

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

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9

## 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<sup>+</sup> fortification  
21 (ISW100K<sup>+</sup>), 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<sup>+</sup> fortification  
26 levels, and there were no deformities in pediveliger larvae reared in ISW100K<sup>+</sup> and in OW. These  
27 results showed that K<sup>+</sup> 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<sup>+</sup>, *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

36 culture various marine seaweeds (Kumar *et al.*, 2010), invertebrates (Fotedar *et al.*, 2008; Prangnell  
37 and Fotedar, 2006b; Tantulo and Fotedar, 2006) and vertebrates (Barman *et al.*, 2005; Doroudi *et al.*,  
38 2006; Fielder *et al.*, 2001). However, commercialisation of ISW aquaculture is constrained due to  
39 salinity fluctuations caused by the alteration of rainfall and high solar radiation (Prangnell, 2007),  
40 fluctuating calcium concentrations (Prangnell and Fotedar, 2006b), and deficiency of potassium ions  
41 relative to ocean water (OW) (Nulsen, 1997; Prangnell and Fotedar, 2006b). Most marine species,  
42 when cultured in ISW, show a low survival rate (Fielder *et al.*, 2001; Partridge and Creeper, 2004;  
43 Roy *et al.*, 2009), growth rate (Partridge and Creeper, 2004; Roy *et al.*, 2009), and a high risk of  
44 skeletal myopathy (Partridge and Creeper, 2004).

45 However, the fortification of potassium to ISW has been shown to improve survival and growth rates  
46 in many adult marine species such as mulloway *Argyrosomus japonicus* (Doroudi *et al.*, 2006),  
47 Australian snapper *Pagrus auratus* (Fielder *et al.*, 2001), grey mullet *Mugil cephalus* (Barman *et al.*,  
48 2005), western king prawn *Penaeus latisulcatus* (Prangnell, 2007; Prangnell and Fotedar, 2006b),  
49 Pacific white shrimp *Litopenaeus vannamei* (Liu *et al.*, 2014; Roy *et al.*, 2010), black tiger prawn  
50 *Penaeus monodon* (Tantulo and Fotedar, 2006), and alga *Gracilaria cliftonii* (Kumar *et al.*, 2010). So  
51 far, these studies mainly focus on the adult stages of marine species, and only a few studies  
52 investigated the effects of potassium fortification in ISW on the development of larval stages of  
53 marine species, e.g. juvenile greenlip abalone *Haliotis laevis* (Fotedar *et al.*, 2008), and the prawns  
54 *P. monodon* (Rahman *et al.*, 2005; Tantulo and Fotedar, 2006) and *P. latisulcatus* (Prangnell, 2007;  
55 Prangnell and Fotedar, 2006b).

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

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

## 71 **2. Materials and methods**

### 72 2.1. Blue mussels

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

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

### 87 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<sup>-1</sup> 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<sup>+</sup>), 40%  
101 (ISW40K<sup>+</sup>), 60% (ISW60K<sup>+</sup>), 80% (ISW80K<sup>+</sup>) and 100% (ISW100K<sup>+</sup>). 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<sup>-1</sup> and 800 mOsm.kg<sup>-1</sup>, 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<sup>+</sup> 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<sup>+</sup> fortification levels were prepared by mixing hydrous potassium chloride (purity > 99%, Sigma-

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

116 The ionic composition of these water treatments used in this experiment was analysed by CSBP Soil  
117 & Plant Laboratory, Bibra Lake, WA using Inductively Coupled Plasma spectroscopy. To measure  
118 the osmolality of the media, 50 µL of water from each of seven stocked waters were collected using a  
119 200 µL pipette. The measurements were performed using a cryoscopic osmometer – Osmomet 030  
120 (Gonotec, Inc, Germany).

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

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

#### 136 2.4. Data analysis

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

138 where S is the survival (%),  $n_t$  is the number of larvae of the blue mussels at time t, and  $n_0$  is the  
139 number of the early larvae of the blue mussels at the commencement of each stage.

