School of Science

Department of Environment and Agriculture

Cultural biology of the blue mussel, *Mytilus edulis* (Linnaeus, 1758) in inland saline water in Western Australia

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DECLARATION

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PREAMBLE

The aim of this research is to evaluate the technical feasibility of culturing blue mussel, *Mytilus edulis* (Linnaeus, 1758) in inland saline water (ISW). In order to achieve this aim the study investigated the effects of ISW and potassium-fortified inland saline water ($ISWK^+$) on osmoregulation, survival, growth, and byssal thread formation of the blue mussel. These effects were evaluated at different early stages of the development, juvenile and the adult stages. This dissertation consists of five chapters. The chapter 1 includes the background information of the subject matter, the aim, objectives and significance of the research. The chapter 2 reviews the origin, distribution and present status of ISW in the world, Australia and Western Australia. The information on the ionic composition of ISW and their fluctuation are also reviewed in this chapter. This chapter also justifies the advantages, disadvantages and the potential of using ISW for the culture of blue mussel. Chapter 3 describes the materials and methods used in the experiments. The chapter 4 presents the results of the experiments. Chapter 5 discusses and compares the results of all the experiments with one another and with the published literature. It is followed by the main conclusions of the study and the future research recommendations.

ABSTRACT

Salinization caused by natural and anthropogenic reasons has rendered more than 80 million hectares of land in more than 100 countries unproductive and has imposed negative impacts on agriculture, environment, infrastructure and water resources. On the other hand, inland saline water (ISW) has the potential to be used as a suitable resource for aquaculture of aquatic species. The blue mussel, *Mytilus edulis* (Linnaeus, 1758), an importantly commercial species around the world was target in the current research for its viability to be cultured in ISW.

A series of five laboratory experiments was conducted to investigate the cultural biology of early larvae and adult blue mussel cultured in K^+-deficient ISW and K^+-fortified ISW. The first experiment investigated the survival and osmoregulatory capacity of the blue mussel exposed to a range of ISW and OW salinities of 5, 10, 15, 20, 25, 30 and 35 ppt for 360 hours. The results showed that the isosmotic point of blue mussel was 700 mOsm kg^{-1} in ISW, equal to a salinity of 27 ppt and stabilized between 770 to 800 mOsm kg^{-1} in OW, equal to salinities of 24 to 25 ppt. The results of the rest of the experiments showed that 100% K^+-fortified ISW significantly improves the survival rate and size, and reduces the intra-moult interval and deformities, of the early life stages. One hundred percent K^+-fortified ISW increases the survival, growth of the juvenile and adult blue mussel after a 4 months of culture, reduces the root shedding and increases their attachment to the substrate. The 100 percent fortification of K^+ in ISW is recommended to culture blue mussel in ISW.

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LIST OF ABBREVIATIONS

A: Artificial
D: day
DO: dissolved oxygen
DW: Dry weight
hpe: hour post exposure
hpf: hour post fertilisation
IMI: Intra-moult interval
IP: Isosmotic point
N: Natural
NA: Not available
OW: Ocean water
OC: Osmoregulatory capacity
PAS: partitioned aquaculture systems
PISW: Potassium-fortified inland saline water
ppt: Past per thousand
ISW: Inland saline water
RAS: recirculating aquaculture systems
SIAS: semi-intensive aquaculture system
SIFTS: semi-intensive floating tanks
SIS: Salinity interception schemes
SW: Seawater
USA: United States of America
SBCS: serial biological concentration system
$: Dollar

Chapter 1 INTRODUCTION

1.1. Introduction

The Australian aquaculture is a rapidly growing industry with the value of A$733 million, accounting for 30 percent of the national fishery production and employs directly 7000 people and indirectly 20,000 people in 2001-2002 (Love & Langenkamp, 2003). In 2012 – 2013, the aquaculture production increased by 43 percent with the total gross value of total fisheries production was $2.4 billion and the average individual consumption of 15 kg (Figure 1.1) (ABARES, 2014). Tasmania accounts for the largest proportion of aquaculture with 29 percent of the total production value, followed by South Australia with 19 percent and Western Australia with 18 percent (ABARES, 2014). The increase in the aquaculture production is due to the higher domestic and international demand.

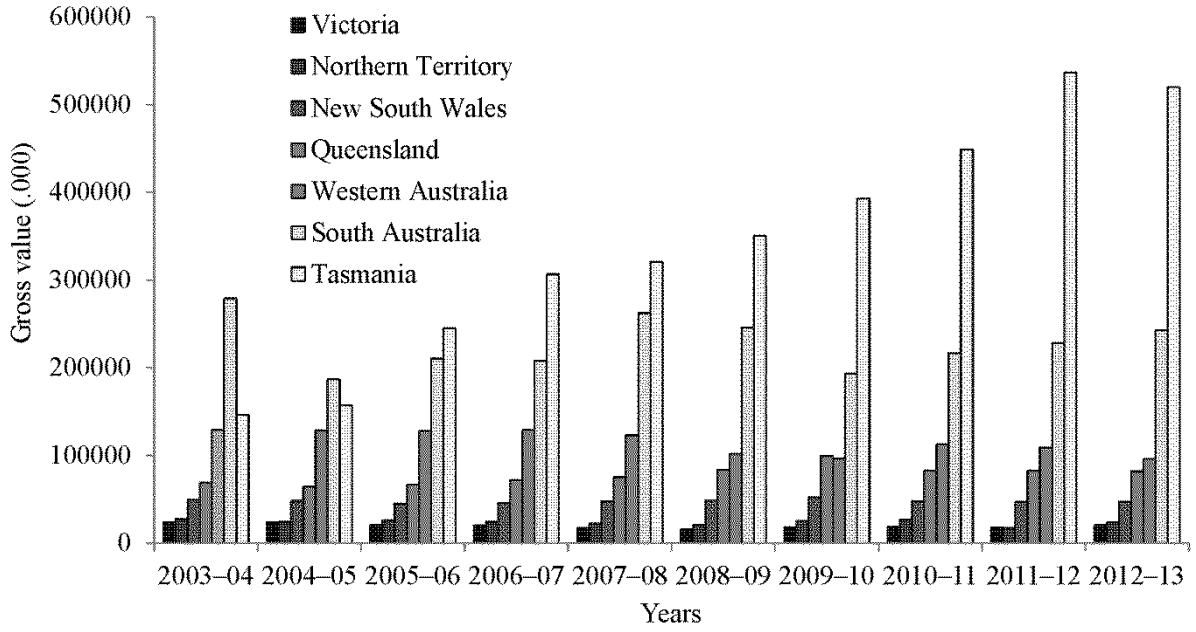


Figure 1.1 Gross value of aquaculture production in Australia (AUD) period 2003 to 2013 (Source: modified from ABARES (2014)).

Main aquacultured species in Australia are fish (salmonids and tuna), crustaceans (prawns, marron and yabby) and molluscs (edible oyster, pearl oyster, abalone and blue mussel)

(ABARES, 2014 ; Love & Langenkamp, 2003). Australian aquaculture production is exporting to major markets: Hong Kong ($371 million), Vietnam ($294 million), Japan ($269 million), China ($48 million) and United States of Amercica ($39 million). Domestic consumption has increased from 13 kg per capita in 2000 – 2001 to 15 kg per capita in 2012 – 2013 (ABARES, 2014).

However, the expansion of aquaculture production in Australia is restrained due to the current legislative limitation with regards to environmental issues, questions about the sustainability of coastal aquaculture and the conflict of interest between coastal aquaculture and other water-based industries such as tourism (Smaal, 2002). In this context, the development of blue mussel aquaculture in ISW may mitigate the environmental impacts on coastal aquaculture (Ogburn, 1998), adding value to ISW aquaculture by offsetting the costs of the negative effects of salinization (Gooley, Ingram, & McKinnon, 1998) and boosting the aquaculture in the rural areas (Allan, Banens, & Fielder, 2001).

There are some major sources of salinized water in Australia including the groundwater areas in the Lower Murray Hydrogeological Basin in Victoria, Eyre Peninsula in South Australia, Central Australia and North Territory, and in the southwest of Western Australia with the salinities ranging from 20 - 40 ppt (Kolkovski, 2010), gas well drills (Brinckerhoff, 2004) and disused open-cut mines (Johnson & Wright, 2003). However, the ionic composition of ISW varies remarkably compare to OW at the same salinity (Table 2.1) or among different locations (Table 1.1) that may impose the challenge to use this type of water for culturing marine species.

Table 1.1 Main ionic composition (mg L^{-1}) of ISW from different locations in Australia

	Wakkool (NSW)[1]	Grong (NSW)[1]	Wakkool (NSW)[2]	Merredin catchment (WA)[3]	Goulburn (Victoria)[4]	Wannamal (WA)[5]	OW (25 ppt)[5]
Na^+	7343	6200	4210	7790	9450	8719	6480
K^+	49	50	9.2	155	87.5	84	226.70
Ca^{2+}	365	1500	504	409	1225	583	231.20
Mg^{2+}	623	27	820	750	1654	1525	749.30

[1]: Allan and Fielder (1998); [2]: (Fielder, Bardsley, & Allan, 2001); [3]: R. J. George and Frantom (1988); [4]: Lee (1998); [5]: This study

To date, various studies have attempted to investigate the possibility of culturing aquatic species in ISW in Australia including fishes (Barman, Jana, Garg, Bhatnagar, & Arasu, 2005; M. Doroudi, Webster, Allan, & Fielder, 2007; M. S. Doroudi, Fielder, Allan, & Webster, 2006; Fielder et al., 2001), crustaceans (Fotedar, Harries, & Savage, 2008; Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006), molluscs (Fotedar et al., 2008; Lee, 1997) and marine seaweeds (Kumar, Fotedar, & Dods, 2010). However, most species cultured in ISW show low survivals, growth rate and risk of skeletal myopathy (Doupe, Sarre, Partridge, Lymbery, & Jenkins, 2005; Fielder et al., 2001; Partridge & Creeper, 2004; Roy, Davis, & Whitis, 2009) as a result of the deficiency of K^+ in ISW. K^+ plays a vital role in physiological processes of aquatic animals, e.g. acid-base balance (Evans, Piermarini, & Choe, 2005; Hadfield, Clayton, Cohrs, & Murphy, 2012) and the Na^+/K^+ ATPase activity (Liu et al., 2014) which directly relates to the osmoregulatory capacity (Charmantier, Charmantier-Daures, & Young-Lai, 1985; Evans et al., 2005; Mantel & Farmer, 1983). Except only three species are successfully cultured in raw ISW: mulloway *Argyrosomus*

japonicas, microalgae *Dunaliella salina* and *Artemia.* The *A. japonicas* shows high survival and growth rate when cultured in ISW intensive and semi-intensive system (Hutchinson & Flowers, 2008) but no commercial scale has been invested for this species. Microalgae *Dunaliella salina* and *Artermia* are successfully cultured on a large scale in Australia (Kolkovski, 2010; Kolkovski, Curnow, & King, 2010).

In attempts to develop aquaculture in ISW, there have been two different ways to improve survival and growth rate of cultured species: the fortification of K^+ or mixing of ISW with ocean water (OW). Many studies have shown that the fortification of potassium to ISW has shown to improve survival and growth rates in many adult marine species such as *A. japonicas* (Doroudi et al., 2006), Australian snapper *Pagrus auratus* (Fielder et al., 2001), grey mullet *Mugil cephalus* (Barman et al., 2005), western king prawns *Penaeus latisulcatus* (Prangnell, 2007; Prangnell & Fotedar, 2006b), Pacific white shrimp *Litopenaeus vannamei* (Liu et al., 2014; Roy et al., 2010), black tiger prawn *Penaeus monodon* (Tantulo & Fotedar, 2006), and agar *Gracilaria cliftonii* (Kumar et al., 2010). The optimal K^+ fortification level depends on species that has been shown varying from 38 to 100% equivalent to the K^+ level in OW (Table 1.2).

Table 1.2 Optimum K^+ fortification (%) in ISW as in OW for marine species cultured in ISW

Species	Salinity (ppt)	K^+	Na^+/K^+ ratio	References
Haliotis laevigata	35	100	23.85	Fotedar et al. (2008)
Penaeus latisulcatus	30	100	31.30	Prangnell and Fotedar (2005)
Penaeus monodon	25	100	23	Tantulo and Fotedar (2006)
Pargrus auratus	21	50 - 100		Fielder et al. (2001)

Argyrosomus japonicus	> 14	> 38	Doroudi et al. (2006)

Among marine species, the blue mussel *Mytilus edulis* is an important candidate for aquaculture (Hickman, 1992) due to their wide distribution, no supplementary feeding requirements, higher nutritional value, and good taste (Gosling, 1992, 2003; Seed, 1992). Blue mussel aquaculture is practised in many European countries and China (Smaal, 2002) with different culture methods (Buck, Ebeling, & Michler-Cieluch, 2010; Smaal, 2002). Blue mussel in one of the main cultured species in Australia with the gross value of A$10 million in 2012 – 2013 (ABARES, 2014). The demand of blue mussel is increasing, yet the expansion of blue mussel culture in coastal areas 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). Therefore, finding an alternative water resource for culturing blue mussel is timely and ISW may be one of the water resources for this purpose. However, there is no available information on the physiological responses and cultural biology of blue mussel cultured in ISW or K^+-fortified ISW.

1.2. Aim

The aim of this study is to investigate the survival, growth and physiological responses of blue mussel when cultured in inland saline water (ISW) and K^+ fortified ISW.

1.3. Objectives

1.3.1. To compare the osmoregulatory capacity of blue mussel when exposed to ISW and OW at different salinities.

1.3.2. To investigate the effect of K^+-fortified ISW on the early larval development of blue mussel.

1.3.3. To determine the effect of K^+-fortified ISW on the survival and growth of juvenile blue mussels.

1.3.4. To determine the effect of K^+-fortified ISW on the survival, growth and biochemical composition of the adult blue mussel.

1.3.5. To study the effect of K^+-fortified ISW on the shedding and renewal rates of the byssus threads of the blue mussel.

Chapter 2 LITERATURE REVIEW

2.1. Inland saline water (ISW)

The demand for food is increasing as a result of the rapidly growing human population in the world, with an increase in average consumption per capita of 9.9 kg in the 1960s to 19.2 kg in 2012. To meet this high demand, it is estimated that the food industry will need to increase its production by 38% in 2050 and by 57% in 2050 (Wild, 2003).

2.1.1. Origin and present status of ISW

The demand for food is increasing as a result of the rapidly growing human population in the world, with an increase in average consumption per capita of 9.9 kg in the 1960s to 19.2 kg in 2012. To meet this high demand, it is estimated that the food industry will need to increase its production by 38% in 2050 and by 57% in 2050 (Wild, 2003). While it is impossible for the capture sector to increase its production capacity to around 90 million tonnes, the global aquacultural production has recently increased from 50 million tonnes in 2007 to 67 million tonnes in 2012. Of which finfish, crustaceans, molluscs and other species account for 44, 6, 15 and 1 million tonnes, respectively (FAO, 2014). The leading countries, with more than one million tonnes of aquacultural production, include China, India, Vietnam, Indonesia, Bangladesh and Egypt (FAO, 2014).

