Development of *Crassostrea belcheri* (Sowerby, 1871), *Crassostrea iredalei* (Faustino, 1932) and inter-specific cross spats at different salinity

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Abstract

Hybrids between *Crassostrea belcheri* (CB) and *C. iredalei* (CI) were produce to evaluate their aquaculture potential. These inter and intra-specific crosses consisted of CB♀XCB♂, CI♀XCI♂, CB♀XCI♂ and CI♀XCB♂. To evaluate their environmental preferences, the spats of 40 day-old were cultured in 10, 20 and 30 ppt for 30 days. Hybrids showed significantly higher (p<0.05) shell increment when cultured in 20 ppt compared to its parental crosses. Subsequently, a short salinity stress experiment was conducted using spat of 140 day-old in 10, 15, 20, 25 and 30 ppt for 10 days. Then followed by the experiment salinity decrements with gradual, intermediate and rapid salinity decrease were carried out on 140 day-old spats for 10 days. In these two experiments, spats of 140 day-old were able to tolerate the different treatments tested evident with the 100% survival for all treatments.

Keywords: inter-specific cross, parental cross, spats, salinity, Crassostrea belcheri, Crassostrea iredalei

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Introduction

Oysters belong to the genus *Crassostrea* offers certain traits and desirable characteristics such as high salinity tolerance, high growth rate, high condition index, and easy harvest from nature (Tan and Wong, 1996). Oyster from the genus *Crassostrea* are being commercialized by the economic sector even with the abundance of other oysters from the genus *Ostrea* and *Saccostrea* in Malaysian water. High demand for oyster from genus the *Crassostrea* is basically due to its desirable taste (Devakie and Ali, 2002). *Crassostrea belcheri* and *C. iredalei* are among the tropical oysters found in Malaysian waters. Between these two oysters, *C. iredalei* is preferred by local consumers for its delicious and creamy flesh. The only disadvantage is being small in size.

Crassostrea belcheri or popularly known as white scar oyster, has golden brown flesh, chewy textures. It is bigger than other oysters, with wide salinity tolerance of 12-30 ppt, and able to withstand low salinity of 3-5 ppt for 7-8 days during monsoonal flood (Tan and Wong, 1996). Based on these significant values, hybridization may produce a new species with good growth and survival, tasteful, wide salinity tolerance, and bigger in size. In shellfish farming, various attempts have been conducted to produce inter-specific or heterosis progenies to offer oyster with superior quality of flesh, as well as high survival and growth. Inter-specific hybridization between same genus and closely related species has been carried out on clam (Bert *et al.,* 1993), mussels (Rawson *et al.,* 1999; Bierne *et al.,* 2002), and oyster (Huo *et al.,* 2014; Huo and Wang, 2014).

Key factors for oyster's growth are temperature and salinity (Soniat, 1985; Soniat *et al.,* 2005). Changes in ecological habitats can cause physical and chemical stresses to marine organism (Holliday *et al.,* 1993). In general, oysters are able to tolerate a wide range of salinity (Galtsoff, 1964). They have the ability to adapt and survive under high salinity fluctuation (Pollack *et al.,* 2011). However, duration and timing of salinity related stress affect the growth of oyster depending on its life phase (Livingston *et al.,* 2000; Peyre *et al.,* 2009). The most vulnerable and sensitive phase is during the planktonic stage. During this critical phase, larvae are highly susceptible and vulnerable to conditions like water quality, predation, disease, and insufficiency of metabolic store before settlement (Mann, 1988; Rumrill, 1990; Fraschetti *et al.,* 2002; Verween *et al.,* 2007).

In bivalve, temperature and salinity stress are two crucial parameters influencing their developments and reproductions. Various studies have evaluated the salinity tolerance of oyster (Davenport and Wong, 1992; Tan and Wong, 1996; Devakie and Ali, 2000a), however, there is none on *C. belcheri*, *C. iredalei* and their hybrids. This study was carried out to determine the salinity tolerance and its decrement

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strategy for oyster spats of parental and inter-specific crosses.

Materials and method

Location of study

The study was carried out at the hatchery of Fisheries Research Institute (FRI) Pulau Sayak, Kedah. Gametes were obtained through mechanical stripping. Oyster spats of inter-specific and pure crosses of *C. belcheri* and *C. iredalei* were produced and used for all experiments.

Salinity tolerance

Three experiments were conducted to determine the salinity tolerance of inter-specific and pure crosses of *C. belcheri* and *C. iredalei* spats. In the first experiment, 40 day-old spats size ranging from 3-9mm from inter-specific and pure crosses of *C. belcheri* and *C. iredalei* were cultured in 10, 20, and 30 ppt to determine their salinity tolerance. All treatments were triplicated, using 300L tank. Experimental period was for 30 days.