140 Sizes of each larval stage were measured at the end of the corresponding development stage when  
141 50% of the larvae had moulted to the next developmental stage. The developmental stages of blue  
142 mussels were identified under the microscopes (SZH and BH-2, Olympus, Japan) based on the  
143 morphological description (His *et al.*, 1997; Redfearn *et al.*, 1986; Saranchova and Flyachinskaya,  
144 2001).

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

148 Morphological deformity was determined based on previous descriptions (Andersen *et al.*, 2013; His  
149 *et al.*, 1997; Kurihara, 2008). Deformity was calculated based on the formula:  $D = 100 \times (nd/n_0)$

150 where D is the deformity (%),  $n_d$  is the number of deformed larvae of the blue mussels at time t, and  
151  $n_0$  is the number of the larvae of the blue mussels at the commencement of each stage.

## 152 2.5. Statistical analysis

153 One-way analysis of variance (ANOVA) and the least significant difference (Tukey's post-hoc tests)  
154 multiple comparisons were used to determine the significant differences ( $p < 0.05$ ) among the means.

155 Percentage values were arcsine-transformed to achieve normality for ANOVA assumption. Linear and  
156 second order regression analyses were performed on the survival, size, DSI and deformity of blue  
157 mussels as a function of  $K^+$  fortification levels in ISW. Data were represented as mean  $\pm$  standard  
158 error (SE). All statistical analyses were performed in SPSS version 22 for Windows.

## 159 **3. Results**

### 160 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 (ISW100 $K^+$ ), 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 trochophore 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  $\mu m$ ), veliger (120–138  $\mu m$ ) and pediveliger (301–331  $\mu m$ ) 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  $\mu m$  at the lowest  $K^+$  level to 610  $\mu m$  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 ISW100 $K^+$  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. 6g).  
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 trochophore, 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; Shiao 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<sup>+</sup>/K<sup>+</sup> ATPase activity (Liu *et al.*, 2014). Na<sup>+</sup>/K<sup>+</sup> ATPase, a sodium  
218 pump that is present in the gill membrane, transports Na<sup>+</sup> and Cl<sup>-</sup> 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<sup>+</sup>/K<sup>+</sup> ATPase activity is dependent on the ratio of Na<sup>+</sup> and K<sup>+</sup> in the  
221 surrounding environment (Tantulo and Fotedar, 2007). The optimal ratio of Na<sup>+</sup>/K<sup>+</sup> for the normal  
222 function of Na<sup>+</sup>/K<sup>+</sup> 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<sup>+</sup> can change the Na<sup>+</sup>/K<sup>+</sup> ratio in a way that can inhibit the ability of Na<sup>+</sup>/K<sup>+</sup>  
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<sup>+</sup> in ISW. The highest survival and growth at Na<sup>+</sup>/K<sup>+</sup> ratio of 28.58 in ISW100K<sup>+</sup> was similar to the  
229 survival in OW25 that also had the Na<sup>+</sup>/K<sup>+</sup> ratio of 28.58. The lowest survival occurred at the Na<sup>+</sup>/K<sup>+</sup>  
230 ratio of 100.27 in ISW27, suggesting that it is possible to add K<sup>+</sup> to ISW to adjust the optimal Na<sup>+</sup>/K<sup>+</sup>  
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<sup>+</sup> 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<sup>+</sup>-deficient waters. Further, the deficiency  
238 of K<sup>+</sup> in the medium can be associated with higher energy investments in the formation and function  
239 of osmoregulatory organs.

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

249 The effects of  $K^+$  on the DSI of the early larvae of blue mussels are not well understood. Possibly, the  
250 shorter DSI of the early larvae in the relatively higher  $K^+$  level (rather than in lower  $K^+$  levels) and the  
251 similar DSI of early larvae in ISW100 $K^+$  and OW25 indicate that the lower  $K^+$  levels (> 80%)  
252 interfere with normal physiological development and function, for example, by limiting the ionic  
253 exchange ability of the gills, as reported in *P. latisulcatus* (Prangnell, 2007), and consequently  
254 lengthening the DSI of the blue mussels at lower  $K^+$  levels. In addition, it is possible that  $K^+$   
255 fortification of ISW influences the size of settling larvae indirectly through the underlying changes in  
256 the DSI. As longer time is spent in a particular developmental stage (longer DSI), more time larvae  
257 would have in increasing their sizes, hence the larger sizes.