Australia, which has the world's largest exclusive economic zone of around 10 million square kilometres, has produced an average fishery value of $2.6 billion per year of both capture and aquaculture, including the main groups of finfish, crustaceans and molluscs (Flood et al., 2012) (Table 2.1). Salinisation has influenced various fields, e.g. agriculture, infrastructure, water resources, and social structures (Kolkovski, 2010; NLWRA, 2000; Pitman & Läuchli, 2002). The estimated cost regarding the effects of salinisation on agriculture is $12 billion $year^{-1}$ (Ghassemi, Jakeman, & Nix, 1995). The salinisation has

worsened as groundwater tables have risen at a fast rate of 10 to 30 cm year^{-1} (Ferdowsian, Pannell, McCarron, Ryder, & Crossing, 2001).

Table 2.1 Regional distribution of salinised areas (million hectares) in the most affected countries (source: Ghassemi *et al*., 1995; Hamdallah, 1997; Szabolcs, 1989).

Region/Country	Cultivated land	Irrigated land	Salinised irrigated land	Percentage of salinised affected land (%)
Commonwealth of Independent States (former USSR)	232.57	20.48	3.70	18.10
Europe			7.80	
North and Central Asia			91.60	
South Asia			83.30	
South east Asia			20.0	
Africa			53.50	
Algeria		0.45		10.00 - 15.00
Argentina	35.75	1.72	0.58	33.70
Australia	47.11	1.83	0.16	8.70
China	96.97	44.83	6.70	15.00
Egypt	2.69	2.69	0.88	33.00
Jordan		0.06		16.00
India	168.99	42.10	7.00	16.60
Iran	14.83	5.74	1.72	30.00
Iraq		3.52		50.00
Mexico				10.00

Pakistan	20.76	16.08	4.22	26.20
Turkmenistan				48.00
Sudan		1.90		20.00
Syria		1.01		16.00
USA	189.91	18.10	4.16	23.00

Australia has a total land area of 7.7 million km^2 (equal to 770 million hectares) (Kolkovski, 2010); however, 5.7 million hectares were affected by salinisation in 2000 (Allan, Heasman, & Bennison, 2008; NLWRA, 2000), and this number is expected to increase to 17 million hectares in 2050 (NLWRA, 2000) (Figure 2.1; Table 2.2), with more than 80% of lakes and wetlands salinised (Timms, 2005). Most of these areas are located in South Australia, e.g. Eyre, Frome and Torrens, and in Western Australia, e.g. Barlle, Ballard, Carey, Cowan, Lefroy, Yindarlgooda and thousands of small lakes in Esperance (Timms, 2005, 2009) (Figure 2.1; Table 2.2).

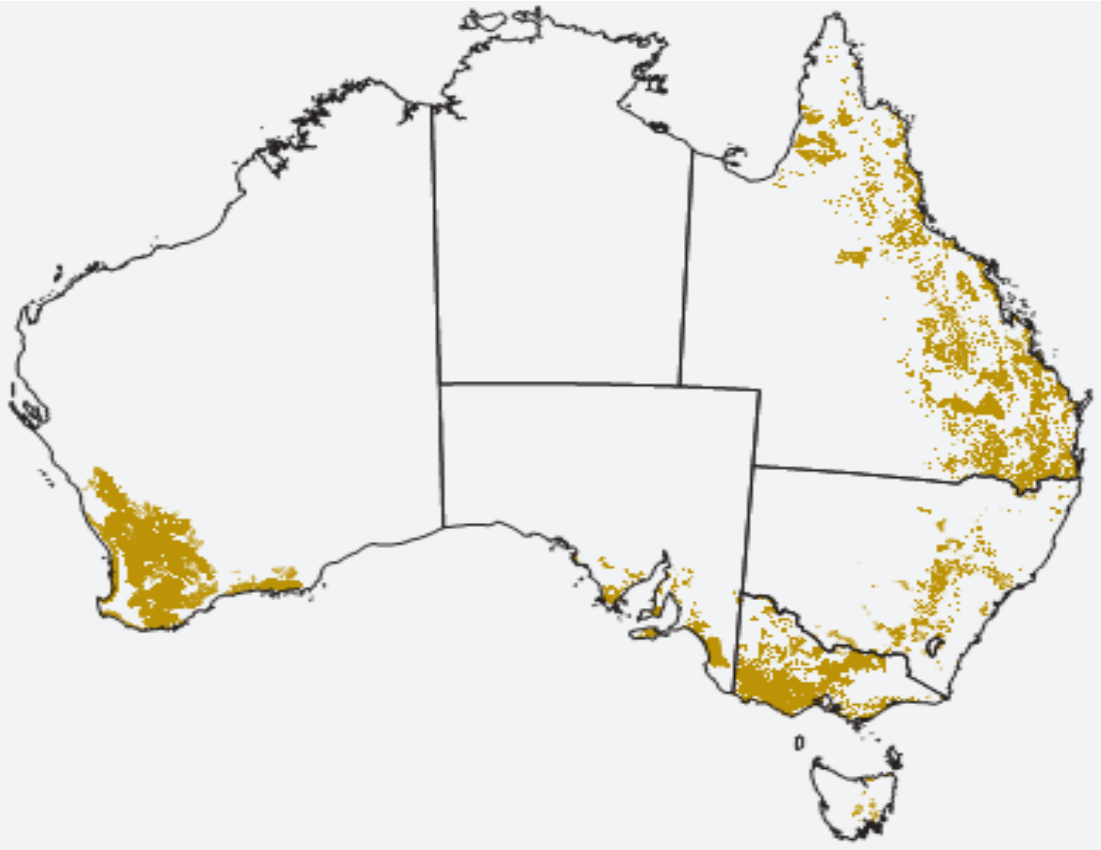


Figure 2.1 Distribution of forecasted salinised areas in Australia in 2050 (Source: NLWRA (2000)).

Table 2.2 Salinisation in different states in Australia (hectares) in 1998/2000, and predicted values for 2050 (Source: NLWRA, 2000). NA: Not available.

State	1998/2000	2050
New South Wales	181,000	1,300,000
Queensland	NA	3,100,000
South Australia	390,000	600,000
Tasmania	54,000	90,000
Victoria	670,000	3,110,000
Western Australia	4,363,000	600,000

Land and water salinisation in Australia have caused severe impacts on agriculture, water resources, infrastructure and biodiversity (Allan, Dignam, & Fielder, 2001; Kolkovski, 2010; NLWRA, 2000) (Table 2.3). Biological diversity in ISW decreases with increasing salinity (Hammer, 1986). Biological richness in these salinised areas (woodland, damp-land and wetland) includes aquatic invertebrates, e.g. Anostraca, Notostraca, Spinicaudata, Cladocera, Copepoda, Ostracoda and Isopoda (Timms, 2009), aquatic insects (Carver et al., 2009) and 450 plant species, which are all at risk of extinction (Halse, Ruprecht, & Pinder, 2003; Keighery, 2000). One-third of aquatic invertebrate species are predicted to disappear in Western Australia WA (Halse et al., 2003).

Table 2.3 Impacts of salinisation on different fields in Australia (Source: NLWRA, 2000).

Field	2000	2020	2050
Agricultural land (*1000 ha)	4650	6371	13660
Remnant and planted perennial vegetation (.000 ha)	631	770	2020
Length of streams and lake perimeters (*1000 km)	11.8	20	41.3
Rail (*1000 km)	1.6	2.1	5.1
Roads (*1000 km)	19.9	26.6	67.4
Towns (number)	68	125	219
Important wetlands (number)	80	81	130

South Australia has a main salinised area in the upper south-east part of the state, but its salinised area is predicted to increase by 60% if current land use and groundwater expansion situations continue (NLWRA, 2000). In response to this, salt interception schemes (SIS) have been developed across the state for salt evaporation (W. Hutchinson, 1998).

Victoria has wide salinised areas in the western, south-western, north-central and north-western parts of the state, which includes the Gippsland, Highlands Province, Murray, Otway, Port Phillip and Western Port main basins (Gooley et al., 1998). Rising water tables have imposed a major problem for the production of irrigated farms. The state has built various pumping stations to mitigate the effect of rising water tables and uses ISW for aquacultural activity (Gooley et al., 1998).

Queensland has small salinised areas located in the eastern part of the state, but has a high risk for the expansion of salinised areas (NLWRA, 2000). New South Wales has large salinised areas in the northern and southern parts of the state, and has the two largest underground basins: Great Artesian and Murray, respectively (Allan & Fielder, 1998).

Western Australia is the largest state and has the largest salinised areas in Australia (Fig. 1.2). It is estimated that 30% of the land and lakes in South-west Western Australia is affected by salinisation (Timms, 2005), which is equivalent to 5.7 million hectares (NLWRA, 2000). The salinity emphasises the remarkable variations (Timms, 2009) due to the alteration of rainfall and high solar radiation (Prangnell, 2007), fluctuating calcium concentrations (Prangnell & Fotedar, 2006b), and a deficiency of potassium ions relative to ocean water (OW) (Nulsen, 1997; Prangnell & Fotedar, 2006b). The largest and most severely salinised region in Australia, the Wheatbelt, accounts for 70% of the state's salinised area (Doupé, Lymbery, & Starcevich, 2003; Kay, Halse, Scanlon, & Smith, 2001). The area includes 38 affected towns (George, K., Hill-T., & N., 2005) and has an annual rainfall of 300–700 mm (Halse et al., 2003; Kay et al., 2001).

Aquatic biodiversity in these salinised ISWs is dominated by 81 invertebrate families (Kay et al., 2001), including brine shrimp *Parartemia* sp., copepod *Calamoecia tilobata*, ostracod *Australocpris bennetti*, *Daphnia (Daphniopsis) truncate*, *Haloniscus searlei* and *Coxiella*

glauerti (Timms, 2009), and diatom communities (Fourtanier & Kociolek, 1999; John, 2000; Lange-Bertalot, Cavancini, Tagliaventi, & Alfinito, 2003; Taukulis & John, 2009; Witkowski, 2000).

2.1.2. Chemistry, ionic composition and the fluctuation of ionic composition

Most of the salinised ISW sources are alkaline (Timms, 2009). The pH ranges from 2.9–10.51 (Kay et al., 2001; Taukulis & John, 2009; Timms, 2009). The salinity remarkably varies from 0.04–258 ppt (Taukulis & John, 2009; Timms, 2009). The conductivity ranges from 0.1–192.2 mS cm^{-1} and the turbidity from 1–290 Nephelometric Turbidity Units (NTU) (Kay et al., 2001). Total N and P vary from 0.05–25.0 mg L^{-1} and 0.01–9.0 mg L^{-1}, respectively (Kay et al., 2001). Dissolved oxygen (DO) and temperature range from 1.11–18.67 mg L^{-1} and from 6.3–28.1^{o}C (Taukulis & John, 2009). The ionic composition of the ISW remarkably varies among locations, with emphasis on the deficiency of K^+ (Table 1.1). Salinised ground water in Australia dominantly consist of Na^+ and Cl^- (Salama, Farrington, Bartle, & Watson, 1993a). Groundwater salinities likely increase at the discharge areas (Salama, Farrington, Bartle, & Watson, 1993b; Timms, 2005) as a result of the addition of salt into water/soil (Bennetts, Webb, Stone, & Hill, 2006). For example, the salt storage in the Cuballing catchment (WA) ranges from 900 to 21000 g m^{-2} depending on the depth of the catchment (Salama et al., 1993b), the evaporation (Bennetts et al., 2006; Prangnell, 2007) and the small proportion of halite (Cl^-/Br^- ratio) (Bennetts et al., 2006). However, the interactions between ions and water result in a slight decrease in salinity (Bennetts et al., 2006). The exchange of Na^+ for Ca^{2+} on smectites results in a decline in Na^+/Cl^-, Mg^{2+}/Cl^-, K^+/Cl^- and HCO_3^-/Cl^- ratios (Bennetts et al., 2006) and an increase in pH (Bennetts et al., 2006).

2.2. Aquaculture in ISW

2.2.1. Potential implication of ISW for aquaculture of marine species

There have been attempts to reduce the negative impact of salinisation, e.g. via developing systems to irrigate and drain the salinised water (Pitman & Läuchli, 2002; Thomas et al., 2005), growing salt tolerant crops and trees to lower the water table in salinised soils (Pitman & Läuchli, 2002; Thomas et al., 2005) and using ISW for aquaculture of aquatic animals (Kolkovski, 2010). The implication of ISW for aquaculture is considered the potential option in terms of economic, social and environmental perspectives. From an economic perceptive, ISW aquaculture adds value to ISW by offsetting the costs of the negative effects of salinisation (Gooley et al., 1998), for ISW treatments (e.g. reduces the cost of building salinity interception schemes (SISs) across Australia), and for land use, as ISW land is cheaper than coastal land (Partridge, 2008).

In addition, most salinised areas are located in remote spaces; therefore, ISW aquaculture reduces the risks of tides, storms and pathogens that coastal aquaculture usually faces (Partridge, 2008). Regarding the social perspective, low agricultural productivity from salinised farms reduces the income of the farmers, with a loss of \$12 billion $year^{-1}$ (Ghassemi et al., 1995), and changes the fabric of the local economy (Beresford, Bekle, Mulcock, & Phillips, 2001; Partridge, Lymbery, & George, 2008). The introduction of ISW aquaculture is one possible way to stabilise local social and economic affairs. Regarding the environmental perspective, ISW aquaculture also directly utilises ISW and directly mitigates the negative impact of ISW on the environment (Partridge, Lymbery, & George, 2008). However, because the characters of ISW vary considerably at different locations, understanding the advantages and disadvantages of ISW (Table 2.4) is one of the crucial steps to creating the optimum medium for cultured candidates and helping reduce risks when using ISW for aquaculture. Utilisation of ISW for aquaculture, which is impractical

for other users, also reduces environmental issues facing coastal aquaculture (Ogburn, 1998).

Table 2.4 Advantages and disadvantages of using inland saline water for aquatic species aquaculture.

