Early development of the blue mussel *Mytilus edulis* (Linnaeus, 1758) cultured in potassium-fortified inland saline water

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10 **Abstract**

11 The low potassium concentration in inland saline water (ISW) restrains the normal development of
12 cultured marine organisms, and thus, possesses challenges for the development of ISW aquaculture.
13 Therefore, assessing the effects of potassium fortification in ISW on the performance of cultured
14 marine species is an important step to determine the feasibility of their culture in ISW. The aim of this
15 research was to investigate the effects of potassium fortification in ISW on the performance of early
16 life stages of the blue mussel *Mytilus edulis* including fertilised eggs, trochophore, veliger and
17 pediveliger larvae. These stages were reared in five different levels of potassium-fortified ISW,
18 namely 20, 40, 60, 80 and 100% of potassium levels equivalent to the potassium level in ocean water
19 (OW) and two controls namely, ISW at 27 ppt (ISW27) and OW at 25 ppt (OW25). The results
20 showed that the higher levels of potassium in ISW, particularly with 100% K^+ fortification
21 (ISW100K^+), invariably improved the survival and size, and reduced the developmental stage interval
22 and deformities of blue mussel larvae. Deformities, such as faulty cell cleavage, abnormal formation
23 of trochophore larvae, protruding mantle in veliger larvae, and indented shell margin in veliger and in
24 pediveliger, were observed when reared in any ISW. However, rearing in ISW did not result in any
25 deformities in settlement larvae. The number of deformities was reduced at higher K^+ fortification
26 levels, and there were no deformities in pediveliger larvae reared in ISW100K^+ and in OW. These
27 results showed that K^+ fortification in ISW improves the performance of the rearing of the larval
28 stages of the blue mussel.

29 Keywords: deformity, fortification, early life stage, inland saline water, K^+, *Mytilus edulis*.

30 **1. Introduction**

31 Salinization caused by natural and anthropogenic reasons (Bennetts *et al.*, 2006; Szabolcs, 1989) has
32 rendered more than 80 million hectares (Ghassemi *et al.*, 1995) of land in more than 100 countries
33 useless for agricultural production (NLWRA, 2000; Rengasamy, 2006). On the other hand, inland
34 saline water (ISW) has the potential to be used as a suitable resource for aquaculture of marine species
35 (Barson and Barrett-Lennard, 1995). Many studies have attempted to investigate the potential to

culture various marine seaweeds (Kumar *et al.*, 2010), invertebrates (Fotedar *et al.*, 2008; Prangnell and Fotedar, 2006b; Tantulo and Fotedar, 2006) and vertebrates (Barman *et al.*, 2005; Doroudi *et al.*, 2006; Fielder *et al.*, 2001). However, commercialisation of ISW aquaculture is constrained due to salinity fluctuations caused by the alteration of rainfall and high solar radiation (Prangnell, 2007), fluctuating calcium concentrations (Prangnell and Fotedar, 2006b), and deficiency of potassium ions relative to ocean water (OW) (Nulsen, 1997; Prangnell and Fotedar, 2006b). Most marine species, when cultured in ISW, show a low survival rate (Fielder *et al.*, 2001; Partridge and Creeper, 2004; Roy *et al.*, 2009), growth rate (Partridge and Creeper, 2004; Roy *et al.*, 2009), and a high risk of skeletal myopathy (Partridge and Creeper, 2004).

However, the fortification of potassium to ISW has been shown to improve survival and growth rates in many adult marine species such as mulloway *Argyrosomus japonicas* (Doroudi *et al.*, 2006), Australian snapper *Pagrus auratus* (Fielder *et al.*, 2001), grey mullet *Mugil cephalus* (Barman *et al.*, 2005), western king prawn *Penaeus latisulcatus* (Prangnell, 2007; Prangnell and Fotedar, 2006b), Pacific white shrimp *Litopenaeus vannamei* (Liu *et al.*, 2014; Roy *et al.*, 2010), black tiger prawn *Penaeus monodon* (Tantulo and Fotedar, 2006), and alga *Gracilaria cliftonii* (Kumar *et al.*, 2010). So far, these studies mainly focus on the adult stages of marine species, and only a few studies investigated the effects of potassium fortification in ISW on the development of larval stages of marine species, e.g. juvenile greenlip abalone *Haliotis laevigata* (Fotedar *et al.*, 2008), and the prawns *P. monodon* (Rahman *et al.*, 2005; Tantulo and Fotedar, 2006) and *P. latisulcatus* (Prangnell, 2007; Prangnell and Fotedar, 2006b).

Among marine species, blue mussels are an important candidate for aquaculture (Hickman, 1992) due to their wide distribution, no supplementary feeding requirements, higher nutritional value, and good taste (Gosling, 1992, 2008; Seed, 1992). Blue mussel aquaculture is practised in many European countries and China (Smaal, 2002) with different culture methods (Buck *et al.*, 2010; Smaal, 2002). In Australia, blue mussels are cultured in Tasmania, Western Australia, Victoria, South Australia and New South Wales with the production of 3585 tonnes in 2013 valued at ca.10 million dollars (Stephan and Hobsbawn, 2014). However, the production of blue mussels is restrained due to the poor seed

supply and the legislative limitations regarding environmental issues and questions with respect to the sustainability of coastal aquaculture (Smaal, 2002). In this context, the development of blue mussel aquaculture in ISW may mitigate the environmental issues facing coastal aquaculture (Ogburn, 1998) and also add value to ISW aquaculture by offsetting the costs of the negative effects of salinization (Gooley *et al.*, 1998). However, it is imperative to investigate the culture potential of early stages in K^+ ISW rather than trying to acclimate the juveniles who were previously cultured in OW into ISW. This study aimed to investigate the effects of potassium fortification in ISW on the performance of the early life stages of blue mussels.