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

266 Previous studies show that  $K^+$  is a metamorphic inducer because of its ability to influence cell  
267 membrane potential (Yool *et al.*, 1986), and also induces larval metamorphosis and settlement of  
268 marine invertebrates (Carpizo-Ituarte and Hadfield, 1998; Sánchez-Lazo and Martínez-Pita, 2012;  
269 Wassnig and Southgate, 2012; Yang *et al.*, 2008; Yang *et al.*, 2011; Young *et al.*, 2011; Yu *et al.*,  
270 2008; Zhao *et al.*, 2003). The addition of  $K^+$  to OW at  $10^{-3}$  to  $5 \times 10^{-2}$  M induced the peak  
271 metamorphosis of *M. galloprovincialis*, and over 90% of the larvae were induced to settle at the  
272 excessive concentrations of 20, 30 and 40 mM (Yang *et al.*, 2011). Therefore, it is good practice to  
273 culture early stages in  $K^+$ -fortified ISW.

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

278 In conclusion, potassium-fortified ISW improves the survival rate and size, and reduces the  
279 developmental stage interval and deformities, of the early life stages of blue mussels. The 100%  $K^+$   
280 fortification of ISW improves the viability of culturing early stages of blue mussels in ISW. The study  
281 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<sup>+</sup> 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<sup>+</sup> fortified inland saline water.

481

482 Figure captions

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

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

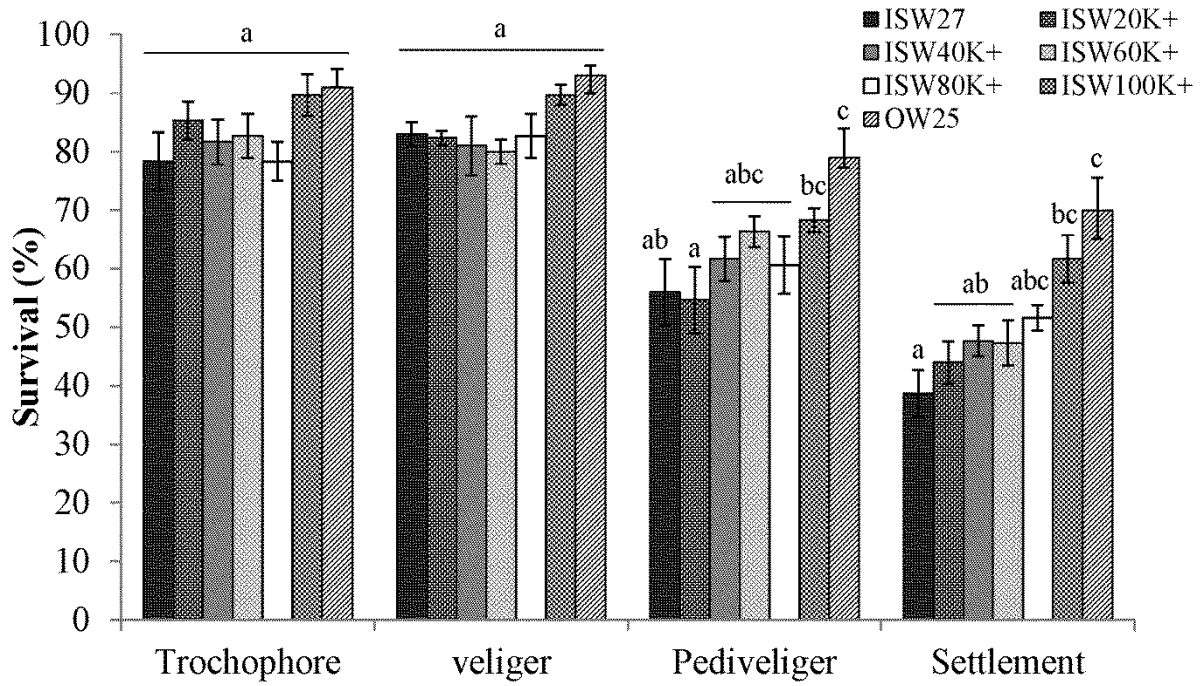
489 Figure 3. Developmental stage interval of early developmental stages of the blue mussel *Mytilus*  
490 *edulis* in response to K<sup>+</sup> addition to ISWs. Data are presented as mean ± SE. Data with different  
491 letters within a stage are significantly different ( $p < 0.05$ ).