In the second experiment, spats were evaluated for their salinity tolerance at 10, 15, 20, 25 and 30 ppt for short term exposure of 10 days. All treatments were triplicated with 8 individual per replicate (Rupp and Parson, 2004; Liang *et al.*, 2009) in 1000ml container.

In experiment 3, all spats were conditioned in 30ppt for 24 hours prior to salinity decrement. Spats were evaluated for their tolerance to salinity decrement of 5, 10, and 15ppt/day, until salinity reaches 5ppt. All treatments were triplicated with 20 spats per replicate in 1000ml container. Control group was using spats cultured in 30 ppt for similar experimental period of 10 days.

For experiment 2 and 3, spats used were of 140 day-old with size ranging from 8-22 mm. Data collected for these 2 experiments was only on survival. Factorial designed for all experiments were CBXCB: *C. belcheri* ♀ X *C. belcheri* ♂, CIXCI: *C. iredalei* ♀ X *C. iredalei* ♂, CBXCI: *C. belcheri* ♀ X *C. iredalei* ♂, and CIXCB: *C. iredalei* ♀ X *C. belcheri* ♂. In all the experiments, spats were fed with 25000 cells per ml of *Isochrysis* sp without salinity changes (Nell and Holliday, 1988).

Survival and growth

Growth performance was evaluated by measuring shell height and width to the nearest mm using vernier caliper (Mitutoyo). Mortality was monitored and recorded throughout the experimental period.

Water quality measurements

Water quality parameters (temperature, pH, salinity) were recorded once a day. Temperature was measured using thermometer, pH with pH pen (Ezodo, Taiwan) and salinity with refractometer (Atago, Japan). Water salinity of 10, 20 and 30 ppt were prepared by addition of freshwater to filtered seawater.

Statistical analysis

Data of shell height and width length were analyzed statistically using one way analysis of variance (ANOVA),

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followed by Duncan's multiple comparison test. Data in percentage were transformed using arcsine before being used for ANOVA.

Results and discussion

Height and width increment of spats of parental and inter-specific crosses of Crassostrea belcheri and Crassostrea iredalei

In Experiment 1, after 30 days of culture, there was no significant different (p>0.05) in spats shell height when cultured in 20 and 30 ppt (Table 1). However, at 10 ppt, the spats of *C. iredalei* parental exhibited significantly (p<0.05) lowest shell height increment compared to others.

Table 1: Increment of shell height of spats of parental and inter-specific crosses of *Crassostrea belcheri* and *Crassostrea iredalei*.

Salinity (ppt)	CB♀CB♂	CI♀CI♂	CB♀CI♂	CI♀CB♂
10	2.47±0.8 ^{ab}	1.60±0.29 ^{b}	3.34±0.08 ^{a}	2.29±0.02 ^{ab}
20	2.41±0.14 ^{c}	1.42±0.07 ^{d}	3.46±0.05 ^{a}	2.54±0.05 ^{b}
30	1.43±0.14^{a}	1.87±0.17 ^{a}	1.85±0.53^{a}	1.87±0.36^{a}

Mean values with the same superscripts within the same row are not significantly different (p>0.05).

Often, the size of *C. belcheri* broodstock is larger compared to *C. iredalei*. Thus, it's not surprising to observe *C. belcheri* having larger spats as compared to *C. iredalei*. However, the inter-specific crosses CB♀CI♂ exhibited significantly higher (p<0.05) increment in height compared *C. iredalei* when cultured in low salinity. This finding indicates that better growth of inter-specific spats can be achieved through the manipulation of salinity.

As for shell width increment of spats (Table 2), at 10ppt, parental *C. belcheri* showed the highest (p<0.05) width increment, followed by both inter-specific crosses and lastly parental *C. iredalei*. At 20 ppt, parental *C. belcheri* showed the highest (p<0.05) width increment, followed by inter-specific CB♀CI♂ and lastly parental *C. iredalei* and inter-specific CI♀CB♂. At 30 ppt, there was no significant different (p>0.05) between parental and inter-specific crosses. However, parental *C. belcheri* showed comparatively the highest width increment as compared to the others. The inter-specific crosses are emulating the size of *C. belcheri* in all salinities. Thus, larger size of inter-specific crosses can be achieved by lowering the salinity during spat culture. Water temperature in the culture of spats of parental and inter-specific crosses showed no drastic fluctuation with temperature ranged of 26.7–27.9°C throughout the 30 days experimental period (Table 3). As for water pH, readings were lower than 6.50 in some of the cultures however no mortality was recorded.