2. Materials and methods

2.1. Blue mussels

Adult blue mussels (shell length 5.30 ± 0.30 cm) were collected from Esplanade Nedlands, Western Australia (31°59'S, 115°48'E) and were transported directly to the Aquatic Research Laboratory, Curtin University. The mussels were cleaned of any epifauna, epiflora and other attached materials with a plastic brush before acclimating them indoors in a glass tank (198 L, 1.1 × 0.6 × 0.3 m; length × width × depth) for 10 days. The tank was supplied with 1 µm-filtered OW at 25 ppt under a static condition and with continuous aeration. During the acclimation, the water temperature was maintained at 20°C (Yaroslavtseva and Sergeeva, 2006) using an automatic heater (Sonpar, HA-200, Zhongshan, Guangdong, China). Twenty percent of the water was exchanged daily before the addition of microalgae (Instant algae, Shellfish Diet 1800, Reed Mariculture, USA).

Microalgae were cultured in 10-L carboys. The seawater was chlorinated (0.1 $mL.L^{-1}$) for 24 h, then neutralised with 0.1 g. L^{-1} sodium thiosulfate and enriched with an F2 algae boost (1 $mL.L^{-1}$) before the addition of microalgae inoculum. Microalgae were cultured under the 12:12 light:dark condition at a pH range of 7.5 to 8 and room temperature of 22°C. During the experiment, larvae from veliger onwards were fed with the microalgae at 80,000 cells. mL^{-1} (Gazeau *et al.*, 2010).

2.2. Spawning induction

88 The mussels were induced to spawn by the temperature shock method (Pronker *et al.*, 2008;
89 Thompson, 1979). Fifteen blue mussels were placed in a spawning tank containing OW at 25 ppt,
90 with continuous aeration. Water temperature was rapidly increased from 20°C to 30°C in
91 approximately 2 hours using the automatic heater. Once the spawning of the mussels had completed,
92 the adults were returned to the acclimation tank. Fertilised eggs were collected using a 30 µm sieve,
93 placed and maintained in a glass beaker (5 litre) filled with OW, filtered through 1-µm filter, with
94 continuous aeration. Fertilised eggs were counted using a Sedgewick-Rafter counting chamber under
95 a microscope (BH-2, Olympus, Japan), diluted to a density of 100 eggs.mL^{-1} in OW (25 ppt) into a
96 glass tank (V = 15 L), namely a stocking tank, before the commencement of the experiment.

97 2.3. Experimental design and testing

98 To test whether the addition of potassium to ISW improved the performance of early life stages of the
99 blue mussel, each of the four early stages, namely fertilised eggs, trochophore, veliger and pediveliger
100 were reared in one of the five different levels of potassium fortification: 20% (ISW20K$^+$), 40%
101 (ISW40K$^+$), 60% (ISW60K$^+$), 80% (ISW80K$^+$) and 100% (ISW100K$^+$). The levels of potassium
102 addition in ISW were equivalent to the typical concentration of potassium in the OW at the same
103 salinity. ISW at salinities of 27 ppt and OW at 25 ppt were used as controls, as our previous results
104 (unpublished) have shown that the iso-osmotic point (the point when the osmolality of the
105 haemolymph and external medium are the same at a particular salinity) of blue mussels in OW and
106 ISW were 700 mOsm.kg^{-1} and 800 mOsm.kg^{-1}, respectively. These osmolalities equate to 25 and 27
107 ppt in OW and ISW, respectively. In order to keep the energy expenditure limited to ionic regulation
108 caused by only K^+ gradients between the haemolymph and external environment and minimise the
109 energy expenditure due to the overall osmoregulation, 25 and 27 ppt of OW and ISW, respectively,
110 were used as two controls in the current trial. OW and ISW were procured from Hillarys (31°49'S,
111 115°45'E) and a lake at Wannamal (31°15'S, 116°05'E), Western Australia, respectively. The
112 salinities of OW and ISW were reduced to 25 and 27 ppt, respectively, by adding deionised water. All
113 K^+ fortification levels were prepared by mixing hydrous potassium chloride (purity > 99%, Sigma-

Aldrich, Germany) with ISW27 to obtain the stock water. These stock waters were stored separately in 125 l plastic containers and were filtered through 1 µm filter before using for the experiment.

The ionic composition of these water treatments used in this experiment was analysed by CSBP Soil & Plant Laboratory, Bibra Lake, WA using Inductively Coupled Plasma spectroscopy. To measure the osmolality of the media, 50 µL of water from each of seven stocked waters were collected using a 200 µL pipette. The measurements were performed using a cryoscopic osmometer – Osmomet 030 (Gonotec, Inc, Germany).

To obtain the trochophore stage, 100 individuals at the two-cell stage were transferred from the stocking tank of OW at 25 ppt to petri dishes (in triplicate) containing 20 mL of one of the water types to observe the appearance of trochophore every 30 minutes. The trochophore stage was marked by the time at which 50% of the fertilised eggs were transformed to the trochophore stage (Bayne, 1965). Similarly, 100 newly transformed larvae at each stage of trochophore and veliger were transferred from the stocking tank to petri dishes containing one of the different water types for the observation of the transformation of these larvae to the next stage of veliger and pediveliger every 6 hours, respectively.

Similarly, to observe the settlement, 100 newly transformed pediveliger larvae from the stock tank were placed into each 40 µm-cell strainer (BD Falcon, BD Biosciences, Bedford, USA). Each cell strainer was placed into 250 mL glass beakers containing one of the different water types with continuous aeration. The development of larvae was observed every 12 hours until they settled. The byssal threads of adult blue mussels were placed into each cell strainer for larvae settlement (Eyster and Pechenik, 1987). Twenty per cent of the water in each beaker was exchanged daily. Each stage was exposed to different water types in triplicate.