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

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

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

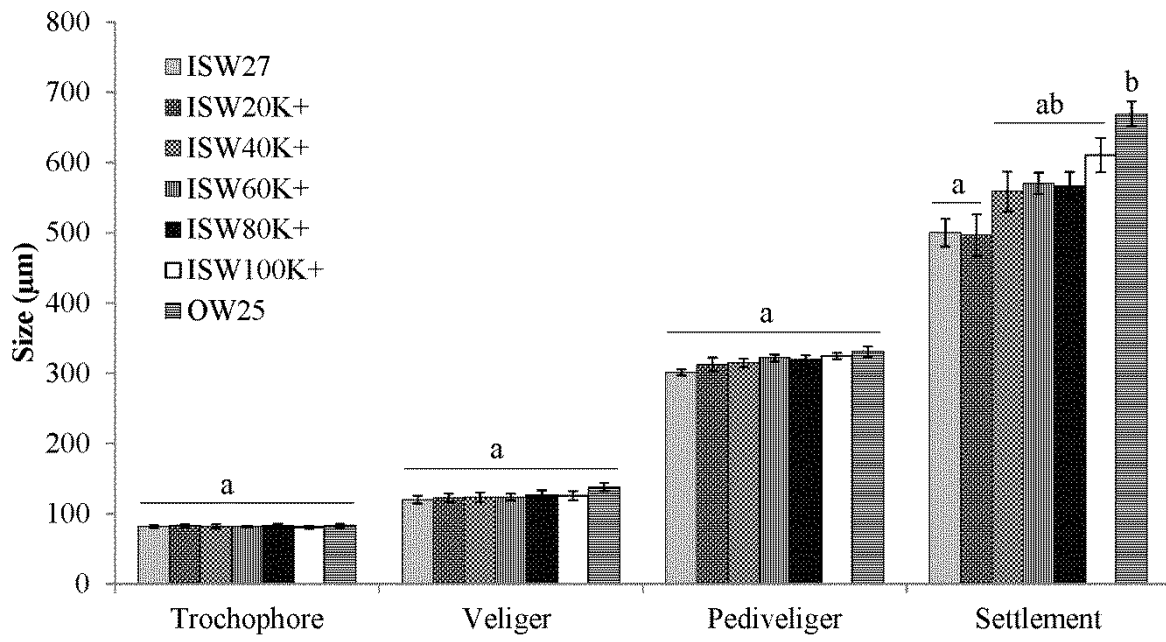
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504 Figure 1