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Table 2: Increment of shell width of spats of parental crosses and inter-specific crosses of *Crassostrea belcheri* and *Crassostrea iredalei*.

Salinity (ppt)	CB♀CB♂	CI♀CI♂	CB♀CI♂	CI♀CB♂
10	3.86±0.87 [a]	1.11±0.26 [b]	2.44±0.13 [ab]	1.97±0.83 [ab]
20	3.57±0.37 [a]	1.20±0.25 [b]	2.05±0.59 [ab]	0.86±0.28 [b]
30	2.18±0.33[a]	0.79±0.32[b]	1.14±0.39[ab]	1.45±0.77[ab]

Mean values with the same superscript within the same row are not significantly different (p>0.05).

Table 3: Range of water temperature and pH of water during the 30 days spat culture at different salinities

Spats	Salinity (ppt)	Range	
		Temperature (°C)	pH
CB♀XCB♂	30	26.9 – 27.6	6.57 – 8.62
CI♀XCI♂	30	27.0 - 27.7	6.17 – 8.92
CB♀XCI♂	30	26.6 – 27.8	6.38 – 8.73
CI♀XCB♂	30	27.0 – 27.8	6.87 – 8.37
CB♀XCB♂	20	27.0 – 27.9	6.52 – 8.37
CI♀XCI♂	20	26.7 – 27.8	6.44 – 8.62
CB♀XCI♂	20	27.0 – 27.9	6.38 - 8.44
CI♀XCB♂	20	26.8 – 27.9	7.03 – 8.37
CB♀XCB♂	10	27.0 – 27.9	6.38 – 8.73
CI♀XCI♂	10	26.9 – 27.9	6.41 – 8.11
CB♀XCI♂	10	27.1 – 27.9	6.17 – 8.44
CB♀XCB♂	10	27.1 – 27.8	6.17 – 8.33

Survival of spats of parental and inter-specific crosses of Crassostrea belcheri and Crassostrea iredalei exposed to different salinity stress and decrement

In Experiment 2, hundred percent survivals were observed for spats from all crosses at 10, 15, 20, 25, and 30 ppt for a short period of 10 days. This study imitates the environmental condition in Malaysia during dry and monsoonal season. During monsoonal season, the salinity can fluctuate to as low as 10 ppt. Salinity plays a vital role to trigger the spawning of tropical oyster of *C. belcheri* (Tan and Wong, 1996). While in Experiment 3, spats from all parental and inter-specific crosses were able to survive when exposed to different salinity decrements. These different levels of salinity decrement are similar to the actual condition of tropical environment during monsoonal season in Malaysia.

In the present study, *Crassostrea* hybrids and parental crosses are able to tolerate wider salinity range of 5-30 ppt during juvenile stage. In Experiment 1, spats of hybrid and parental crosses cultured for 30 days in different salinities were evaluated based on tropical environment. At higher salinity, oyster valves opened to facilitate osmoregulation (Nell and Holliday, 1988). At lower range of 28 to 30 ppt, exposure to pathogen and other contaminant (Bagenda *et al.*, 2019) is considerably reduced. At lower salinity, the filtration rate of oyster is very much

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reduced and this often caused stress which results in mortality (Southworth *et al.,* 2017; Chang *et al.,* 2016). The valves usually opened for oxygen exchange and to accommodate metabolic activities.

Prolonged period of undesirable salinity will cause stress and subsequently mortality to shellfish (Bagenda *et al.*, 2019; Hand and Stickle, 1977). Lower salinity can cause the degeneration of spat's shell, suppress growth and induce abnormal activities (Christophersen and Strand, 2003; Navarro and Gonzales 1998). Tan and Wong (1996) reported that *C. belcheri* from embryo to D-stage can tolerate salinity of 12 to 30 ppt. However, there is no report on salinity tolerance of spats of tropical *Crassostrea* species and its hybrid in Malaysia. The optimal salinity for conducive growth of *Crassostrea virginica* is between 10-28 ppt (Wilson *et al*., 2005). Nevertheless, adult *C. virginica* can tolerate salinity up to 35 ppt (Buroker, 1983). Prolonged period of unsuitable salinity will lead stress and eventually mortality (Christophersen and Strand, 2003). The optimum salinity requirement varies depending on life stages such as during eggs development, growth, and gonadal maturation (Davis, 1958).