2.4. Data analysis

Survival was calculated based on the formula: $S = 100 \times (nt/no)$

where S is the survival (%), nt is the number of larvae of the blue mussels at time t, and no is the number of the early larvae of the blue mussels at the commencement of each stage.

Sizes of each larval stage were measured at the end of the corresponding development stage when 50% of the larvae had moulted to the next developmental stage. The developmental stages of blue mussels were identified under the microscopes (SZH and BH-2, Olympus, Japan) based on the morphological description (His *et al.*, 1997; Redfearn *et al.*, 1986; Saranchova and Flyachinskaya, 2001).

Developmental stage interval (DSI, hours) was estimated by subtracting the time when 50% of larvae moulted to the next developmental stage from the time when they were newly moulted from the previous development stage.

Morphological deformity was determined based on previous descriptions (Andersen *et al.*, 2013; His *et al.*, 1997; Kurihara, 2008). Deformity was calculated based on the formula: $D = 100 \times (nd/no)$

where D is the deformity (%), nd is the number of deformed larvae of the blue mussels at time t, and no is the number of the larvae of the blue mussels at the commencement of each stage.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) and the least significant difference (Tukey's post-hoc tests) multiple comparisons were used to determine the significant differences ($p < 0.05$) among the means. Percentage values were arcsine-transformed to achieve normality for ANOVA assumption. Linear and second order regression analyses were performed on the survival, size, DSI and deformity of blue mussels as a function of K^+ fortification levels in ISW. Data were represented as mean ± standard error (SE). All statistical analyses were performed in SPSS version 22 for Windows.

3. Results

3.1. Environmental parameters and haemolymph osmolality

161 The addition of K^+ to ISW brought the K^+ concentrations closer to K^+ concentrations in OW without
162 changing the concentrations of other ions. The Na^+/K^+ ratios decreased with the elevated K^+
163 concentrations (Table 1).

164 3.2. Survival

165 Over 78 per cent of the fertilised eggs transformed successfully to trochophore, and K^+ fortification
166 had no effect ($p > 0.05$) on the hatching success of fertilised eggs. Similarly trochophore larvae were
167 transformed to veliger with ca. 80% of success. Higher K^+ levels significantly ($p < 0.05$) increased the
168 survival of pediveliger from ca. 55% to 68% (Fig. 1). Similarly, the number of the newly settling
169 larvae was significantly ($p < 0.05$) higher at higher K^+ (Fig. 1), wherein, the percentage of settling
170 larvae reached ca. 62% in the highest K^+ levels ($ISW100K^+$), 24% higher than the ISW control,
171 showing the high sensitivity of pediveliger and settlement stages to the increased K^+ fortification.
172 Stronger linear correlations were shown between survival rate with pediveliger and settling larvae.
173 However, survival of trocophore exhibited stronger ($R^2 = 0.95$) second order relationship with K^+
174 fortification levels in ISW. The survival of veliger stage of blue mussels was independent of K^+ levels
175 as shown by R^2 value of 0.53.

176 3.3. Size

177 Size of trochophore (81–84 µm), veliger (120–138 µm) and pediveliger (301–331 µm) were not
178 affected ($p > 0.05$, Fig. 2) by K^+ levels (Fig. 2). Fortification of K^+, (Fig. 2) significantly ($p < 0.05$)
179 increased the size of settling larvae from 497 µm at the lowest K^+ level to 610 µm at the highest K^+
180 level (25 % increase in size). This also highlighted the sensitivity of settling larvae to the increase in
181 K^+ fortification levels. There was no difference in the size of settling larvae when exposed to
182 $ISW100K^+$ than when reared in OW25 (Fig. 2). Linear regression analysis between K^+ concentrations
183 and the size of early larval blue mussels showed strong correlations in pediveliger ($R^2 = 0.89$) and
184 settlement stages ($R^2 = 0.87$). Size of veliger larvae was weakly correlated ($R^2 = 0.65$) with K^+
185 concentrations, whereas no correlation ($R^2 = 0.01$) was observed in trochophore stage (Table 2).

186 3.4. Developmental stage interval (DSI)

187 DSI of all larval stages were shorter ($p < 0.05$) under higher K^+ levels (Fig. 3). Fertilised eggs lasted
188 10.33 to 12.5 hours before hatching to trochophore. It took 42.0 to 44.5 hours for trochophore larvae
189 to develop into veliger larvae. DSI for pediveliger varied from 675.3 to 721.7 hours to settle using
190 byssal threads. DSI was strongly negatively correlated with K^+ fortification levels in ISW at all
191 studied development stages. However, this negative correlation was linear only in settlement stages
192 (Table 2).

193 3.5. Morphological deformity

194 Normal and abnormal formation of each early stages of blue mussel were shown in Figure 5 and 6,
195 respectively. Four types of deformities were observed during the larval stages, namely faulty cell
196 cleavage (Fig. 6a, b, c), abnormal formation in trochophore larvae (Fig. 6d), protruding mantle in
197 veliger larvae (Fig. 6e), and indented shell margin in veliger (Fig. 6f) and in pediveliger (Fig. 6 g).
198 Deformities occurred in larval stages from trochophore to pediveliger, but were not detected at the
199 settlement stage. Overall, the deformity percentage was low (lower than 5% in all larval stages in any
200 water types). The highest deformity of 4.67% occurred in ISW with no K^+ fortification. The K^+
201 fortification in ISW did not influence ($p > 0.05$) the deformity rate of trochophore and veliger larvae.
202 The deformity rate of pediveliger larvae decreased ($p < 0.05$) with the increase in K^+ levels. K^+
203 concentrations showed strong negative linear correlations with percentages of deformities in
204 trocophore, veliger and pediveliger larvae but stronger positive second order correlation was observed
205 between K^+ levels and number of deformities in fertilised eggs (Table 2).