	Character	Reference
Potential implication	Various source of salinised water (underground water, gas well drills, disused open-cut mines)	Kolkovski (2010); Johnson and Wright (2003); Brinckerhoff (2004)
	National policies have been developed for aquaculture in ISW	Trendall, Aldert, and Lymberyt (1997)
	Sufficient funds and resources have been allocated by the government, research institutes and corporations	Smith and Barlow (1999)
	Various species have been successfully experimentally cultured using ISW	Fotedar et al. (2008); Prangnell and Fotedar (2006b); Fotedar et al. (2008); Liu et al. (2014); Roy et al. (2010); Tantulo and Fotedar (2006); Doroudi et al. (2006); Fielder et al. (2001); Barman et al. (2005); Kumar et al. (2010)
	Suitable systems have been developed for ISW aquaculture	Hutchinson and Flowers (2008); Kolkovski (2010); Allan et al. (2008); Partridge, Sarre, Ginbey, Kay, and Jenkins (2006); Doupe et

		al. (2005); Ingram, McKinnon, and Gooley (2002)
Constraint	Deficit or unstable water source due to severe or long-term drought	Kolkovski (2010); Allan et al. (2008); Allan et al. (2009)
	Deficiency in ionic composition (K^+)	Kolkovski (2010); Prangnell and Fotedar (2006b)
	High variation in environmental quality and quantity in water temperature, salinity and pH	Allan et al. (2009); Kumar, McCullough, and Lund (2009); Kolkovski (2010)
	High labour cost	Kolkovski (2010)
	High operation cost as locations of ground water sources are usually in rural areas	Kolkovski (2010)
	Small local market, with a population of 22 million, and a low rate of seafood consumption	Kolkovski (2010)
	Environmental concerns	Butler (1998)
	Contains chemical and biological contaminants	Kolkovski (2010); Nott, Pridham, Roe, Ibbott, and Leeson (2004); Fitzpatrick et al. (2005); Partridge, Lymbery, and George (2008)

2.2.2. Present status of ISW aquaculture in the world and Australia

2.2.2.1. Worldwide ISW aquaculture

ISW aquaculture have been practised worldwide (Roy et al., 2010), which was inspired by the success of shrimp aquaculture in Thailand using a brine solution (Roy et al., 2010), and then expanded worldwide to include Brazil, China, Ecuador, Mexico, Thailand, the United States of America and Vietnam (Roy & Davis, 2010). To date, the list of aquatic species cultured in ISW has mussel continuously expanded (Table 2.5), and the successful commercial scale of white shrimp *Litopenaeus vannamei* in several countries is the consolidating evidence for the use of ISW for aquaculture.

However, common ionic concentrations, e.g. potassium, magnesium and sulphate, are typically low when compared to the ionic concentration of OW at the same salinity (Boyd, Boyd, & Rouse, 2007), particularly K^+, except in the ISW in the Netherlands, where the K^+ concentration is higher than that of OW (Van der Hiele, Rijstenbil, Creemers, & Heringa, 2014). To optimise conditions for ISW aquaculture, two methods have been employed to modify the potassium concentration for the aquaculture of marine species: i; through ISW, such as supplementing fertilisers to change the ionic composition of ISW and ii; through ionically nutritional supplements (Romano & Zeng, 2012; Roy & Davis, 2010; Saoud, Roy, & Davis, 2007). The former has been widely used (Fisher, Bodinier, Kuhl, & Green, 2013; Fotedar et al., 2008; McNevin, Boyd, Silapajarn, & Silapajarn, 2004; Prangnell, 2007; Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006).

Table 2.5 Cultured species in inland saline water worldwide.

Common name	Scientific name	Country	Reference
Manila clam	*Venerupis (Ruditapes) philippinarum*	Netherlands	Van der Hiele et al. (2014)

Pearl spot	*Etroplus suratensis*	India	Swivedi and Lingaraju (1986)
Chinese white shrimp	*Fenneropenaeus chinensis*	China	Liu (2001)
Tiger prawn	*P. monodon*	India	Dwivedi and Trombetta (2006); Rahman, Jain, Reddy, Kumar, and Raju (2005)
Pacific white shrimp	*Litopenaeus vannamei*	USA	Gong, Jiang, Lightner, Collins, and Brock (2004); Roy et al. (2009)
		China	Liu et al. (2014)
Giant freshwater prawn	*Macrobrachium rosenbergii*	India	Jain, Raju, Kumar, Ojha, and Reddy (2007)
Brine shrimp	*Artemia salina*	USA	Brune, Reach, and O'Connor (1981)
Barramundi	*Lates calcarifer*	India	Jain, Kumar, and Mukherjee (2006)
European carp	*Cyprinus carpio*	India	Swivedi and Lingaraju (1986)
Fathead minnow	*Pimephales promela*	USA	Burnham and Peterka (1975)
Red drum	*Sciaenops ocellatus*	USA	Forsberg, Dorsett, and Neill (1996)
Grey mullet	*Mugil cephalus*	India	Barman et al. (2005); Swivedi and Lingaraju (1986)
Marine diatom	*Phaeodacrylum tricomutum*	USA	Brune et al. (1981)

2.2.2.2. Australian ISW aquaculture

The potential of using ISW for aquaculture of aquatic species emerges with solid foundations which were mentioned above. Various species of different marine groups have been tested in ISW (Table 2.6) such as black bream *Acanthopagrus butcheri* (Doupe et al., 2005), barramundi *Lates calcarifer* (Partridge, Lymbery, & Bourke, 2008), mulloway *Argyrosomus japonicas* (Doroudi et al., 2006; Hutchinson & Flowers, 2008), Australian snapper *Pagrus auratus* (Fielder et al., 2001), western king prawns *Penaeus latisulcatus* (Prangnell, 2007; Prangnell & Fotedar, 2006b), Pacific white shrimp *Litopenaeus vannamei* (Liu et al., 2014; Roy et al., 2010), black tiger prawn *Penaeus monodon* (Tantulo & Fotedar, 2006), and agar *Gracilaria cliftonii* (Kumar et al., 2010).

Table 2.6 Cultured species in inland saline water in Australia.

Common name	Scientific name	State	Reference
Trochus	*Trochus niloticus*	NT	Lee (1997)
Greenlip abalone	*Haliotis laevigata*	WA	Fotedar et al. (2008)
Pacific oyster	*Crassostrea gigas*	Vic	Ingram et al. (2002)
Sydney rock oyster	*Saccostrea glomerata*	Vic	Ingram et al. (2002)
Blue mussel	*Mytilus edulis*	WA	Dinh *et al.* (unpublished)
Brine shrimp	*Artemia salina*	Vic	Gooley et al. (1998)
Banana prawn	*Penaeus merguiensis*	Qld	Collins, Russell, Walls, and Hoang (2005)
Kuruma prawn	*P. japonicus*	Vic	Ingram et al. (2002)

Tiger prawn	*P. monodon*	WA	Tantulo and Fotedar (2006)
Western king prawn	*P. latisulcatus*	WA	Prangnell and Fotedar (2005); Prangnell and Fotedar (2006b)
Western rock lobster	*Panulirus cygnus*	WA	Tantulo (2007)
Atlantic salmon	*Salmo salar*	Vic	Ingram et al. (2002)
Australian bass	*Macquaria novemaculeata*	Vic	Ingram et al. (2002)
Australian snapper	*Pagrus auratus*		Fielder et al. (2001)
Barramundi	*Lates calcarifer*	WA	Partridge et al. (2006); Partridge, Lymbery, and Bourke (2008)
Black bream	*Acanthopagrus butcheri*	WA	Doupe et al. (2005)
European carp	*Cyprinus carpio*	Vic	McKinnon, Ingram, and Gooley (1998)
Greenback flounder	*Rhombosolea tapirina*	SA, Vic	Hutchinson (1998); Ingram et al. (2002)
Mulloway	*Argyrosomus japonicas*	NSW, SA, WA	Doroudi et al. (2006); Hutchinson and Flowers (2008); Partridge et al. (2006)

Rainbow trout	*Oncorhynchus mykiss*	Vic, WA	Ingram et al. (2002); Partridge et al. (2006)
Sand whiting	*Sillago ciliata*	Vic	Ingram et al. (2002)
Silver perch	*Bidyanus bidyanus*	Vic	Ingram et al. (2002); Doroudi et al. (2007)
Snapper	*Pagrus auratus*	SA, Vic, WA	Hutchinson and Flowers (2008); Ingram et al. (2002); Partridge et al. (2006)
Tommy rough/Australian herring	*Arripis georgiana*	SA	Hutchinson (1998)
Yellow-fin whiting	*Sillago schombergkii*		Hutchinson (1998)
Yellowtail kingfish	*Seriola lalandi*		Hutchinson and Flowers (2008)
Microalgae	*Dunaliella salina*		Kolkovski (2010)
Agar	*Gracilaria cliftonii*		Kumar et al. (2010)

NSW: New South Wales; NT: Northern Territory; Qld: Queensland; SA: South Australia; Vic: Victoria; WA: Western Australia.

In the past decade, several organisations have been established nationwide to investigate and support the potential for inland saline aquaculture in most of the ISW-affected states in Australia. For example, the Australian Centre for International Agricultural Research (ACIAR), the Fisheries Research and Development Corporation (FRCD), the Department of

Primary Industries (Victorian Government), Marine and Freshwater Resources Institute (MAFRI) (Victoria), and the South Australian Research and Development Institute (New South Wales). New systems and models have been developed for the ISW culture of marine species, for example: semi-intensive floating tank system (SIFTS) (Allan et al., 2008; Partridge et al., 2006), SIS (Hutchinson & Flowers, 2008), cages (Doupe et al., 2005) and earthen ponds (Allan et al., 2008; Fielder et al., 2001). Advantages and disadvantages of these systems are described in Table 2.7.

Table 2.7 Advantages and disadvantages of the systems developed for inland saline water aquaculture (modified from Partridge *et al*. (2008b).

System	Advantage	Disadvantage	Reference
SIFTS	Increase stock management High stock density Effective operation and waste management	High cost	Allan et al. (2008); Partridge et al. (2006)
SIAS	Effective environmental management (temperature and salinity)		Hutchinson and Flowers (2008); Kolkovski (2010); Allan et al. (2008)
SBCS	Multi-species culture (integrated aquaculture) Possible salt harvest at the ending of the system	Ineffective water quality management, e.g. water temperature, hydrogen sulphide Risk of predators (rats)	Ingram et al. (2002)
PAS	High stock density Effective stock management	High light requirement Complex management	
Static earthen	Low cost	Low stock density	Partridge, Lymbery, and George

pond			(2008)
Flow through pond	Higher stock density (compared to static earthen pond)	Ineffective waste and water temperature management	
Cage	Effective stock management Low cost	Ineffective waste and water quality management Risk of predators (birds, rats)	Doupe et al. (2005)
Raceway	High stock density Effective stock management	Decrease in water quality at the end of the system Causes stress on fish Ineffective waste management	
RAS	Reuse of water	High cost Complex management	Piedrahita (2003)
Flow through tank	Low cost Effective management of water temperature	Ineffective waste management Cost for ionic supplementation	

SBCS: serial biological concentration system; SIFTS: semi-intensive floating tanks; SIAS: semi-intensive aquaculture system; PAS: partitioned aquaculture systems; RAS: recirculating aquaculture systems.

2.3. Osmoregulation and osmoregulatory related issues

2.3.1. Osmoregulation

Marine and fresh waters are the home of various aquatic vertebrates and invertebrates. Aquatic animals employ different physiological mechanisms when facing environmental variations (salinity, temperature and oxygen). Some species simply migrate or bury themselves to avoid environmental changes, e.g. *Mya arenaria* (Deaton, 1992) or gape their shells (Costa & Pritchard, 1978; Davenport, 1979a, 1981), while others gradually change their body fluid according to the cyclical change in the environment and return to their normal condition when the cyclical environment finishes the fluctuation cycle (Willmer, 2006). Some species employ osmoregulatory processes to regulate the difference between their body and the external environment, e.g. *Geukensia demissa* (Deaton, 1992).

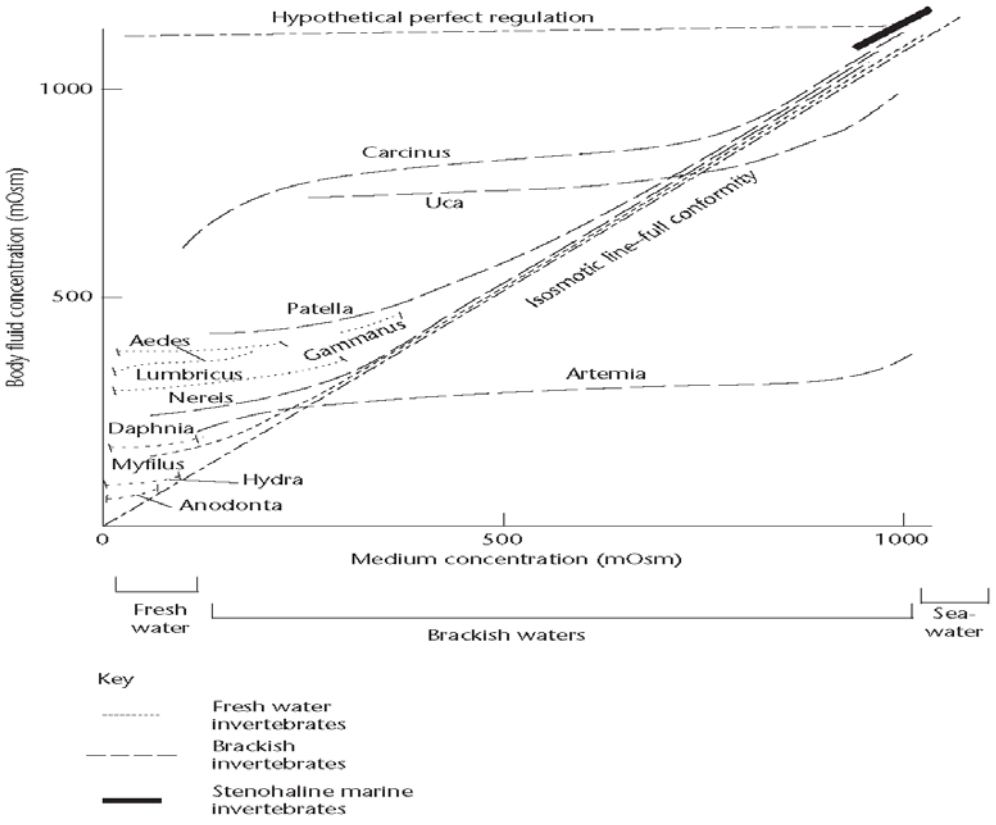


Figure 2.2 Osmoregulatory patterns in aquatic invertebrates (Source: Willmer, 2006).

Osmoregulation is an essential process for freshwater and OW invertebrates (Figure 2.2) (Willmer, 2006), and is defined as the balance state of the osmotic gradient between internal and external media (Bourque, 2008; Burton, 1980). However, the osmoregulatory process is still active even when haemolymph and environmental osmolality are equal (Willmer, 2006). Osmoregulation is costly in terms of energy (Willmer, 2006). Molluscs allocate more energy to the osmoregulatory process to adapt to the environment when they live in unstable environmental conditions (estuaries, tidal variation) and vice versa (Willmer, 2006).

