



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



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ABSTRACT

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

Statement of relevance: This study is the first attempt to culture larval stages of blue mussel *Mytilus edulis* in potassium-fortified inland saline water. The results have shown that the larval development is feasible only if ISW is fortified with K⁺. By initiating larval culture in inland saline water, we expect better results will be reflected by adult stages.

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1. Introduction

Salinization caused by natural and anthropogenic reasons (Bennett et al., 2006; Szabolcs, 1989) has rendered more than 80 million hectares (Ghassemi et al., 1995) of land in more than 100 countries useless for agricultural production (NLWRA, 2000; Rengasamy, 2006). On the other hand, inland saline water (ISW) has the potential to be used as a suitable resource for aquaculture of marine species (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 japonicus* (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*

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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 ($31^\circ 59'S$, $115^\circ 48'E$) 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, $1.1 \times 0.6 \times 0.3$ m; length $\times$ width $\times$ depth) for 10 days. The tank was supplied with $1 \mu\text{m}$ -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 ($0.1 \text{ mL} \cdot \text{L}^{-1}$) for 24 h, then neutralised with $0.1 \text{ g} \cdot \text{L}^{-1}$ sodium thiosulfate and enriched with an F2 algae boost ($1 \text{ mL} \cdot \text{L}^{-1}$) 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 $80,000 \text{ cells} \cdot \text{mL}^{-1}$ (Gazeau et al., 2010).

2.2. Spawning induction

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

2.3. Experimental design and testing

To test whether the addition of potassium to ISW improved the performance of early life stages of the blue mussel, each of the four early stages, namely fertilised eggs, trochophore, veliger and pediveliger were reared in one of the five different levels of potassium fortification: 20% (ISW20 K^+), 40% (ISW40 K^+), 60% (ISW60 K^+), 80% (ISW80 K^+) and 100% (ISW100 K^+). The levels of potassium addition in ISW were equivalent to the typical concentration of potassium in the OW at the same salinity. ISW at salinities of 27 ppt and OW at 25 ppt were used as controls, as our previous results (unpublished) have shown that the iso-osmotic point (the point when the osmolality of the haemolymph and external medium are the same at a particular salinity) of blue mussels in OW and ISW were $700 \text{ mOsm} \cdot \text{kg}^{-1}$ and $800 \text{ mOsm} \cdot \text{kg}^{-1}$, respectively. These osmolalities equate to 25 and 27 ppt in OW and ISW, respectively. In order to keep the energy expenditure limited to ionic regulation caused by only K^+ gradients between the haemolymph and external environment and minimise the energy expenditure due to the overall osmoregulation, 25 and 27 ppt of OW and ISW, respectively, were used as two controls in the current trial. OW and ISW were procured from Hillarys ($31^\circ 49'S$, $115^\circ 45'E$) and a lake at Wannamal ($31^\circ 15'S$, $116^\circ 05'E$), Western Australia, respectively. The salinities of OW and ISW were reduced to 25 and 27 ppt, respectively, by adding deionised water. All 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 $1 \mu\text{m}$ 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, $50 \mu\text{L}$ of water from each of seven stocked waters were collected using a $200 \mu\text{L}$ 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 min. 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 h, respectively.

Similarly, to observe the settlement, 100 newly transformed pediveliger larvae from the stock tank were placed into each $40 \mu\text{m}$ -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 h 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: $S = 100 \times (\text{nt}/\text{no})$ —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).

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