206 **4. Discussion**

207 Marine species can be successfully cultured in ISW after ISW is either modified by fortifying
208 it with K^+ salts (KCl or potassium fertilizers) (Fisher *et al.*, 2013; Fotedar *et al.*, 2008;
209 McNevin *et al.*, 2004; Prangnell, 2007; Prangnell and Fotedar, 2006b; Tantulo and Fotedar,
210 2006) or formulated feed (Romano and Zeng, 2012; Roy and Davis, 2010; Saoud *et al.*,
211 2007b) for the target species is supplemented with K salts. More studies aiming to culture and
212 improve the feasibility of the hatchery production of marine species in ISW and potassium-fortified

213 ISW are needed. The lack of studies on the hatchery development of molluscs, including blue
214 mussels, in ISW warrants further investigation.

215 Potassium is a primary intracellular ion in aquatic animals (Roy *et al.*, 2010; Shiau and Hsieh, 2001)
216 and plays a crucial role in acid-base balance, osmoregulation, maintaining membrane potentials
217 (Hadfield *et al.*, 2012) and the Na^+/K^+ ATPase activity (Liu *et al.*, 2014). Na^+/K^+ ATPase, a sodium
218 pump that is present in the gill membrane, transports Na^+ and Cl^- ions between the gill epithelial cells
219 and haemolymph to maintain a stable osmoregulation in invertebrates (Charmantier *et al.*, 1985;
220 Mantel and Farmer, 1983). Na^+/K^+ ATPase activity is dependent on the ratio of Na^+ and K^+ in the
221 surrounding environment (Tantulo and Fotedar, 2007). The optimal ratio of Na^+/K^+ for the normal
222 function of Na^+/K^+ ATPase in marine animals varies from 23.85 to 85.20 in juvenile *H. laevigata*
223 (Fotedar *et al.*, 2008), *P. latisulcatus* (Prangnell and Fotedar, 2005) and *L. vannamei* (Zhu *et al.*,
224 2004). A deficiency of K^+ can change the Na^+/K^+ ratio in a way that can inhibit the ability of Na^+/K^+
225 ATPase to function. This may eventually result in the poor survival of marine species (Fisher *et al.*,
226 2013; Prangnell and Fotedar, 2005, 2006a; Tantulo and Fotedar, 2007; Zhu *et al.*, 2004). In line with
227 this, early developmental stages of blue mussels showed higher survival rates when exposed to higher
228 K^+ in ISW. The highest survival and growth at Na^+/K^+ ratio of 28.58 in ISW100K^+ was similar to the
229 survival in OW25 that also had the Na^+/K^+ ratio of 28.58. The lowest survival occurred at the Na^+/K^+
230 ratio of 100.27 in ISW27, suggesting that it is possible to add K^+ to ISW to adjust the optimal Na^+/K^+
231 ratio for better survival of early larvae of the blue mussels.

232 The osmoregulation is a high energy demanding process (Chong-Robles *et al.*, 2014; Saoud *et al.*,
233 2007a), and the deficiency of K^+ results in a significant imbalance of ions between internal and
234 external media (Panikkar, 1968) and forces the pediveliger and settlement larvae to allocate more
235 energy to fix the imbalance through ion-regulatory mechanisms (Deaton, 2001; Silva and Wright,
236 1994). Consequently, energy allocated for growth is reduced (Zhu *et al.*, 2004), resulting in induced
237 reduction in sizes of pediveliger and settlement larvae in K^+-deficient waters. Further, the deficiency
238 of K^+ in the medium can be associated with higher energy investments in the formation and function
239 of osmoregulatory organs.

In our study, K^+ did not influence the size of early larvae, except during the settlement stage, suggesting that the effects of K^+ on the size of early larvae of the blue mussel is related to the formation and functionality of osmoregulatory organs during the development of early larvae of the blue mussel (Bayne, 1971). Stages prior to pediveliger show no developed osmoregulatory organs such as ctenidia (Bayne, 1971), thus, K^+ have no influence on the sizes of these earlier stages. Although the first ctenidial filaments are formed during the pediveliger stage, these ctenidia are not fully functional until the settlement stage (Bayne, 1971), when they are fully responsive to the ionic profile of the external medium. Hence, the K^+ levels in ISW could only have an impact at the settlement stage of the blue mussels.

The effects of K^+ on the DSI of the early larvae of blue mussels are not well understood. Possibly, the shorter DSI of the early larvae in the relatively higher K^+ level (rather than in lower K^+ levels) and the similar DSI of early larvae in ISW100K^+ and OW25 indicate that the lower K^+ levels (> 80%) interfere with normal physiological development and function, for example, by limiting the ionic exchange ability of the gills, as reported in *P. latisulcatus* (Prangnell, 2007), and consequently lengthening the DSI of the blue mussels at lower K^+ levels. In addition, it is possible that K^+ fortification of ISW influences the size of settling larvae indirectly through the underlying changes in the DSI. As longer time is spent in a particular developmental stage (longer DSI), more time larvae would have in increasing their sizes, hence the larger sizes.

Types of morphological deformities of the early larvae that were exposed to different K^+ fortifications in this study were similar to the deformity types found previously in the blue mussel embryos exposed to copper (Hoare *et al.*, 1995) or early larval mussels *Mytilus galloprovincialis* exposed to different pCO_2 (Kurihara, 2008), artificial OW (His *et al.*, 1997), and OW (His *et al.*, 1997) with four deformity types. Trochophore and veliger larvae of the scallop *Pecten maximus* show similar deformities, two days after the exposure to elevated pCO_2 levels (Andersen *et al.*, 2013). In our study, the deformity rate of blue mussel larvae in all water types, even in ISW27, was under 10%, an acceptable rate as recommended by His *et al.* (1997).