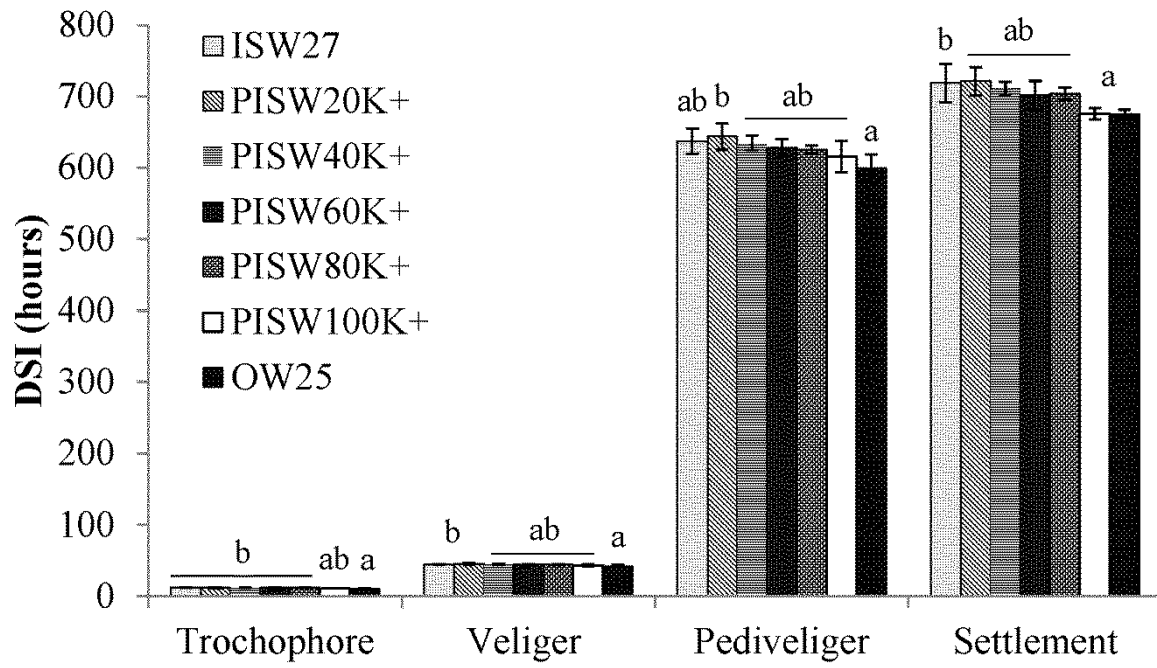
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507 Figure 2

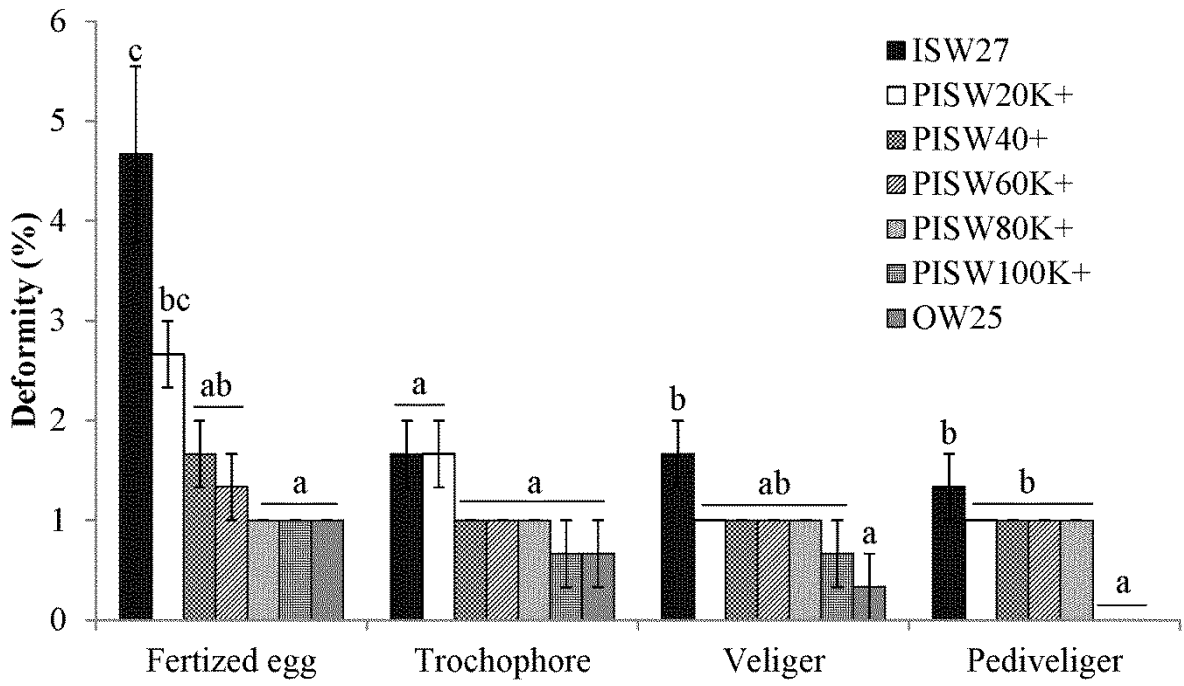
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510 Figure 3