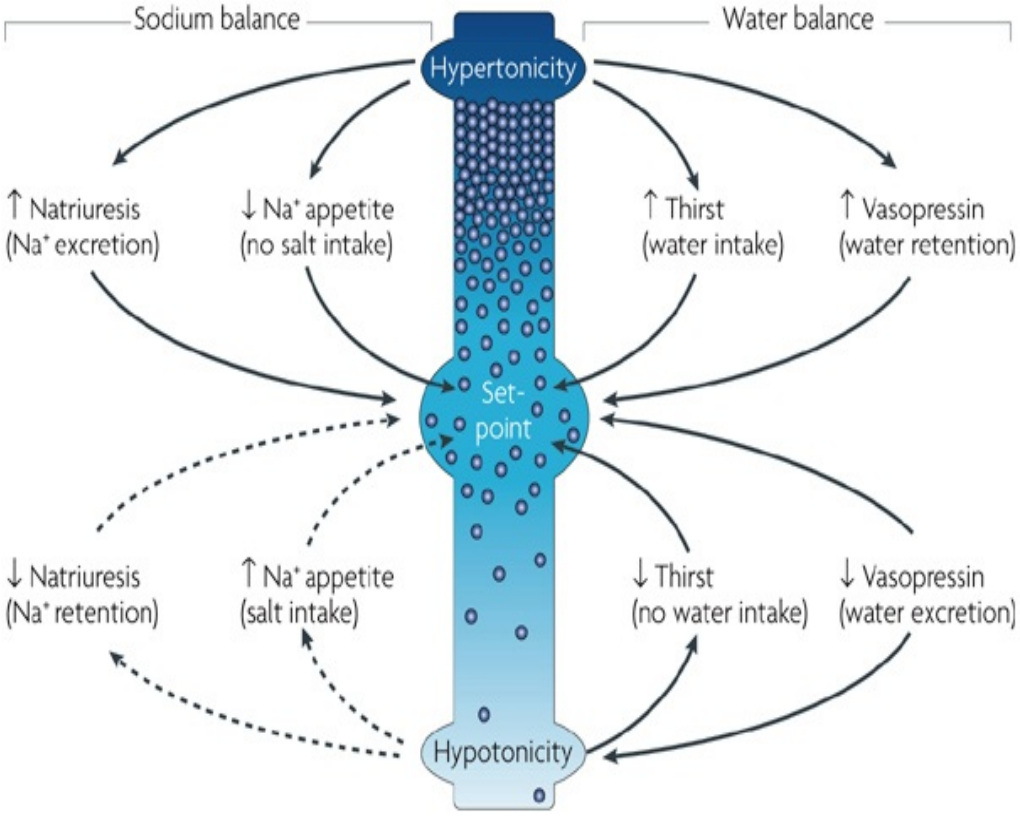


Figure 2.3 Basic concept of osmoregulation (Source: Bourque, 2008).

In invertebrates, organs, such as skin, gills and kidneys, are involved in ionic exchanges in the osmoregulatory process (Table 2.3) (Hiscock, 1953; Mantel & Farmer, 1983; Pequeux, 1995; Willmer, 2006). Of which, gills are the main organ that regulate ions, e.g. Na^+ and Cl^- (Mantel & Farmer, 1983; Pequeux, 1995). The presence of a sodium pump in the gill membrane, namely Na^+/K^+ ATPase, transports Na^+ and Cl^- ions between the gill epithelial

cells and haemolymph to maintain osmoregulation in invertebrates (Charmantier et al., 1985; Mantel & Farmer, 1983). Na^+/K^+ ATPase activity is dependent on the ratio of Na^+ and K^+ in the surrounding environment (Tantulo & Fotedar, 2007). Changes in osmotic concentration in the extracellular fluid may disturb the distribution of water and ion gradients across the cell membrane (Deaton, 2008) and eventually disable cellular function (Deaton, 2008). The osmotic concentrations of haemolymph in most marine mussel and external environments is equilibrium (Figure 2.4) (Deaton, 2008; Pierce, 1971).

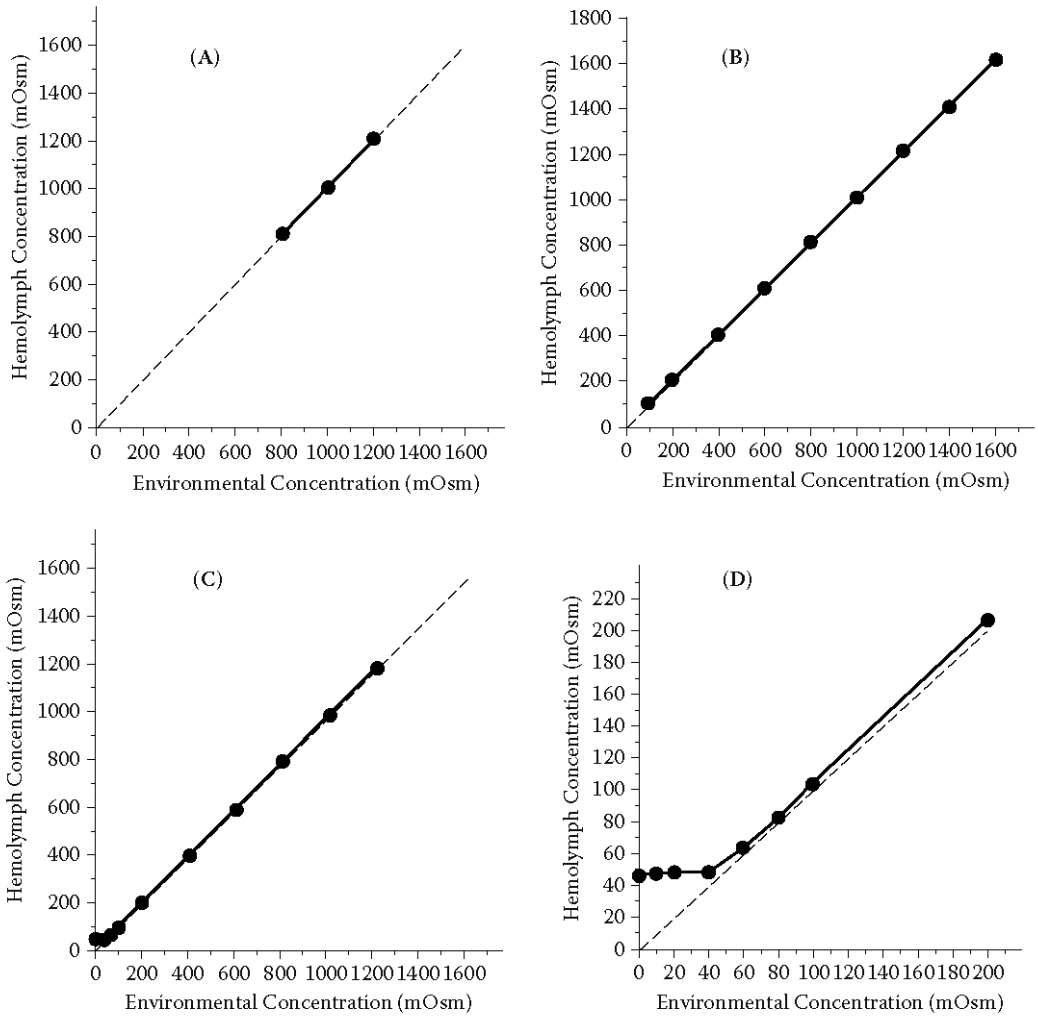


Figure 2.4 Relationship between haemolymph osmolality and environmental osmolality of mollusc groups in different environments. (A) stenohaline; (B) euryhaline; (C) oligohaline; and (D) freshwater (Source: Deaton, 2008).

2.3.2. Mechanisms of osmoregulation

Mainly, three mechanisms of osmoregulation have been clarified: i, reduction in external permeability; ii, ion regulation exchange between the haemolymph and surrounding environment; and iii, cellular osmoregulation (Figure 2.5) (Willmer, 2006).

Reduction in external permeability:

Invertebrates adapt to an unstable or changeable environment by reducing their surface permeability (Rainbow & Black, 2001; Willmer, 2006). For example, when the euryhaline molluscs are exposed to low salinity environments, their body permeability likely declines, they excrete body water via renal organs and they decrease their osmotic pressure (Pequeux, 1995). The decrease in osmotic pressure results in a decrease in osmolality, causing the cells to swell, which decreases the intracellular space, resulting in an increase in tonicity and a slowing of the water influx into the body (Roy et al., 2010). Levels of osmoregulation via excretory organs depend on the levels of environmental changes (Willmer, 2006). Excretory organs function based on ultrafiltration (Willmer, 2006) via a semi-permeable membrane (the first line of defence in invertebrates). However, other physiological mechanisms are required to regulate environmental changes, as membrane activity is limited since the membrane cannot function fully when the external environment changes (Willmer, 2006).

Ion regulation:

Ion regulation is the mechanism for the active exchange of ions based on the activities of the skin, gills and kidneys (Hiscock, 1953; Mantel & Farmer, 1983; Pequeux, 1995; Willmer, 2006) and the sodium ion pump Na^+/K^+ ATPase (Liu et al., 2014). Changes in salinities

force invertebrates to regulate osmolality via ionic composition and body fluid (Deaton, 2008; Willmer, 2006) and transports Na^+ and Cl^- ions between the gill epithelial cells and haemolymph to maintain a stable osmoregulation in invertebrates (Charmantier et al., 1985; Mantel & Farmer, 1983). Na^+/K^+ ATPase activity is dependent on the ratio of Na^+ and K^+ in the surrounding environment (Tantulo & Fotedar, 2007). The suitable ratio of Na^+/K^+ for the normal function of Na^+/K^+ ATPase in marine animals varies from 23.85 to 85.20 in juvenile *H. laevigata* (Fotedar et al., 2008), *P. latisulcatus* (Prangnell & Fotedar, 2005) and *L. vannamei* (Zhu, Dong, Wang, & Huang, 2004). A deficiency of K^+ can change the Na^+/K^+ ratio in a way that can inhibit the ability of Na^+/K^+ ATPase to function, which can result in the poor survival of marine species (Fisher et al., 2013; Prangnell & Fotedar, 2005, 2006a; Tantulo & Fotedar, 2007; Zhu et al., 2004).

Cellular osmoregulation:

Another adaption to the environmental variations and environmental contaminants is the intracellular regulation (Deaton, 2008; Willmer, 2006) via the management of free amino acids (Babarro & Fernández Reiriz, 2006; Babarro, Reiriz, Garrido, & Labarta, 2006; Hummel et al., 1996; Hummel, Bogaards, de Wolf, Sinke, & Poortvliet, 1994; Kube, Gerber, Jansen, & Schiedek, 2006; Kube, Sokolowski, Jansen, & Schiedek, 2007; Sokolowski, Wolowicz, & Hummel, 2003; Zurburg, Hummel, Bogaards, De Wolf, & Ravestein, 1989), which is termed “osmotic effectors” (Willmer, 2006). Of which, the taurine/glycine ratio (Hummel et al., 1994; Zurburg et al., 1989) or/and the sum of threonine and serine are used as environmental indicators (Zurburg et al., 1989). In the blue mussel, glycine (25 to 100 mmol kg^{-1}) and taurine (4 to 180 mmol kg^{-1}) are significantly correlated with environmental osmolality (Kube et al., 2006). The sum of threonine and taurine decreases when the blue mussel exposed to increased temperatures (Livingstone, 1985). When exposed to decreased salinity, the aquatic animals are likely to keep the ions and

release amino acids from cells into the blood and, consequently, reduce the osmotic gradient (Willmer, 2006) (Fig. 2.5). However, the constraints for the use of free amino acid as a stress indicator are the salinity and considerable seasonal variation (Livingstone, 1985; Zandee, Kluytmans, Zurburg, & Pieters, 1980).

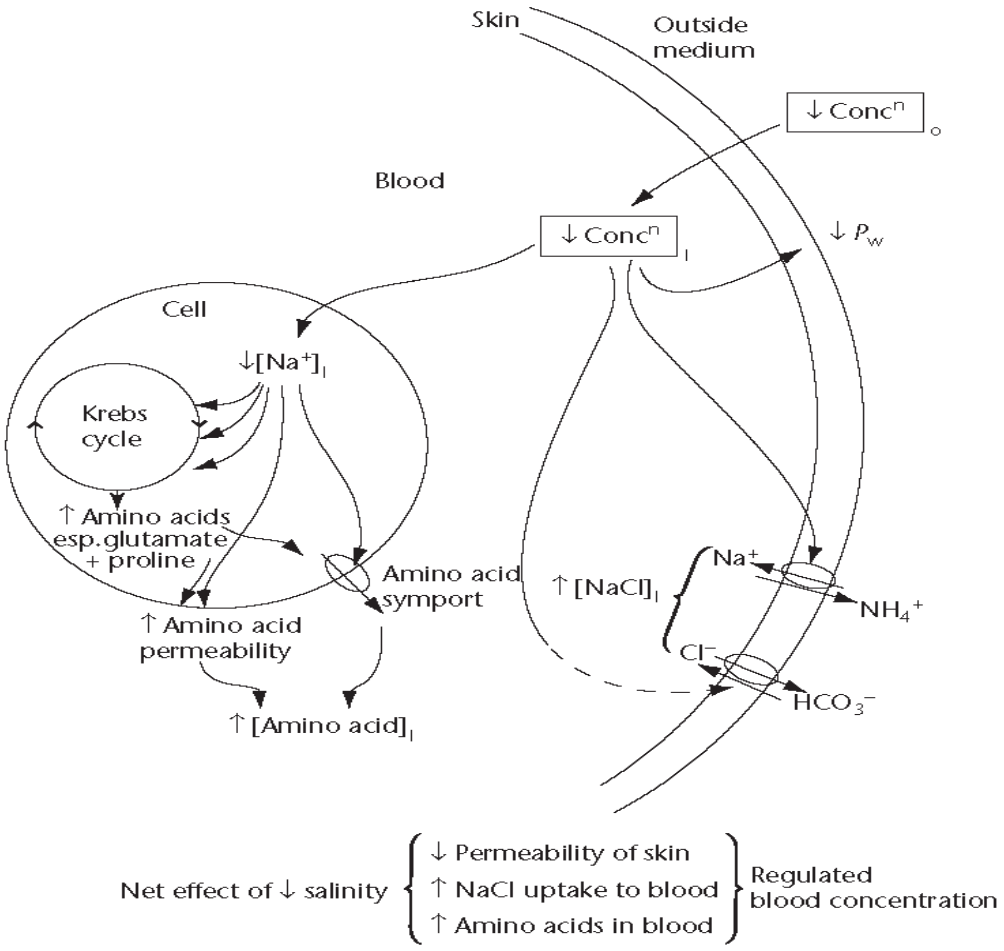


Figure 2.5 Major mechanisms for haemolymph – cell regulation of the littoral estuary in invertebrates (Source: Willmer, 2006).

