (1990)
<i>Crassostrea iredalei</i>	10–30	15–30	This study

The highest larval survival occurred at salinity 15 ppt throughout the 11 days experiment with exception of day 5 (Fig. 3). At day 7, larvae in all salinities except 10 ppt showed early umbo formation. Larval survival at salinity 10 ppt plummeted 62.3% to only 27.3% at day 7 and eventually less than 1.0% at day 11. High amount of ciliated trochophores were observed at salinity 10 ppt after 24 hours of fertilisation indicating a delay in embryonic development for this species at salinity lower than 15 ppt. This study result agrees to previous studies findings that exposure to low salinity delays the larval growth and reduce survival rate of the species (Madrones-Ladja 2002; Dove & O'Connor 2007). During termination on day 11, the highest survival observed at 15 ppt (50.9±8.45%). Upon termination, the greatest size was observed at salinity 20 ppt (SH: 136.07±27.07

μm ; SL: $108.64 \pm 21.77 \mu\text{m}$) (Fig. 4). Larvae size has no significant differences with all salinities except for 10 ppt. Nevertheless, early larvae of *C. iridalei* is expected to survive better in natural estuarine due to variation of high and low salinity throughout the day compare to continuous submergence in low salinity as in this experiment (Qiu *et al.* 2002).

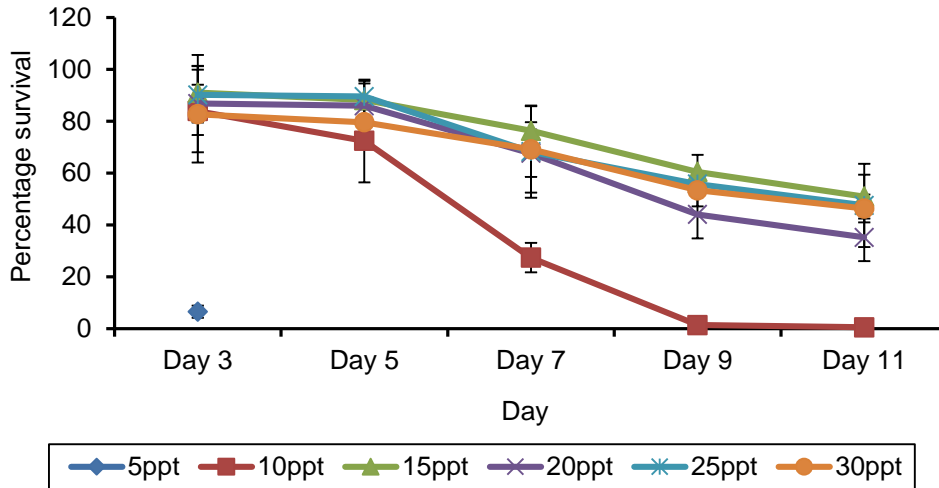


Figure 3: Percentage of *C. iridalei* larval survival at different salinities.

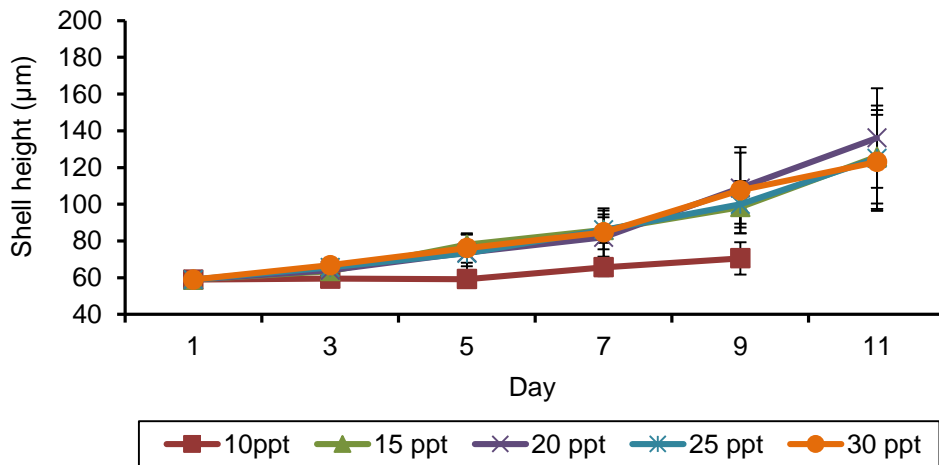


Figure 4: Mean shell height of *C. iridalei* larvae at different salinities.

CONCLUSION

Overall, this study suggests that lower salinity exhibited negative consequences on survival and performance of oyster, *C. iredalei*. This study provides salinity data to improve successful embryonic and larvae development through optimising conditions for the hatchery culture of the species and evaluate the possibility of oyster natural recruitment in the estuarine. The extent of broodstock habitat influencing the salinity tolerance range of this species needs to be further studied.

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