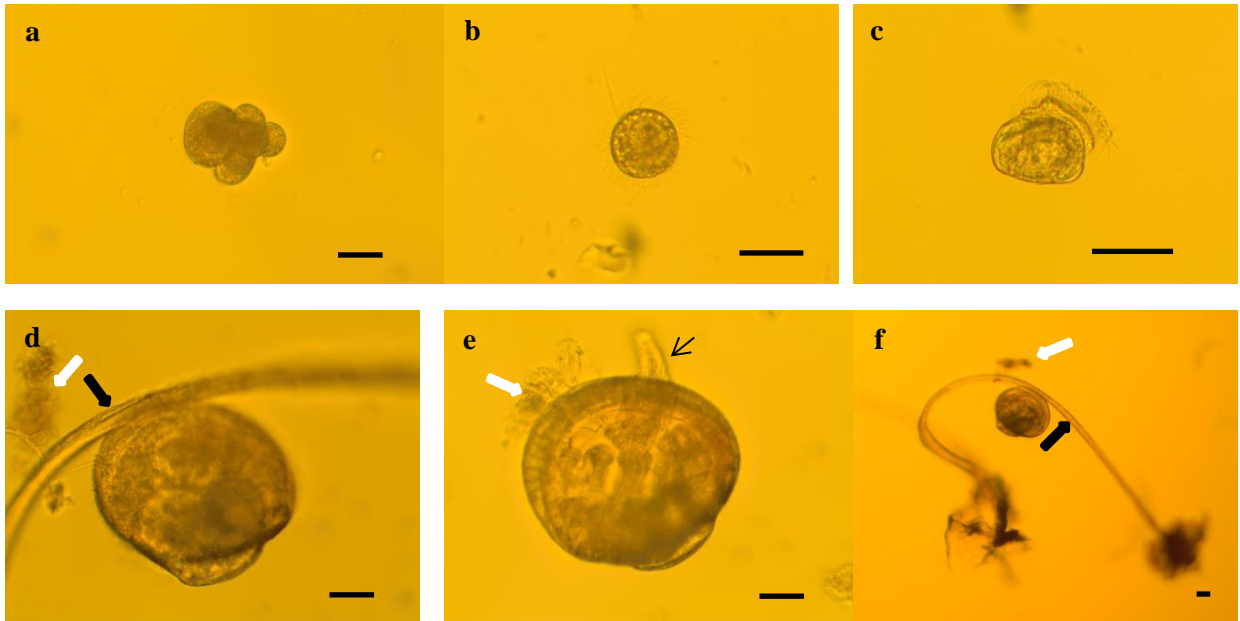
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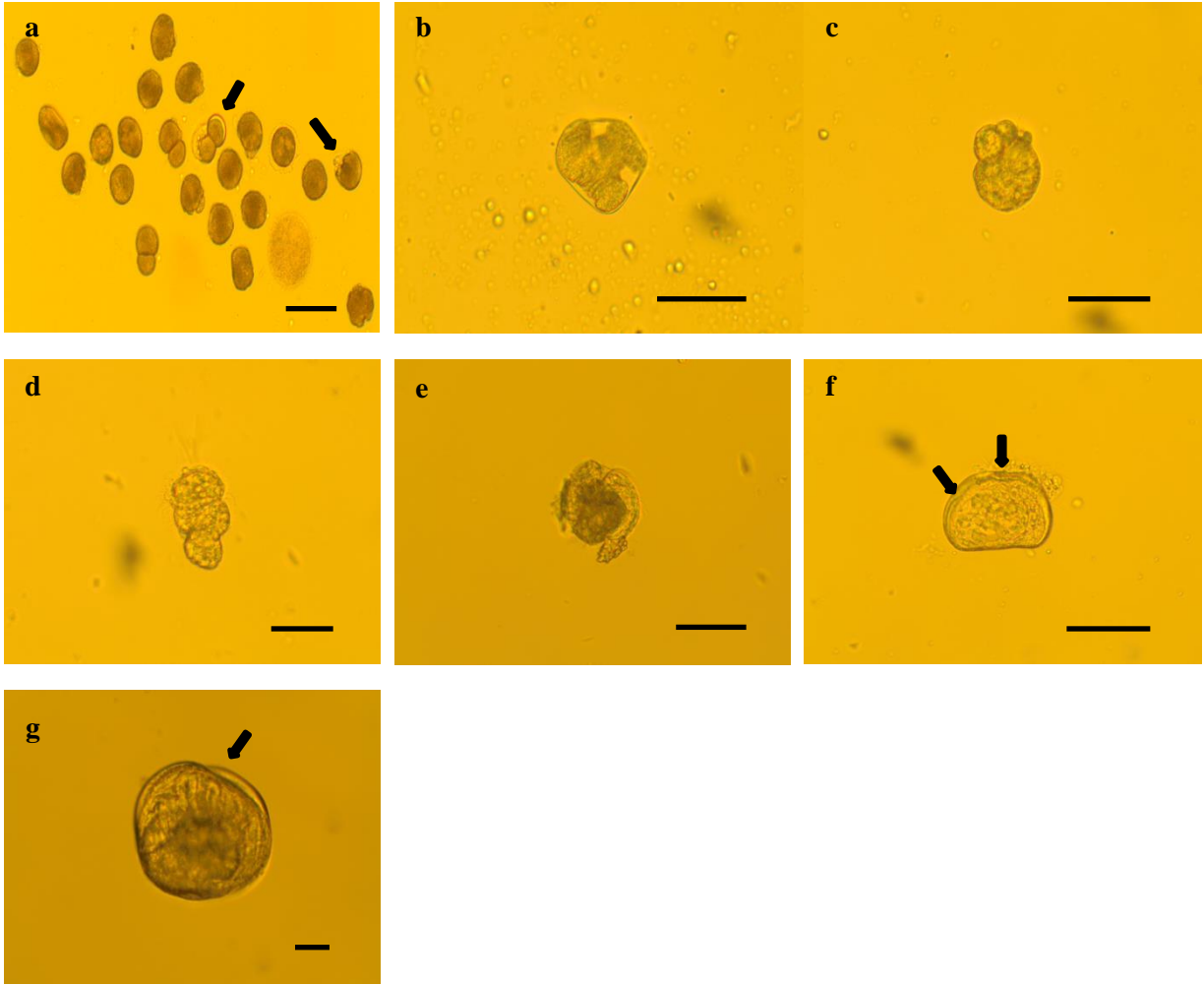
513 Figure 4