2.4. Blue mussel

Documents on the blue mussel in the last decades have been really intensive in reflecting the interest of researchers as well the economic value that this species possesses regarding

reproduction biology, physiology, genetics, hatchery and aquaculture technology, market demand.

2.4.1. Taxonomy, distribution and life cycle

Blue mussel consists of a group of four close-related taxonomies, namely the *Mytilus edulis* complex. The origin of the blue mussel is extremely complex due to different hypotheses (Hilbish et al., 2000) that make it impossible to identify them based on morphology (Wonham, 2004). A gene-related technique has been recruited to identify their origin and distribution (Heath, Rawson, & Hilbish, 1995; Hilbish et al., 2000; Ladoukakis, Saavedra, Magoulas, & Zouros, 2002; Martínez-Lage et al., 2002; Rawson & Hilbish, 1995; Śmietanka, Burzyński, & Wenne, 2009). The taxonomy of species in the genus *Mytilus* is as follows:

Phylum: Mollusca

Class: Bivalvia

Order: Mytiloida

Family: Mytilidea

Genus: *Mytilus*

Species: *Mytilus edulis, M. californianus, M. galloprovincialis, M. trossulus*

Blue mussel is a common species and are distributed worldwide, throughout temperate northern and southern hemispheres (Figure 2.6) (Buck et al., 2010; Gérard, Bierne, Borsa, Chenuil, & Féral, 2008; Gosling, 1992; Hilbish et al., 2000; McDonald, Seed, & Koehn, 1991; Rawson & Hilbish, 1995; Seed, 1992; Sukhotin, Strelkov, Maximovich, & Hummel,

2007) due to their high fecundity and mobile dispersal characteristics (Hickman, 1992). The distribution of the blue mussel is principally affected by water temperature (Hickman, 1992; Seed, 1992). The presence of blue mussel in the northern hemisphere resulted from a migration from the southern hemisphere during the Pleistocene via an Atlantic route (Hilbish et al., 2000). The specific distribution of the blue mussel is as follows:

- *Mytilus californianus* is from the Pacific coast of North America (Gosling, 1992)
- *M. edulis* is widely distributed in the southern hemisphere (Borsa, Rolland, & Daguin-Thiébaut, 2012), northern temperate (Gosling, 1992) and north-east Europe (Sukhotin et al., 2007). This euryhaline species lives in estuarine conditions, as they are able to adapt to fluctuating salinities in mesohaline and ocean conditions (Gardner & Thompson, 2001; Kube et al., 2006)
- *M. galloprovincialis* is native to the Mediterranean, the Black Sea and Western Europe. They were introduced to the temperate North Pacific, South Africa and elsewhere in the Southern Hemisphere (Gosling, 1992)
- *M. trossulus* is restricted to the brackish water (5 to 15 ppt) in the North Pacific, northern parts of the North Atlantic, the Baltic Sea (Europe) and the Pacific coast of North America (Gosling, 1992; Śmietanka, Zbawicka, Wołowicz, & Wenne, 2004)

The blue mussel habitat is on the hard substrate of intertidal and subtidal areas of many shorelines (Hickman, 1992).

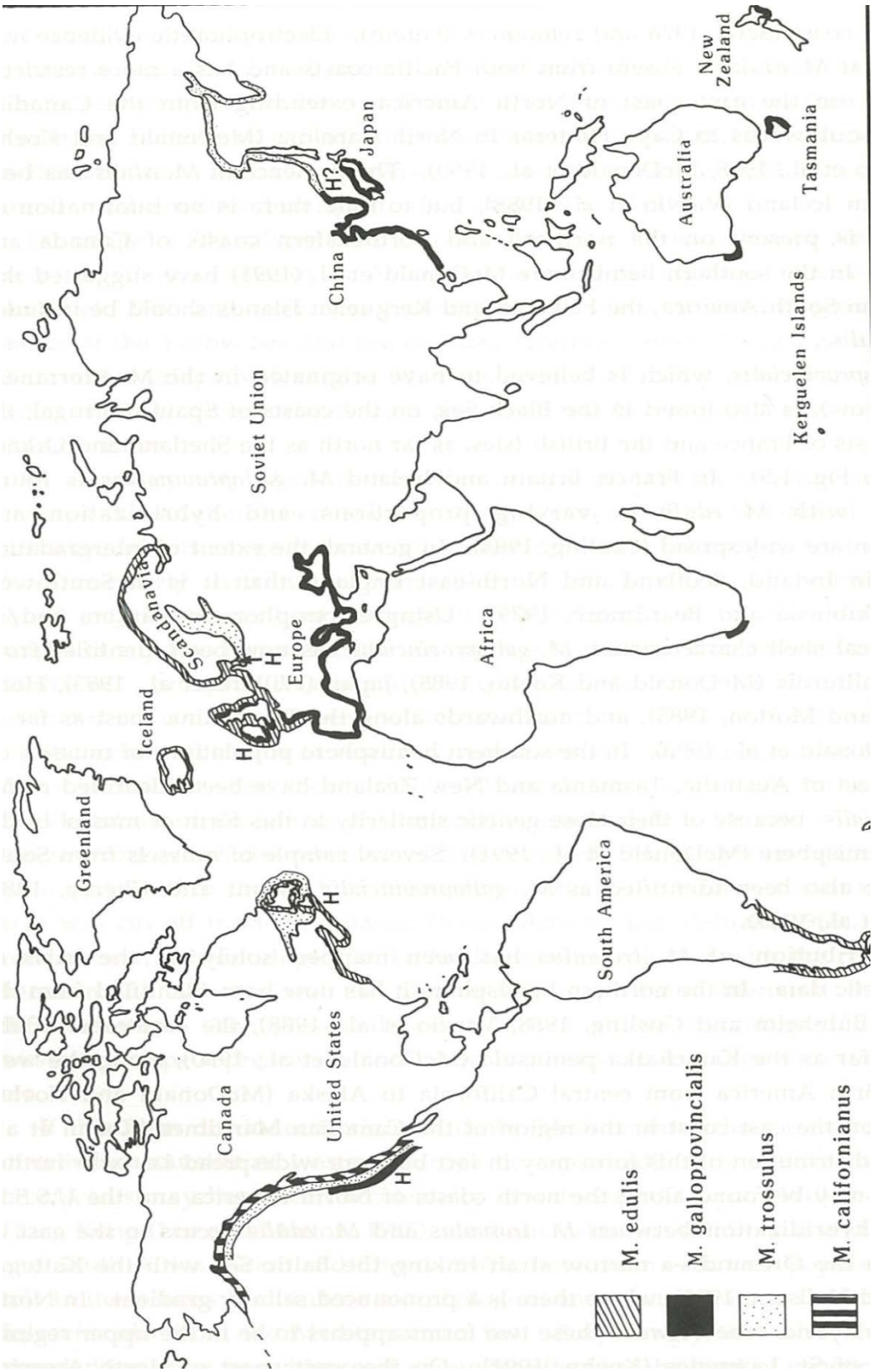


Figure 2.6 The worldwide distribution of mussel of the genus *Mytilus* (Source: Gosling, 1992).

2.4.2. Reproduction

The reproductive cycle of the blue mussel has been well documented (Figure 2.7) (Chipperfield, 1953; Seed, 1969; Thompson, 1979; Thorarinsdóttir & Gunnarsson, 2003; Toro, Thompson, & Innes, 2002).

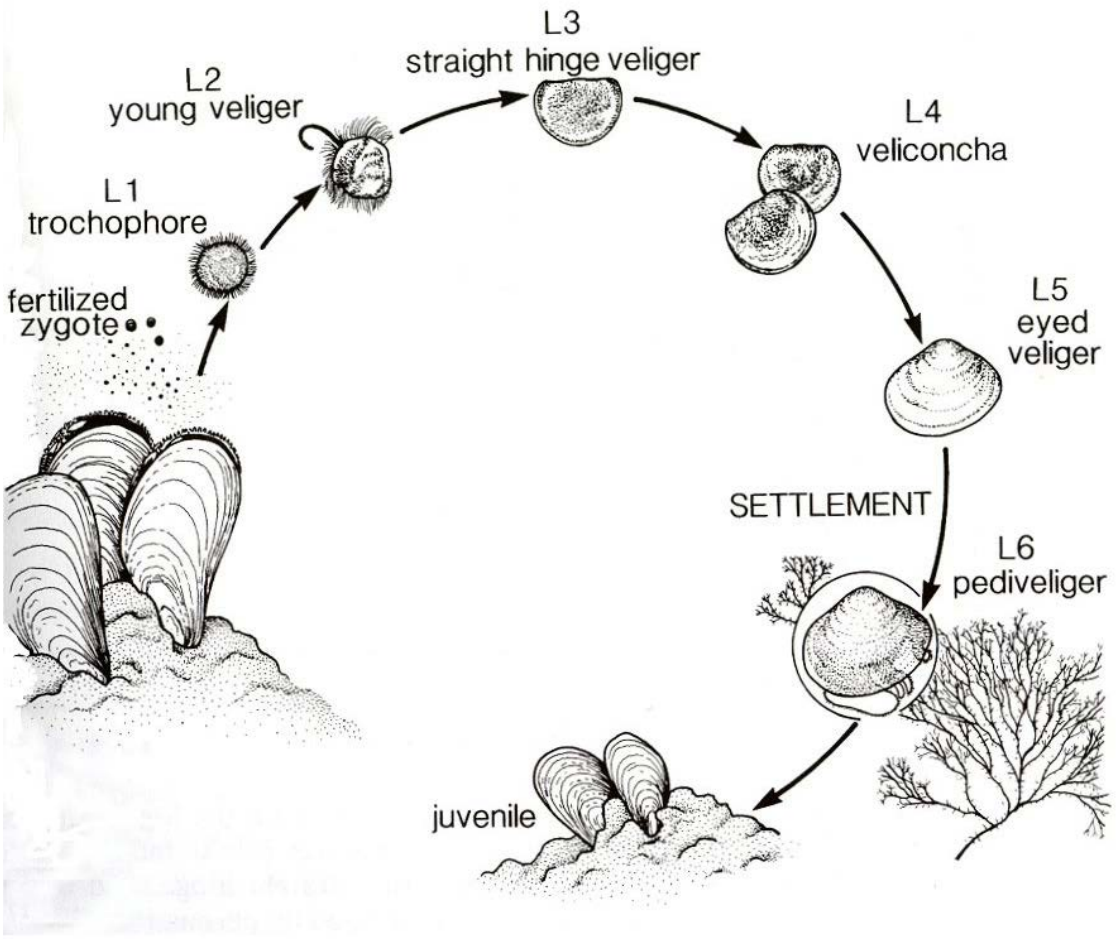


Figure 2.7 The life cycle of the blue mussel (Source: Saurel *et al.*, 2004).

Blue mussel have two distinct genders, but it is difficult to distinguish between male and females through the external sharp (R. Seed, 1969). Based on previous studies, five stages of the gonad have been determined (Table 2.8) (Chipperfield, 1953; Sprung, 1983). The blue mussel is mature and first spawning at the size of 12.15 mm (Toro et al., 2002).

Table 2.8 Gonadal stages of the blue mussel.

Stage	Characteristic	Reference
1	Development of adipogranular and vesicular connective tissue cells	(Chipperfield, 1953; King, McGrath, & Gosling, 1989; Lowe, Moore, & Bayne, 1982; Rodhouse et al., 1984; Seed, 1969; Sprung, 1983; Thorarinsdóttir & Gunnarsson, 2003)
2	Early development of the gonad with the first appearance (or not) of the gametes and the gradual thickness of the mantle	
3	Further development of the gonad with the presence of unripe gametes in the mantle, and immobile sperm (maturation of gametes)	
4	Full development of the gonad with ripe gametes, mobile sperm, and the release of the gametes into the water	
5	Regression of the follicle and the rest of the gametes in the gonad	

The blue mussel shows year-round spawning activity geographically: March to August (Chipperfield, 1953; Pieters, Kluytmans, Zandee, & Cadee, 1980), and June to November (Thorarinsdóttir & Gunnarsson, 2003). Reproduction of blue mussel consists of two seasons annually based on the appearance of the a settlement: May to August and September to October (Pulfrich, 1996). Reproduction is related directly to the fluctuation of the biochemical composition of the tissues such as proteins, glycogens and lipids (Pieters, Kluytmans, Zurburg, & Zandee, 1979; Zandee et al., 1980). Accordingly, the release of the

gametes into the water reduces the protein and lipid levels in the tissues (Pieters et al., 1979; Zandee et al., 1980). Reproduction of blue mussel can be delayed intentionally via low temperatures (5–6^{o}C) and low food (Bayne & Thompson, 1970).

The fecundity of blue mussel varies yearly, and reflects the energy allocation through the availability of the surrounding environment (Thompson, 1979). Among the genus *Mytilus*, *M. trossulus* has a higher fecundity than *M. edulis*, which is of a similar shell length (Toro et al., 2002). The fecundity of the blue mussel is shown in Table 2.9.

Table 2.9 The fecundity of the blue mussel.

Broodstock size (shell length, mm)	Egg	Sperm	Reference
	10^6	10^{10}	Thompson (1979)
68	7.6×10^6		Bayne, Holland, Moore, Lowe, and Widdows (1978)
42	2×10^2		Mateeva (1948)

2.4.3. Larval development

The fertilisation of *M. edulis* occurs at salinities ranging from 15 to 40 ppt, and temperatures ranging from 5 to 22^{o}C (Bayne, 1965). Early cleavage starts one hour post fertilisation (hpf) (Lutz, Chalermwat, Figueras, Gustafson, & Newell, 1991) and transformation to a trochophore is at 24 to 48 hpf, followed by the appearance of veliger at 288 to 384 hpf. Pediveliger larvae occur 720 hpf. The settlement of the blue mussel marks a significant change in morphology and the appearance of byssal threads 672 hpf (Table 2.10).

Table 2.10 Early larval stages, size (µm) and duration (hours) of the blue mussel.