Previous studies show that K^+ is a metamorphic inducer because of its ability to influence cell membrane potential (Yool *et al.*, 1986), and also induces larval metamorphosis and settlement of marine invertebrates (Carpizo-Ituarte and Hadfield, 1998; Sánchez-Lazo and Martínez-Pita, 2012; Wassnig and Southgate, 2012; Yang *et al.*, 2008; Yang *et al.*, 2011; Young *et al.*, 2011; Yu *et al.*, 2008; Zhao *et al.*, 2003). The addition of K^+ to OW at 10^{-3} to 5×10^{-2} M induced the peak metamorphosis of *M. galloprovincialis*, and over 90% of the larvae were induced to settle at the excessive concentrations of 20, 30 and 40 mM (Yang *et al.*, 2011). Therefore, it is good practice to culture early stages in K^+-fortified ISW.

From the aquaculture point of view, closing the entire life cycle of any target species in only one type of water is an important proposition to avoid further costs associated with the acclimation process to a different type of water. Therefore, successful hatchery production of blue mussel spats in K^+ fortified ISW is a positive step towards the ISW culture of blue mussels.

In conclusion, potassium-fortified ISW improves the survival rate and size, and reduces the developmental stage interval and deformities, of the early life stages of blue mussels. The 100% K^+ fortification of ISW improves the viability of culturing early stages of blue mussels in ISW. The study shows the feasibility of using ISW fortified K^+ for culturing blue mussels in their early stages.

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475 Highlights:

476 - Information on the potential of culturing early life stages of blue mussel Mytilus edulis in
477 inland saline water is lacking

478 - Fortifying ISW with K+ increases the feasibility of culturing early stages of blue mussels.

479 - Early stages of blue mussels, except settling larvae show four types of deformities.

480 - It is feasible to culture early stages of blue mussels in K+ fortified inland saline water.

481

Figure captions

Figure 1. Survival of early developmental stages of the blue mussel *Mytilus edulis* in response to K^+ fortification to ISWs. Data are presented as mean ± SE. Data with different letters are significantly different ($p < 0.05$).

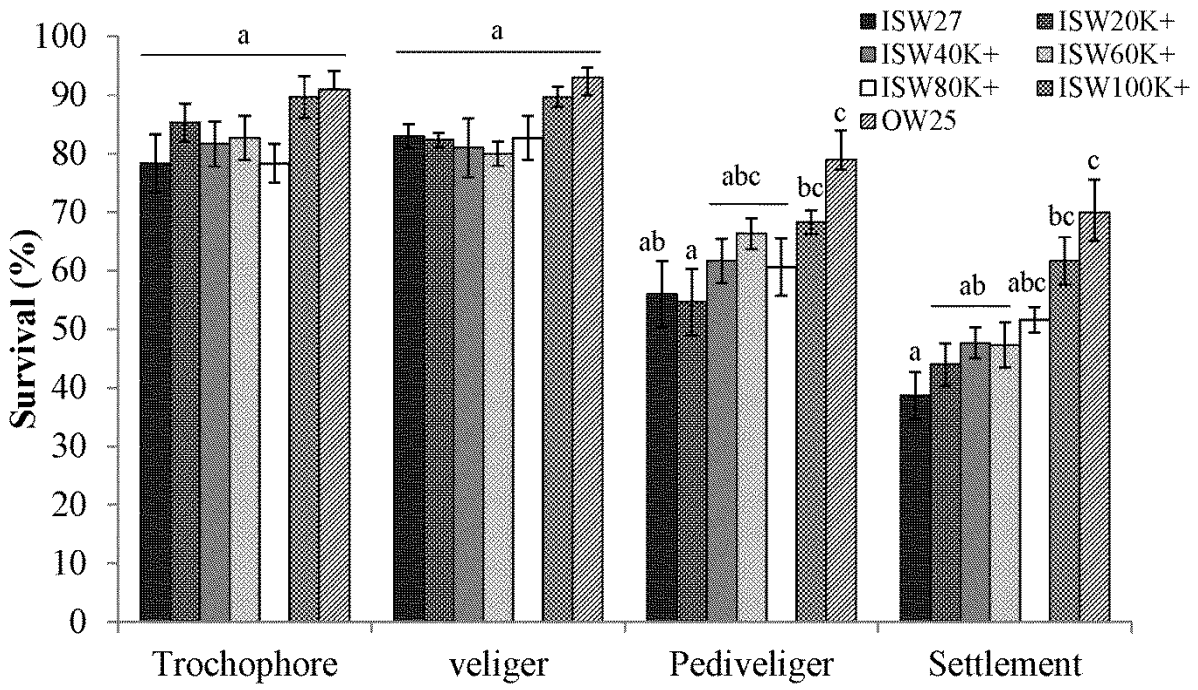
Figure 2. Sizes of early developmental stages of the blue mussel *Mytilus edulis* in response to K^+ addition to ISWs. Data are presented as mean ± SE. Data with different letters within a stage are significantly different ($p < 0.05$).

Figure 3. Developmental stage interval of early developmental stages of the blue mussel *Mytilus edulis* in response to K^+ addition to ISWs. Data are presented as mean ± SE. Data with different letters within a stage are significantly different ($p < 0.05$).

Figure 4. Morphological deformity of early developmental stages of the blue mussel *Mytilus edulis* in response to K^+ addition to ISWs. Data are presented as mean ± SE. Data with different letters within a stage are significantly different ($p < 0.05$).

Figure 5. Development of early stages of blue mussels *Mytilus edulis* in response to K^+ addition to ISWs. (a) eight cell stage; (b) trochophore larva; (c) veliger larva; (d, e, f) settlement larvae; thin arrow: foot; black arrows: byssal thread of adult blue mussels; white arrows: byssal thread of settlement larva of the blue mussels. Scale bar = 100 µm.