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515 Figure 5.

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517 Figure 6

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519 Table 1. The ionic composition of ISWs and OW

Parameters	ISW27	ISW20K <sup>+</sup>	ISW40K <sup>+</sup>	ISW60K <sup>+</sup>	ISW80K <sup>+</sup>	ISW100K <sup>+</sup>	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 <sup>+</sup> (mg.L <sup>-1</sup> )	6584.00	6824.00	6816.00	6872.00	6943.00	6774.00	6480.00
K <sup>+</sup> (mg.L <sup>-1</sup> )	65.66	96.25	127.00	152.40	182.30	217.50	226.70
Ca <sup>2+</sup> (mg.L <sup>-1</sup> )	431.10	465.20	462.50	456.90	461.50	451.60	231.20
Mg <sup>2+</sup> (mg.L <sup>-1</sup> )	1145.00	1202.00	1197.00	1189.00	1198.00	1173.00	749.30
S <sup>2+</sup> (mg.L <sup>-1</sup> )	453.40	483.50	475.90	471.50	477.20	464.70	515.90
Na <sup>+</sup> : K <sup>+</sup> ratio	100.27:1	70.90:1	53.67:1	45.21:1	38.09:1	28.58:1	28.58:1
Mg <sup>2+</sup> : Ca <sup>2+</sup> ratio	2.66:1	2.58:1	2.59:1	2.60:1	2.60:1	2.60:1	3.24:1

520

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<sup>+</sup> fortification levels in ISW

Parameter	Developmental stage	Equation	R <sup>2</sup>
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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525