Stage	Measurement	Reference
Fertilised egg	60–90	Lutz et al. (1991)
Trochophore		
Size	65–70	Saranchova and Flyachinskaya (2001)
Duration		
Veliger		
Size	92–252	Bayne (1964b); Sprung (1984)
Duration	288–384	Bayne (1964b)
Pediveliger		
Size	250–300	Bayne (1964b); Sprung (1984); Dobretsov and Wahl (2008)
Duration	> 720	Saranchova and Flyachinskaya (2001)
Settlement		
Size	250–1000	Chipperfield (1953); Pechenik, Eyster, Widdows, and Bayne (1990); McGrath, King, and Gosling (1988); Bayne (1964a)
Duration	> 672	Chipperfield (1953)

The settlement of blue mussel is influenced by the biological, physical and ionic composition of the media (Eyster & Pechenik, 1987; McGrath et al., 1988; Ompi, 2011; Pulfrich, 1996; Satuito, Natoyama, Yamazaki, & Fusetani, 1995). Larvae of blue mussel primarily attach to filamentous algae (Dobretsov & Wahl, 2008; Eyster & Pechenik, 1987; Grizzle, Short, Newell, Hoven, & Kindblom, 1996) and hydroids (Genzano, Excoffon, Acuña, & Zamponi, 2003) for a period before detaching (Dobretsov & Wahl, 2008; Lane,

Beaumont, & Hunter, 1985) and secondarily resettling (Dobretsov & Wahl, 2008) on the blue mussel beds (Bayne, 1964a). Filamentous algae play dual roles in inducing the primary settlement of blue mussel larvae: by secreting the soluble metabolites (Dobretsov, 1999) and protecting settled larvae from predators (Genzano et al., 2003).

However, while Bayne (1964a), Dare, Edwards, and Davies (1983) and Lane et al. (1985) concluded that larvae of blue mussel experienced two stages of settlement, other studies have found contrary results, in which, blue mussel experiences secondary settlement by attaching to adult mussel beds directly without the primary settlement (McGrath et al., 1988; Svane & Ompi, 1993). This pattern of settlement has also been found in other species of the genus *Mytilus* such as *M. galloprovincialis* (Caceres-Martinez, Robledo, & Figueras, 1994; Ompi, 2011). However, development in early stages is influenced by the combination and interaction of biological, physical and chemical factors rather than a single factor (Pernet, Tremblay, & Bourget, 2003; Rodriguez, Ojeda, & Inestrosa, 1993). For example, Pechenik et al. (1990) found that blue mussel larvae with the combined food concentration of 30 x 10^4 cells/mL and temperature of 16^{o}C resulted in the highest growth rate of 8.1 µm/day compared to the lowest growth rate of only 1.0 µm/day at the combined food concentration of 0.5 x 10^4 cells/mL and temperature of 16^{o}C.

Sizes and developmental stage interval (DSI) of blue mussel larval cultured under different conditions from other studies are found in Table 2.11.

Table 2.11 Developmental stage interval (h) and size (µm) of early larvae of blue mussel.

Stage	DSI	Size	Reference
Fertilised egg		60–90	Lutz et al. (1991)
Trochophore			

	20–24	70	Bayne (1964b)
	12	65–70	Saranchova and Flyachinskaya (2001)
Veliger			
	96–120	240–245	Bayne (1964b)
	96	75–250	Saranchova and Flyachinskaya (2001)
Pediveliger			
		260–265	Bayne (1964b)
	384–744	260	Bayne (1965)
	936–1680	220	Bayne (1965)
	>720	250–400	Saranchova and Flyachinskaya (2001)
Settlement			
	>672	400–1000	Chipperfield (1953)
		301–341	Pechenik et al. (1990)
		260	McGrath et al. (1988)
		250–350	Bayne (1964a)

DSI: developmental stage interval

2.4.4. Byssus and attachment capacity of mussel

Many bivalvia species, for example, zebra mussel *Dreissena polymorpha* (Rajagopal, Van der Velde, Jenner, Van der Gaag, & Kempers, 1996), pygmy mussel *Xenostrobus securis*

(Babarro & Comeau, 2014), green-lipped mussel *Perna viridis* (Cheung, Luk, & Shin, 2006), mussel *Brachidontes variabilis* (Cheung et al., 2006), ribbed mussel *Geukensia demissa* (Brodsky, Walters, Schneider, & Hoffman, 2011) mussel *Mytella charruana* (Brodsky et al., 2011), mussel *Mytilus galloprovincialis* (Babarro & Comeau, 2014), California sea mussel *M. californianus* (Dowd & Somero, 2013), mussel *M. trossulus* (Dowd & Somero, 2013) and blue mussel *M. edulis,* produce byssus threads to secure themselves to the substrate (Lachance, Myrand, Tremblay, Koutitonsky, & Carrington, 2008). Byssal threads play various roles in locomotion (Allen, Cook, Jackson, Preston, & Worth, 1976), orientation (Allen et al., 1976), attachment to a substratum (Aldred et al., 2006; Allen et al., 1976; Kishore, Hunter, Zeng, & Southgate, 2014), the reduction of the dislodgement rate of mussel (Thieltges & Buschbaum, 2007), and defence against epibionts (Thieltges & Buschbaum, 2007) and predators (Cheung et al., 2006; Côté, 1995; Garner & Litvaitis, 2013).

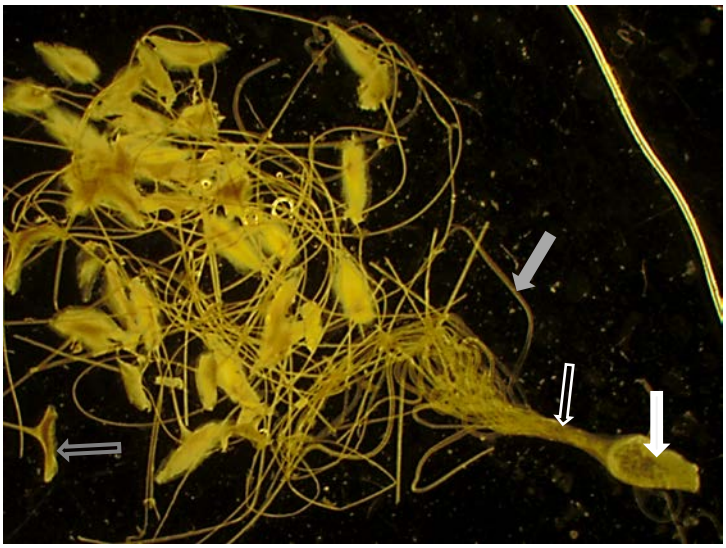


Figure 2.8. Byssus morphology of the blue mussel. Solid white arrow: root; empty white arrow: stem; grey solid arrow: byssal thread and grey empty arrow: plaque (adhesive disc).

Byssus apparatus appears at the pediveliger stage (Ackerman, Sim, Nichols, & Claudi, 1994; Bayne, 1971), while the larvae are undergoing metamorphosis (Yonge, 1962) with the

formation of the specific gland located at the foot of blue mussel being responsible for the formation of byssal threads (Winkle, 1970) through a so-called process of injection moulding (Waite, Qin, & Coyne, 1998). Generally, a byssal thread consists of four parts (Price, 1981; Wiegemann, 2005): root, stem, thread and adhesive plaque (disc) (Figure 2.8). Byssal threads of blue mussel are regularly formed (Lachance et al., 2008) with a duration of 2–5 min thread^{-1} (Waite, 1992). The threads decompose after 4–6 weeks (Carrington, 2002; Moeser & Carrington, 2006). The formation of byssus threads is influenced by various factors such as water velocity (Hunt & Scheibling, 1996; Moeser, Leba, & Carrington, 2006; Price, 1980, 1982), seasons (Young, 1985), temperature (Selin & Vekhova, 2004; Young, 1985), salinity (Young, 1985), pesticides (Roberts, 1975) and predators (Christensen, Dolmer, Petersen, & Tørring, 2012; Côté, 1995; Côté & Jelnikar, 1999; Garner & Litvaitis, 2013; Lowen, Innes, & Thompson, 2013). Ions, such as Ca^{2+} and Mg^{2+}, are reported to influence the formation of byssal threads of the blue mussel (Winkle, 1970).

2.4.5. Present status of blue mussel aquaculture

To date, 80% of globally consumed shellfish is from aquaculture (FAO, 2014). Blue mussel is becoming an ideal candidate for aquaculture due to outstanding characteristics such as the fast speed at which they grow, their pleasant taste (Seed & Suchanek, 1992), the year-round availability of the seed (FAO, 2004-2015; Hickman, 1992) and their high density (Hickman, 1992). Blue mussel plays a vital role in coastal ecosystems (Lindahl et al., 2005; Śmietanka et al., 2004) and the aquaculture industry (Śmietanka et al., 2004). Blue mussel is cultured worldwide, with main producers in China, Spain, Italy, France, New Zealand and Thailand, and a global production of 197.831 tonnes in 2013 (Figure 2.9). In Australia, the production of marine mollusc species was 16.838 tonnes, and the total aquaculture production was 80.066 tonnes (Stephan & Hobsbawn, 2014). Various methods have been employed to

culture blue mussel, for example, on-bottom, longline, raft, hanging park, bouchot, pole and fish trap (FAO, 2004-2015; Hickman, 1992).

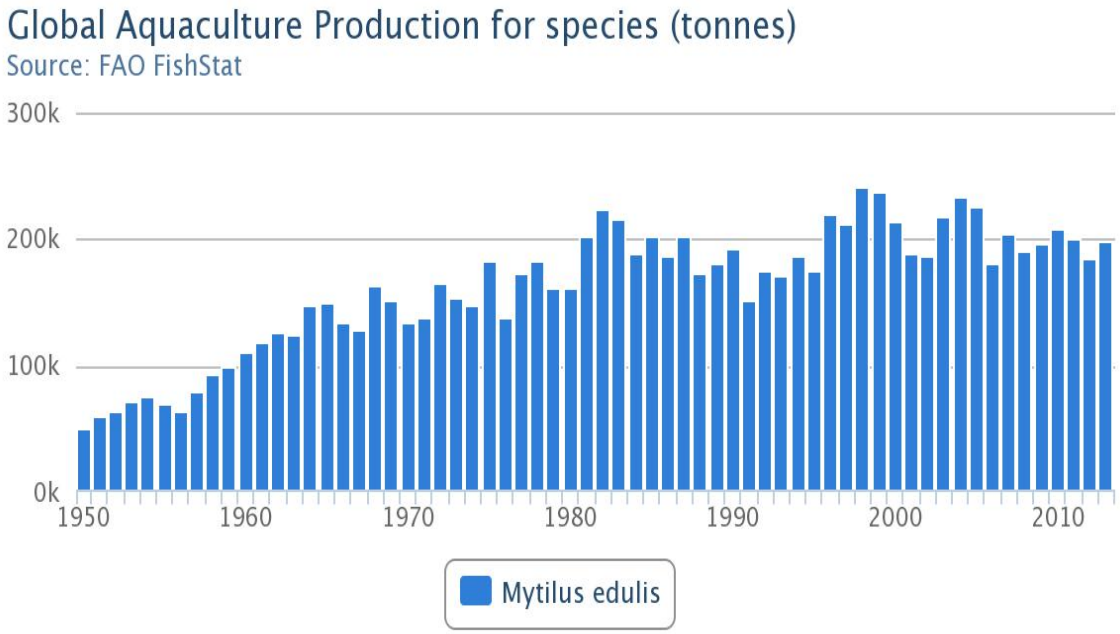


Figure 2.9 Global aquaculture production of blue mussel (tonnes). Source: FAO, 2004–2015).

However, the development of mussel aquaculture is facing rising issues, such as the demand exceeding the supply due to poor seed supply, outbreaks of diseases (Smaal, 2002), conflicts between cultures (or capture) and management (Smaal, 2002), legislative limitations regarding environmental issues, and questions with respect to sustainability of coastal aquaculture (Ogburn, 1998).

Nonetheless, increasing global demand has exceeded the supply from fishery and aquaculture production (FAO, 2004-2015), encouraging the development of new aquaculture methods and technologies with an emphasis on using ISW as a medium to culture blue mussel.

It is now established that ISW, after ionic manipulations, can be used to culture various marine species of Western Australia, including molluscs. Only abalone has been investigated for its cultural potential in ISW of Western Australia, no attempts have been made to evaluate its cultural feasibility in ISW of Western Australia.

Chapter 3 MATERIALS AND METHODS

3.1. Site of the studies

All experiments were conducted at the Curtin Aquatic Research Laboratory (CARL), Curtin University, Western Australia.

3.2. Water, blue mussel and microalgae

OW and ISW were procured from Hillarys (31°49'S, 115°45'E) and a lake at Wannamal (31°15'S, 116°05'E), Western Australia, respectively. The water was transported to the CARL where it was stored in two 10000-ton circular thanks to precipitate suspended solids for the further dilution by adding deionised water to achieve different water types at different salinities used in the experiments. A comparison of ionic composition of the ISW and OW at 35 ppt is shown in Table 3.1.

Table 3.1 The ionic compositions (mg L^{-1}) of inland saline water (ISW) and ocean water (OW) at 35 ppt.

Parameter	OW	ISW
pH	7.83	7.85
DO	5.65	5.37
Osmolality (mOsm kg^{-1})	984.00	853.00
Na^+	10010.00	8719.00
K^+	351.10	84.40
Ca^{2+}	371.60	583.00

Mg^{2+}	1168.00	1525.00
S^{2+}	805.40	602.40
B^{3+}	3.95	0.66
P^{3+}	< 0.05	0.07
Cu^{2+}	< 0.05	< 0.05
Fe	< 0.05	< 0.05
Zn	< 0.05	< 0.05
Mn^{2+}	< 0.05	< 0.05
Na^{+}: K^{+} ratio	28.51:1	103.31:1
Mg^{2+}: Ca^{2+} ratio	3.14:1	2.62:1

In the chapter three, fourteen different water types were used in the experiment, deionised water was added to ISW and OW separately to obtain seven different salinities: 5, 10, 15, 20, 25, 30, and 35 ppt of ISW and OW. All water types were filtered through 1 µm filter before the commencement of the experiment.

In the remaining experiments, five different levels of potassium fortification: 20% ($ISW20K^{+}$), 40% ($ISW40K^{+}$), 60% ($ISW60K^{+}$), 80% ($ISW80K^{+}$) and 100% ($ISW100K^{+}$) were prepared by mixing hydrous potassium chloride (purity > 99%, Sigma-Aldrich, Germany) with ISW27. ISW at 27 ppt (ISW27) and OW at 25 ppt (OW25) were used as controls.

The ionic composition of all the water types used in this experiment was analysed by CSBP Soil & Plant Laboratory, Bibra Lake, WA using Inductively Coupled Plasma spectroscopy.

Adult blue mussel at different sizes according to the respective experiments 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 & 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).

3.3. Equipment and blue mussel

Two glass tanks (198 L, 1.1 × 0.6 × 0.3 m; length × width × depth) containing OW at salinity ranging from 25 to 26 ppt were used to acclimatise of blue mussel collected from the Esplanade Nedlands, Western Australia (31°59‘S, 115°48‘E) for all experiments in this thesis.

In the experiment one to investigate the osmolality, osmoregulatory capacity (OC) and isosmotic point (IP), the blue mussels were exposed to 7 different ISW and OW salinities of 5, 10, 15, 20, 25, 30 and 35 ppt for 360 hours. Experimental units were 1.75-liter glass beakers (PYREX, USA) filled 1.5 liter of each of fourteen water types (7 from ISW and 7 from OW) with continuous aeration. Each beaker contained 15 adult mussels. Each water type had triplicates (a total of 42 experimental units).