Figure 6. Morphological deformity in early larval stages of the blue mussel *Mytilus edulis* in response to K^+ addition to ISWs. (a, b, c) deformed cell division; (d) deformed trochophore; (e, f) deformed veliger and (g) deformed pediveliger. Scale bar = 100 µm.



503

504 Figure 1

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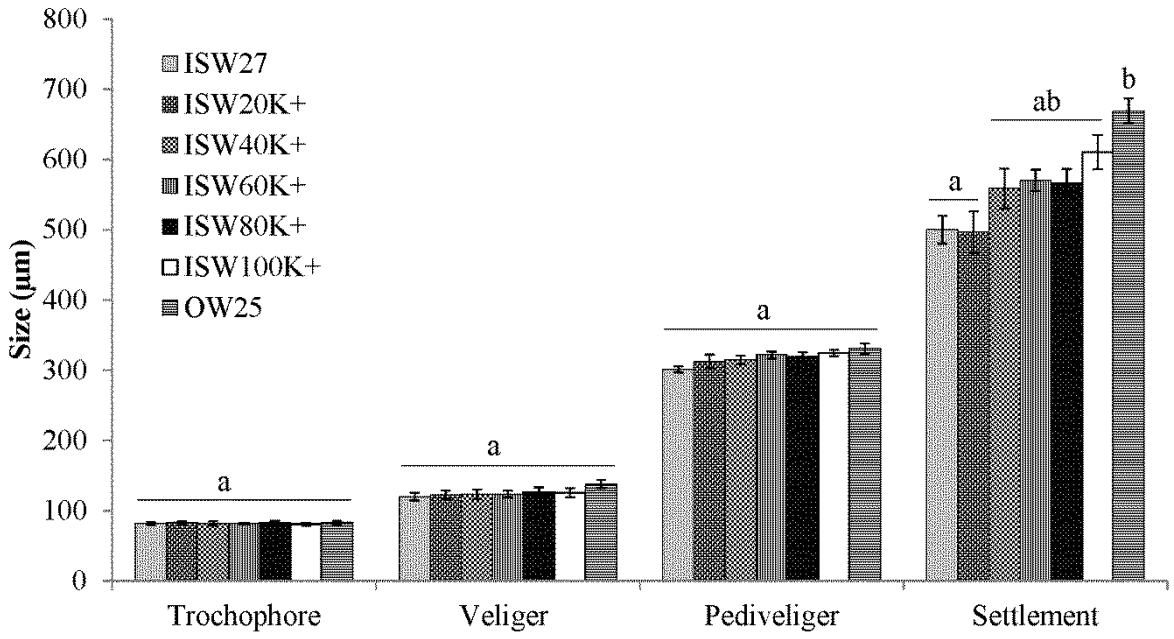

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507 Figure 2

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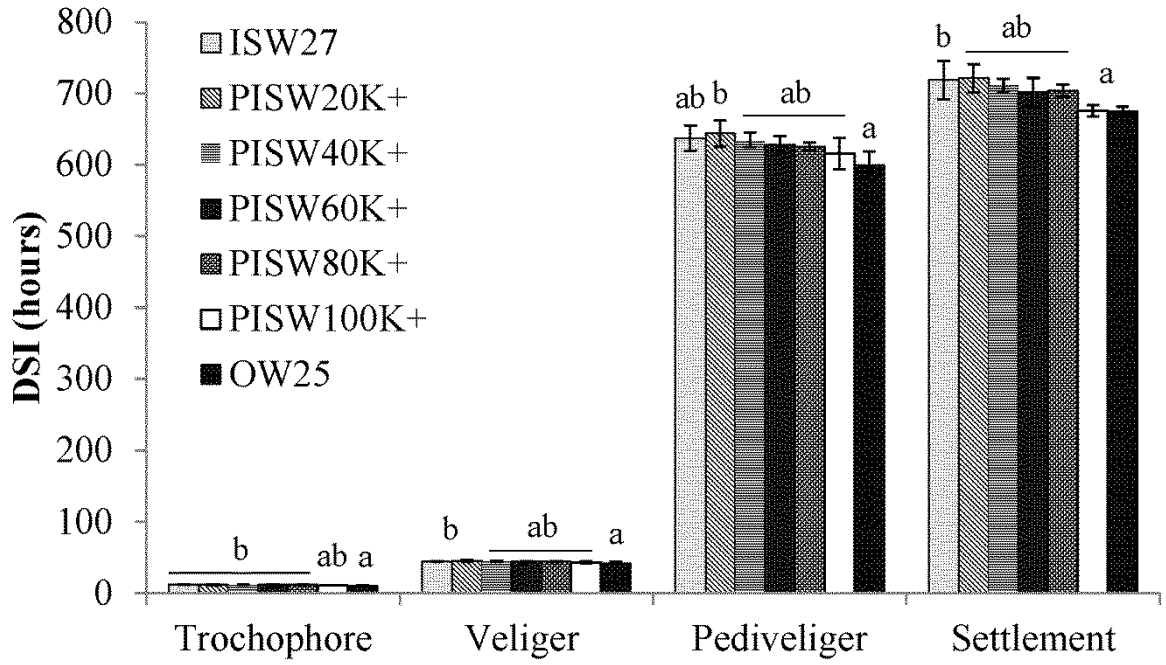