According to Loosanoff (1953), spats are able to tolerate wide salinity range almost similar to adult oyster. However, due to the thin shell, spat is less tolerant to salinity fluctuation. Therefore, these spats are susceptible to extreme temperature (Table 4) (Cáceres-Puig *et al*., 2007; Child and Laing, 1998; Flores-Vergara *et al.*, 2004). Retarded shell growth was observed in *C. virginica* spats when exposed to high salinities (Paynter *et al*., 1995). According to Nell and Holliday (1988), the size of *Crassostrea gigas* spats influence its tolerance to different salinity level. The larger the spats, the better ability to adapt to broader salinity range.

Table 4: Temperature tolerance of various oysters spats.

Species	Temperature (°C)		References
	Tolerance range	optimum	
			Flores-Vergara *et al.,* (2004)
C. gigas	3-32	30	Child, A. R., and Laing, I., (1998)
O. edulis	3- 32	-	Child, A. R., and Laing, I., (1998)
C. corteziensis	16-32	24-30	Cáceres-Puig *et al*., (2007)

Bivalve has two life phases, planktonic at early stage, then later become benthic dweller. Adults of *C. gigas* are able to tolerate up to 55% salinity but the spats only up to 45% (Nell and Holliday, 1988). As such, spats have lower tolerance compared to adult oyster. Hybridization can produced highly tolerant larvae. Hybrid of *Crassostrea ariakensis* and *C. hongkongensis* can tolerate salinity higher than 30 ppt as compared to *C. hongkongensis* (Huo and Wang, 2014). In estuaries, salinity, temperature, pH, dissolved oxygen and sedimentation always fluctuate. When these fluctuations become extreme, the oyster will closed their valve so as to reduce osmotic stress, and in turn will limits feeding, oxygen uptakes, and other metabolic activities (Hand and Stickle, 1977).

In this study, inter-specific spats with size range from 8-18 mm are able to tolerate different salinity levels and decrement. Spats of CIXCB are small in size compared to its parental crosses. However, they able to survive and adapt well to different salinities.

Thus these hybrid spats are able to survive in a broad salinity range similar to its parental crosses. In comparison to other oyster species, *C. belcheri* and *C. iredalei* spats and its hybrids displayed the widest tolerance limits of 5-30 ppt (Table 5). D-stage hybrid larvae of *Crassostrea ariakensis* and *C. sikamea* hybrid (Table 6) were able to withstand salinity up to 35 ppt (Xu *et al.*, 2011). This suggests that the larvae are able to adapt and withstand high salinity due to genome which has better fitness compared to their parental species.

Table 5: Salinities tolerance of various oysters spats in comparison to the present study.

Species	Salinity (ppt)		References
	Tolerance range	optimum	
C. belcheri	5-30	30	Present study
C. iredalei	5-30	30	Present study
C. belcheri♀ X *C. iredalei*♂	5-30	30	Present study
C. iredalei ♀X *C. belcheri* ♂	5-30	30	Present study
C. gigas	15-45	15-30	Nell and Holliday, (1988)
C. hongkongensis	15-30	15	Huo *et al*. (2014)
S. commerciallis	20-40	23-39	Nell and Holliday (1988)

Table 6: Salinities tolerance of various oysters larvae

Species	Salinity (ppt)		References
	Tolerance range	optimum	
C. belcheri	12-30	12-24	Tan and Wong (1996)
C. iredalei	13-28	N	Devakie and Ali (2000b)
C. gigas	15-34	25	Helm and Millican (1977)
C. ariakensis	20-35	N	Xu *et al.* (2011)
C. sikamea	20-35	N	Xu *et al.* (2011)
C. hongkongensis	15-30	15	Huo *et al*. (2014)
C. rhizophorae	25-37	N	Santos and Nascimento (1985)
O. edulis	12-27	N	Davis and Ansell (1962)

N: Not mention

According to Grabowski *et al.* (2004), the optimum growth of *C. ariakensis* occurred at 15-25 ppt. However, the optimum salinity was ambiguous. *Crassostrea ariakensis* can only be found near the lower tidal level since

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this species cannot survive without water for a long period of time. High salinity stress of 35 ppt negatively impacted the survival and growth of larvae aged 1- 7day both in inter-specific and parental crosses (Xu *et al.*, 2011).

Conclusion

Based on the findings of this study, the spats of all inter and intra-specific crosses of *C. belcheri* and *C. iredalei* are able to tolerate all the tested salinities and the different salinity decrements. The hybrid exhibited highest shell increment at 10 ppt as compared to all other crosses. The level and method of salinity decrement are two crucial factors limiting the growth and survival of parental and inter-specific crosses of *C. belcheri* and *C. iredalei* from larvae to spat stage. Overall, *C. belcheri*, *C. iredalei* and their hybrids are able to tolerate salinity decrement between 5 to 30 ppt.

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