In the experiment two to investigate the developmentally early stages of blue mussel in K^+ fortified ISW, 100 individuals at each early stage (fertilised eggs, trochophore and veliger) of blue mussel was transferred from the stoking tank to a series of petri dishes (in triplicate) containing 20 mL of one of the water types to observe the next development stage. To observe the development from pediveliger to 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 until they settled. The byssal threads of adult blue mussels were placed into each cell strainer for larvae settlement (Eyster & Pechenik, 1987). Twenty per cent of the water in each beaker was exchanged daily. Each stage was exposed to different water types in triplicate.

In the experiment three to test whether the addition of potassium to ISW improved the survival and growth rate of the juvenile blue mussel during the culture period in various water types, the mussels (initial length and weight were 15.7 ± 0.29 mm and 0.42 ± 0.02 g, respectively) were cultured for 4 months in ISW at 27 ppt fortified with one of five different potassium levels, namely 20% ($ISW20K^+$), 40% ($ISW40K^+$), 60% ($ISW60K^+$), 80% ($ISW80K^+$) and 100% ($ISW100K^+$). The levels of potassium addition in ISW were equivalent to the typical concentration of potassium in the OW. The original ISW and OW

were used as controls. The experiment was conducted in triplicate with a total of 21 experimental glass tanks (34.5 × 20.5 × 26.0 cm) and each tank contained 30 individuals.

In the experiment four to test whether the addition of potassium to ISW improved the survival, growth rate, and biochemical composition of the blue mussels during the culture period in various water types, the mussels were cultured for 4 months in ISW, at 27 ppt fortified with one of the five different potassium levels: 20% ($ISW20K^+$), 40% ($ISW40K^+$), 60% ($ISW60K^+$), 80% ($ISW80K^+$), and 100% ($ISW100K^+$). The levels of potassium addition in ISW were equivalent to the typical concentration of potassium in the OW. The original ISW and OW were used as controls. Two different initial sizes of blue mussels were included in this study. The smaller mussels had the initial size of 34.25 ± 0.59 mm and the larger mussels had the initial size of 45.12 ± 0.47 mm. The water types were used in triplicates with a total of 42 experimental glass tanks (34.5 × 20.5 × 26.0 cm), and each tank contained 13 individuals.

In the experiment to investigate the byssus thread renewal capacity and root shedding of the blue mussel, a total of 81 blue mussels in three size classes, 27 mussels in each size class, 3 blue mussels in each water type were used in the experiment. To start the exposure period, blue mussels were placed individually into petri dishes and introduced into a glass beaker filled with 1.5 L of one of the different water types. The mussels were placed individually to avoid the formation of cluster of the blue mussels, making it difficult to quantify the number of byssus thread for each individual (Winkle, 1970). Blue mussels were exposed to one of the five water types with different levels of potassium fortification: 20% ($ISW20K^+$), 40% ($ISW40K^+$), 60% ($ISW60K^+$), 80% ($ISW80K^+$) and 100% ($ISW100K^+$) for 35 days. The levels of potassium addition in ISW were equivalent to the typical concentration of potassium in the OW of the same salinity. ISW at 27 ppt and OW at 25 ppt were used as controls. To see the difference in the number of byssus thread renewal capacity under

different impacts of K^+ fortifications, blue mussels in OW25 were categorized into two sub-categories of (i) daily cuts of byssus threads (OW25c) where the byssus threads were cut daily and (ii) uncuts (OW25u) where the byssus threads of the blue mussels were cut only at the end of the experiment (Rajagopal et al., 1996; Young, 1985). Similar procedure was applied to the blue mussels, exposed to another control of ISW27, yielding two further sub-categories ISW27cut (ISW27c) and ISW27uncut (ISW27u).

3.4. Data collection

3.4.1. Water ionic composition and parameters

Water temperature was maintained at 27^{o}C using an automatic heater (Sonpar. Model: HA-200, Zhongshan, Guangdong, China). DO and temperature were recorded using a dissolved oxygen meter (YSI model 58, Yellow Springs Instrument Co., Ohio, USA). pH and salinity were measured one a day using a pH meter (Cyber Scan pH 300, Eutech Instruments, Singapore) and a hand-held refractometer (RHS-10ATC, Xiamen Ming Xin Instrument, Xiamen, Fujian, China), respectively.

3.4.2. Survival

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

Where S is the survival (%), nt is the number of mussels at time t and no is the number of mussels at the commencement.

3.4.3. Growth

Blue mussels were dried with paper before the measurement. Then shell length was measured using a caliper, the wet weight was measured with an electronic balance (AW220, d=0.1 mg, Shimazu, Japan).

Specific growth rates in length (SGR_L) and weight (SGR_W) were calculated based on the fomulae:

$$SGR_{L\ or\ W}\ (\%\ d^{-1}) = ((\ln_{(Lt\ or\ Wt)} - \ln_{(Lo\ or\ Wo)})/t) \times 100$$

Where SGR is the specific growth rate (% day^{-1}), L is length, W is weight, L_t and W_t are total length (mm) and weight (g) at time t, and L_0 and W_0 are total length and weight at the commencement.

3.4.4. Early larval development and settlement

To obtain the trochophore stage, 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. The trochophore stage was marked by the time at which 50% of the fertilised eggs were transformed to the trochophore stage (Bayne, 1965).

Similarly, 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, respectively.

To determine the settlement of early blue mussel larvae, 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 until they settled. The byssal threads of adult blue mussels were placed into each cell strainer for larvae settlement (Eyster & Pechenik, 1987).

3.4.5. Deformity

Morphological deformity was determined based on previous descriptions (Andersen, Grefsrud, & Harboe, 2013; His, Seaman, & Beiras, 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 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.

3.4.6. Developmental stage interval (DSI, hours)

IMI was estimated by subtracting the time when 50% of larvae moulted to the next developmental stage to the time when they were newly moulted from the previous development stage.

3.4.7. Haemolymph and medium osmolality

Fifty µL of haemolymph (Aarset & Zachariassen, 1982) was collected using a 2 mL syringe connected to a 25 G needle from the posterior adductor muscle sinus (Livingstone, Widdows, & Fieth, 1979) of each of 3 randomly sampled mussels. The haemolymph was then transferred into a 0.5 mL plastic Eppendorf tube and chilled on iced-brick for immediate measurement of haemolymph osmolality using Cryoscopic Osmometer – Osmomet 030 (Gonotec, Inc, Germany).

The measurement of the medium osmolalities (ISW and OW) was performed using the same procedure as the haemolymph osmolality, except for 50 µL haemolymph was replaced by 50 µL of the corresponding media.50 µL of haemolymph.

3.4.8. Byssus thread renewal capacity and root shedding

In all water types, byssus threads of each mussel were cut to the edge of the external shells using a pair of scissors and the number of byssus threads was recorded daily. The blue mussels were placed back to the corresponding water types after the collection of byssus threads. The new formation of byssus threads (renewal capacity) was presented as a mean number of byssus threads produced by a blue mussel per day for the entire exposure duration. At the end of the experiment, the average number of byssus threads was either calculated as a mean value of 'per day basis' or by 'every 5 days interval'.

During the experiment, any byssus roots were completely dislodged from the blue mussels and left in the petri dish were also counted and termed as root shedding (Fig. 1). The number of shed byssus roots was calculated as the mean number of shed roots for 35 days.

During the experiment, if any byssus root which was completely dislodged from the blue mussel and left in the petri dish was also counted and termed as root shedding (Figure 4.18). The shed number of byssus root was calculated as the mean number of shed root for 35 days.

3.4.9. Biochemical composition analyses

After measuring the length and weight, the flesh of survived blue mussel was taken out of the shell with a surgical blade and was weighted. Shells and flesh were dried separately at 105^{o}C to a constant weight (5 hours) in a drying oven (Thermotec 2000, Contherm Scientific Ltd, New Zealand) for biochemical analysis. The dried mass of the blue mussel flesh was pooled and finely ground to powder with a mortar and pestle. Protein, lipid, moisture and ash were analysed according to the standard methods described in Association of Official Analytical Chemists (Helrich, 1990). Crude protein was analysed using the Kjeldahl method with a Kjeltec 8200 Auto distillation and Tecator digestor 8 (FOSS

Analytical AB, Sweden). Lipid content was analysed by petroleum ether extraction using the Soxhlet method. Ash was analysed by combustion at 550°C for 24 hours using an electric furnace (Carbolite Ltd, UK). Gross energy was determined using a bomb calorimeter (C2000, IKA, Staufen, Germany).

3.5. Data 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. T-test two samples for means for different tested parameters was used to determine the significant difference ($p < 0.05$) between the two sized mussels. Percentage values were arcsine-transformed to achieve normality for ANOVA assumption. Linear and second order regression analysis was performed on the experimental parameters as a function of K^+ fortification levels in ISW where appropriate. Data were presented as means and standard errors (SE). All statistical analyses were performed in SPSS version 22 for Windows (IBM Corporation, USA).

Chapter 4 RESULTS

4.1. The survival and osmoregulatory capacities of blue mussel cultured in ISW and OW

4.1.1. Ionic compositions of ISW and OW

Ion concentrations in both ISW and OW increased proportionally with increased salinity. Ca^{2+} and Mg^{2+} levels were higher in ISW than in OW, whereas S^{2+} and particularly K^{+} were lower in ISW than in OW. The lower K^{+} level in ISW resulted in a much higher Na^{+}/K^{+} ratio in ISW (more than 3 times, Table 4.1) than in OW. The Mg^{2+}/Ca^{2+} ratio was relatively stable across all water types.

4.1.2. Survival

Survival was 100% at salinities from 20 to 30 ppt in ISW and from 20 to 35 ppt in OW. In both ISW and OW, mortalities significantly ($p < 0.05$) increased with a decrease in salinities below 20 ppt. Mortalities showed a negative linear relationship with exposure time (R^2 = 0.87 – 0.99 for ISW and 0.92 – 0.99 for OW, Table 4.2), resulting in 100% mortality at 72, 120, 144 and 168 hours post exposure (hpe), in ISW5, ISW10, ISW15 and ISW35 (Figure 4.1) and at 96 hpe, 192 hpe and 312 hpe in OW5, OW10 and OW15 (Figure 4.2), respectively.

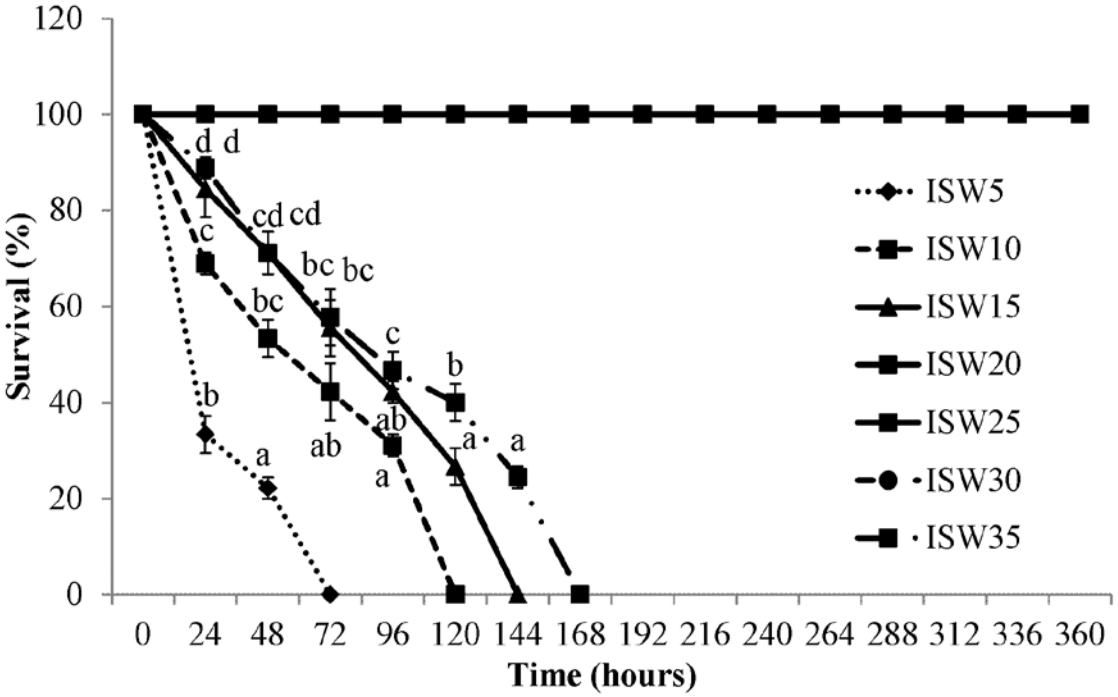


Figure 4.1 Survival (mean ± SE) of blue mussel in ISW at different salinities. Data points marked with different letters are significantly different ($p < 0.05$).

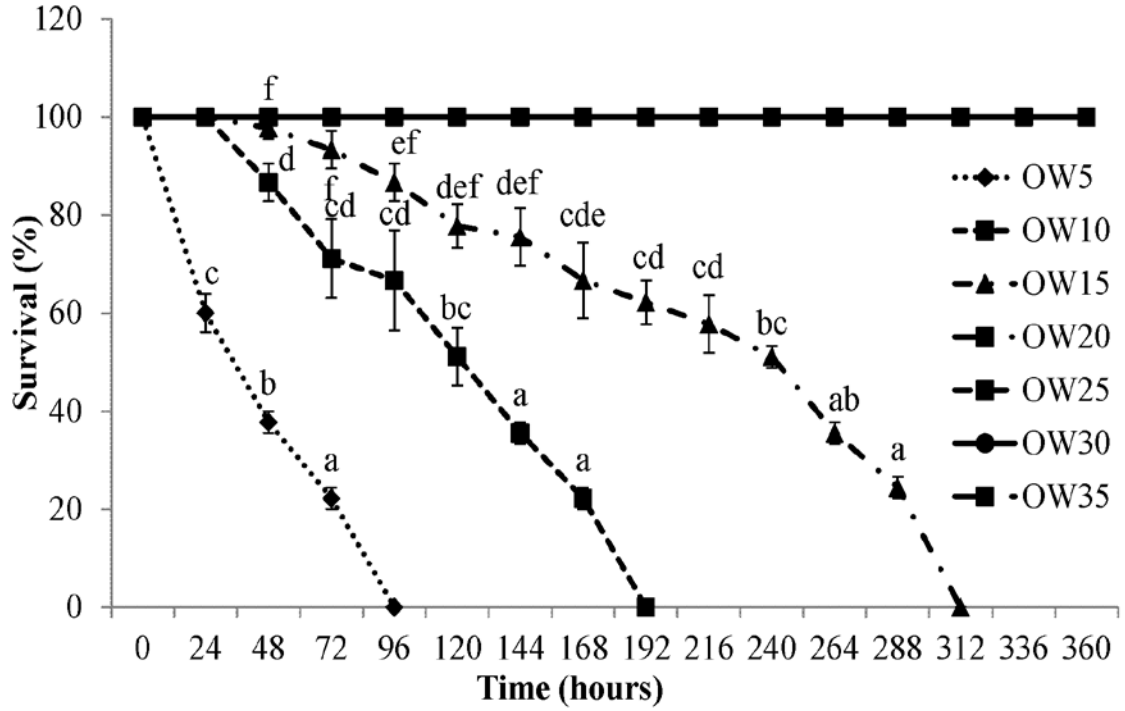


Figure 4.2 Survival (means ± SE) of blue mussel in OW at different salinities. Data with different letters are significantly different ($p < 0.05$).