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510 Figure 3

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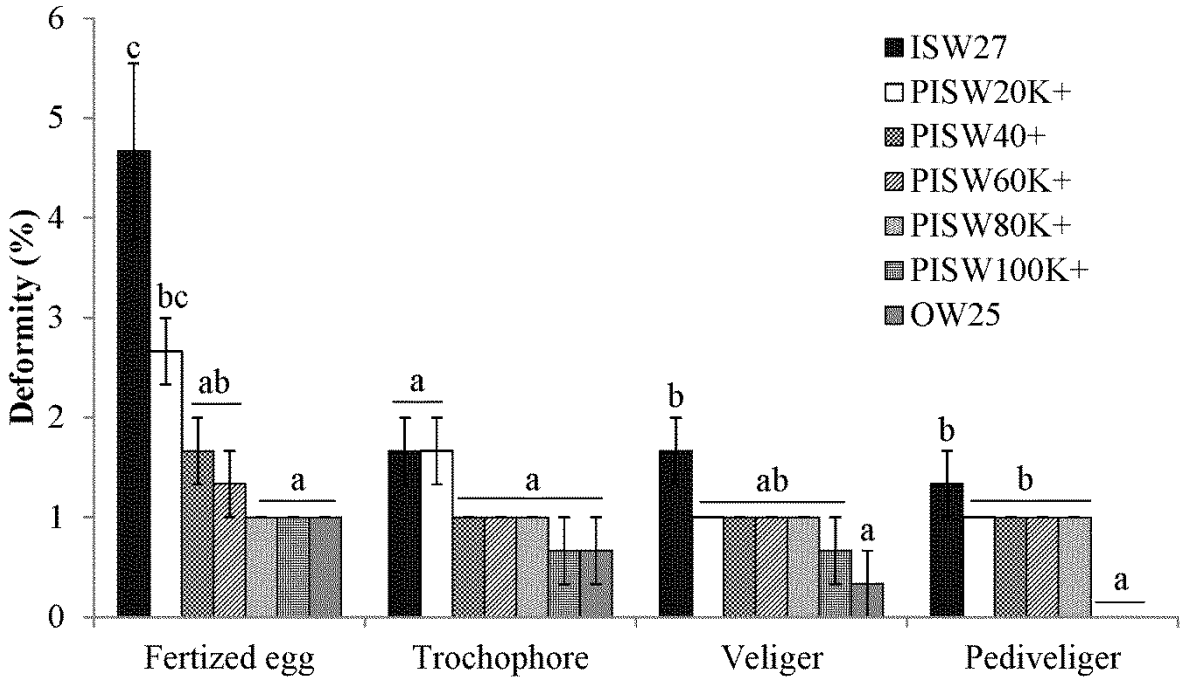

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513 Figure 4

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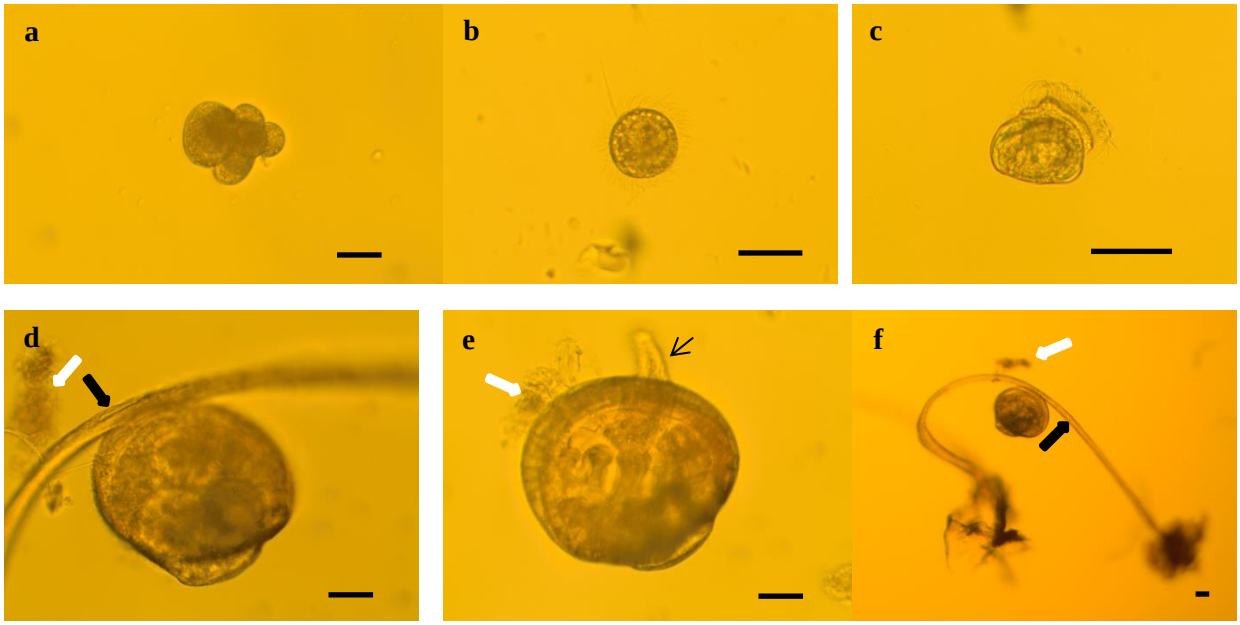
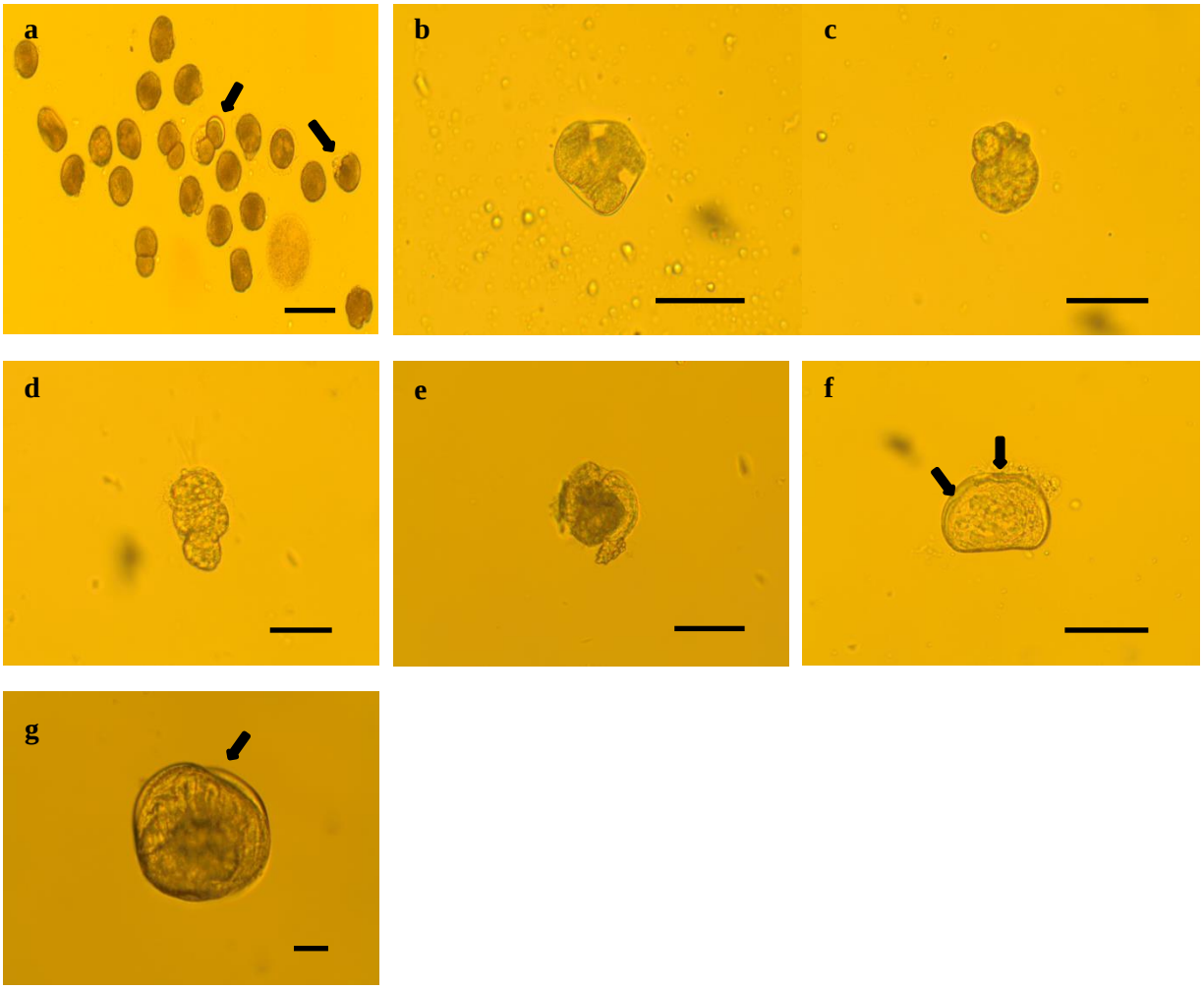


Figure 5.



517 Figure 6

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519 Table 1. The ionic composition of ISWs and OW

Parameters	ISW27	ISW20K^+	ISW40K^+	ISW60K^+	ISW80K^+	ISW100K^+	OW25
Salinity (ppt)	27	27	27	27	27	27	25
Osmolality (mOsm/kg)	719.00	671.33	680.33	669.67	675.67	662.33	659.67
Na^+ ($mg.L^{-1}$)	6584.00	6824.00	6816.00	6872.00	6943.00	6774.00	6480.00
K^+ ($mg.L^{-1}$)	65.66	96.25	127.00	152.40	182.30	217.50	226.70
Ca^{2+} ($mg.L^{-1}$)	431.10	465.20	462.50	456.90	461.50	451.60	231.20
Mg^{2+} ($mg.L^{-1}$)	1145.00	1202.00	1197.00	1189.00	1198.00	1173.00	749.30
S^{2+} ($mg.L^{-1}$)	453.40	483.50	475.90	471.50	477.20	464.70	515.90
Na^+: K^+ ratio	100.27:1	70.90:1	53.67:1	45.21:1	38.09:1	28.58:1	28.58:1
Mg^{2+}: Ca^{2+} ratio	2.66:1	2.58:1	2.59:1	2.60:1	2.60:1	2.60:1	3.24:1

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521

522 Table 2. Linear (shown by *) and second order regressions of the survival, size, DSI and deformity
523 numbers of the blue mussels as a function of K^+ fortification levels in ISW

Parameter	Developmental stage	Equation	R^2
Survival (%)	Trochophore	$y = 0.001x^2 - 0.291x + 98.140$	0.95
	Veliger	$y = 0.001x^2 - 0.127x + 87.200$	0.53
	Pediveliger	$y = 0.117x + 45.938$	0.72*
	Settlement	$y = 0.167x + 26.074$	0.88*
Size (µm)	Trochophore	$y = 0.000x^2 + 0.007x + 81.69$	0.01
	Veliger	$y = 0.001x^2 - 0.073x + 123.590$	0.69
	Pediveliger	$y = 0.149x + 295.070$	0.89*
	Settlement	$y = 0.928x + 425.950$	0.87*
DSI (hours)	Trochophore	$y = -0.000x^2 + 0.028x + 10.960$	0.83
	Veliger	$y = -0.000x^2 + 0.047x + 42.370$	0.83
	Pediveliger	$y = -0.0008x^2 + 0.314x + 626.240$	0.91
	Settlement	$y = -0.293x + 746.070$	0.87*
Deformity (%)	Fertilised eggs	$y = 0.000x^2 - 0.089x + 9.310$	0.98
	Trochophore	$y = -0.006x + 2.080$	0.87*
	Veliger	$y = -0.006x + 1.848$	0.77*
	Pediveliger	$y = -0.008x + 1.929$	0.75*

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