1 Table 4.1 The ionic compositions (mg L^{-1}) of inland saline water (ISW) and ocean water (OW).

Parameter	5 ppt		10 ppt		15 ppt		20 ppt		25 ppt		30 ppt		35 ppt	
	ISW5	OW5	ISW10	OW10	ISW15	OW15	ISW20	OW20	ISW25	OW25	ISW30	OW30	ISW35	OW35
Osmolality (mOsm kg^{-1})	187.33	211.33	290.00	326.67	426.67	507.33	482.33	659.33	651.67	781.00	805.67	941.00	927.67	1140.33
Na^+	1416.00	1671.00	2488.00	3109.00	3574.00	4235.00	4890.00	5876.00	6125.00	7315.00	7720.00	8803.00	9385.00	10190.00
K^+	19.90	59.51	28.82	110.30	38.68	150.50	49.63	206.80	63.16	258.80	77.26	313.00	92.32	356.10
Ca^{2+}	106.30	75.76	176.20	121.70	247.90	159.90	330.40	216.50	419.50	266.90	530.80	320.90	640.60	363.90
Mg^{2+}	253.80	199.60	449.40	361.10	636.40	493.10	868.30	681.30	1088.00	843.40	1375.00	1015.00	1674.00	1172.00
S^{2+}	108.20	138.60	184.60	253.10	261.10	342.00	359.70	427.50	447.20	591.80	560.00	706.30	647.40	819.30
Na^+: K^+ ratio	71.16:1	28.08:1	86.33:1	28.17:1	92.40:1	28.14:1	98.52:1	28.41:1	96.98:1	28.26:1	99.92:1	28.12:1	101.66:1	28.62:1
Mg^{2+}: Ca^{2+} ratio	2.39:1	2.63:1	2.55:1	2.97:1	2.57:1	3.08:1	2.63:1	3.15:1	2.59:1	3.16:1	2.59:1	3.16:1	2.61:1	3.22:1

Table 4.2 Linear regression of the survival of the blue mussel at each salinity versus exposure time. (Note that the regressions were not established for water types where survival was 100% over the entire exposure period, since the $R^2 = 1$).

Water types	Regression equations	R^2 value
ISW5	y = -31.111x + 116.67	0.87
IW10	y = -17.841x + 111.7	0.96
ISW15	y = -15.873x + 117.78	0.99
ISW35	y = -13.413x + 113.97	0.98
OW5	y = -23.778x + 115.33	0.97
OW10	y = -12.593x + 122.22	0.97
OW15	y = -7.0037x + 118.88	0.92

4.1.3. Osmolality

Haemolymph osmolality of the blue mussel increased from 24 to 72 hours in ISW at different salinities and from 24 to 96 hpe in OW, and maintained a stable osmolality until the end of the exposure period. All blue mussels exposed to ISW35 died at 168 hpe after their haemolymph showed an osmolality ca. 870 mOsm kg^{-1} (Figure 4.3). Surviving individuals had osmolalities between 650 and 750 mOsm kg^{-1} in ISW and 730 to 950 mOsm kg^{-1} in OW (Figure 4.4). Overall, haemolymph osmolality of blue mussel in OW was higher than in ISW at a given salinity and exposure time.

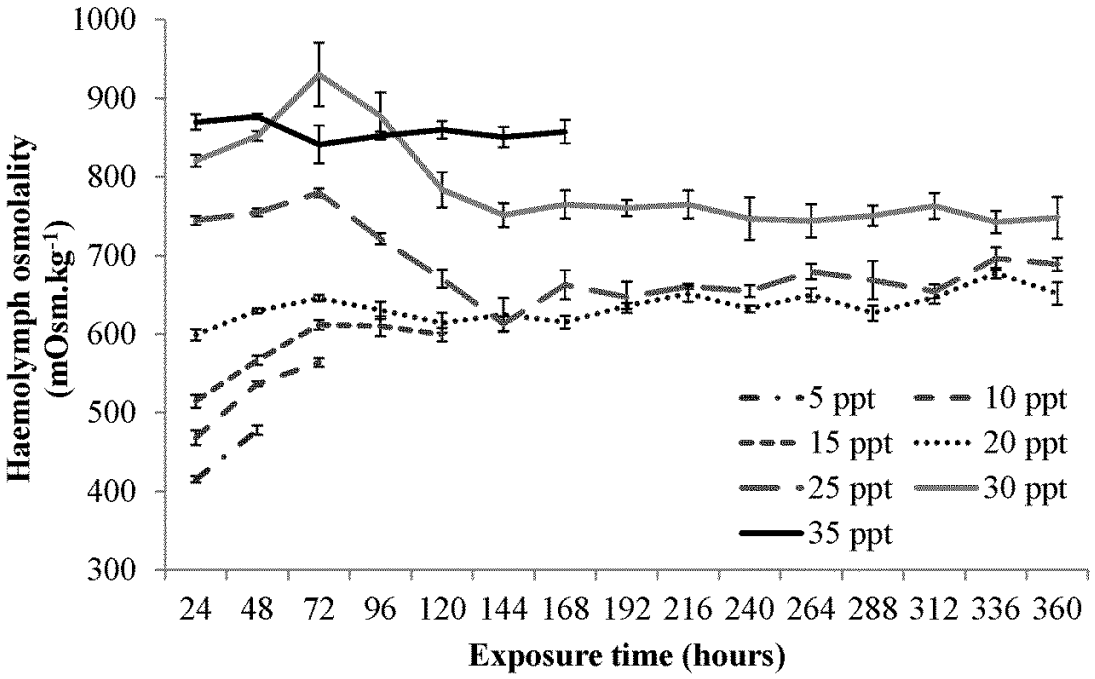


Figure 4.3 Haemolymph osmolality (mean ± SE) of blue mussel (*Mytilus edulis*) reared in ISW at different salinities.

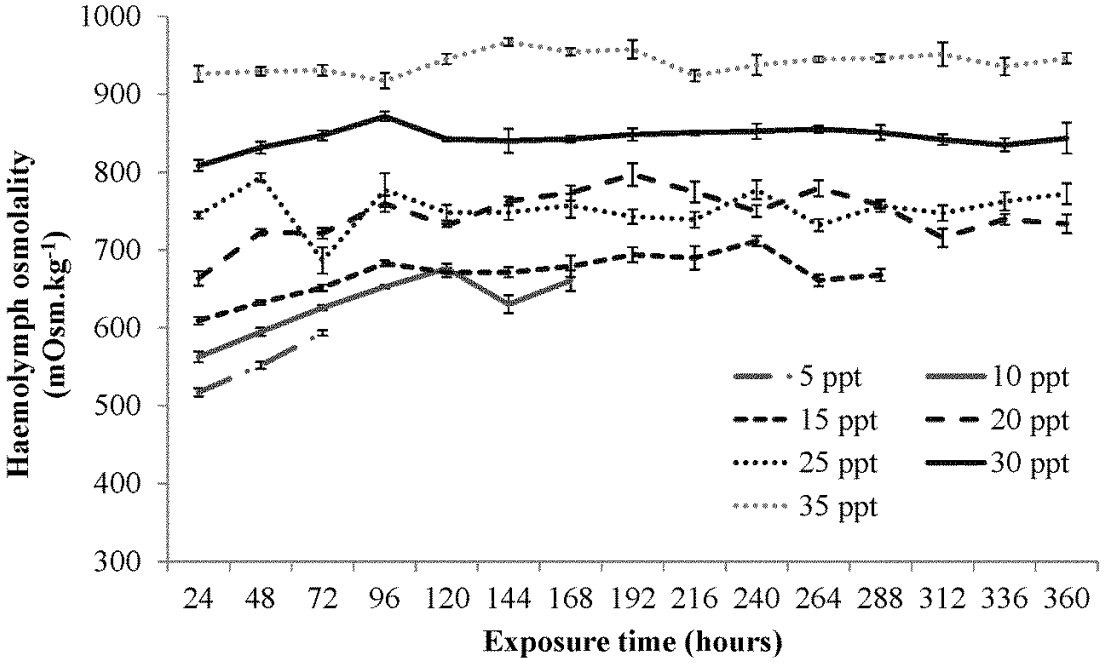
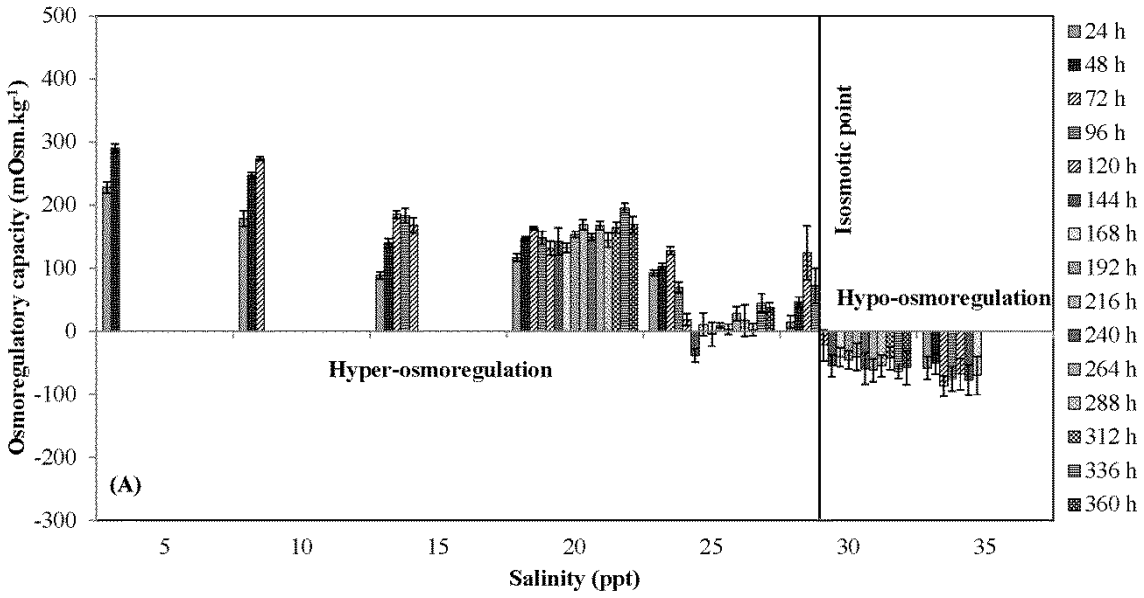


Figure 4.4 Haemolymph osmolality (mean ± SE) of blue mussel (*Mytilus edulis*) reared in OW at different salinities.

4.1.4. Osmoregulatory capacity (OC)

Overall, the OC of blue mussel showed more deviations from zero when they were exposed to salinities further away from their IP (Figure 4.5 A and B). In ISW, positive OCs (hypo-osmoregulatory pattern) were observed in the blue mussel in salinities from 5 to 25 ppt at 360 hpe and in 30 ppt at 24–96 hpe, while negative (hyper-osmoregulatory pattern) OC was observed at salinities of 30 and 35 ppt (Figure 4.5 A). In OW, blue mussel showed a hypo-osmoregulatory pattern in salinities from 5–20 ppt, whereas they showed a hyper-osmoregulatory pattern in salinities above 25 ppt (Figure 4.5 B). The hypo- and hyper-osmoregulatory patterns in the blue mussel were confirmed by regression analyses (Table 4.3).

Slopes from regression analysis between haemolymph osmolality and medium osmolality in ISW (Figure 4.6) and in OW (Figure 4.7) increased with the increase in salinities. The blue mussel showed hyper-osmoregulatory capacity at hypo-salinities and vice versa (Figure 4.5 A and B, Figure 4.6, and Figure 4.7).



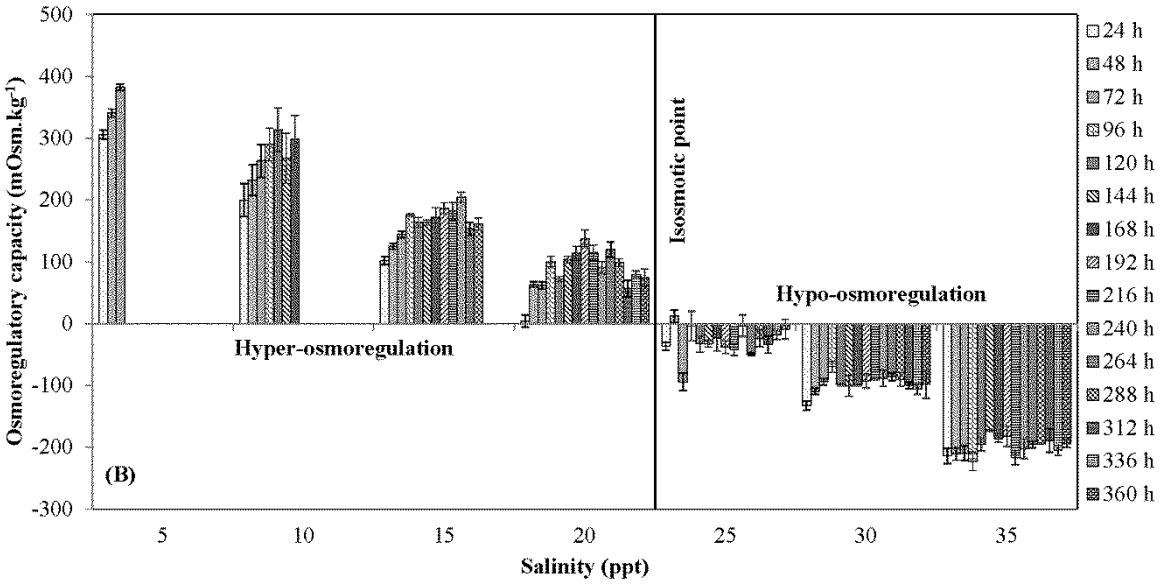


Figure 4.5 Osmoregulatory capacity (mean ± SE) of blue mussel (*Mytilus edulis*) reared in different salinities of ISW (A) and OW (B).

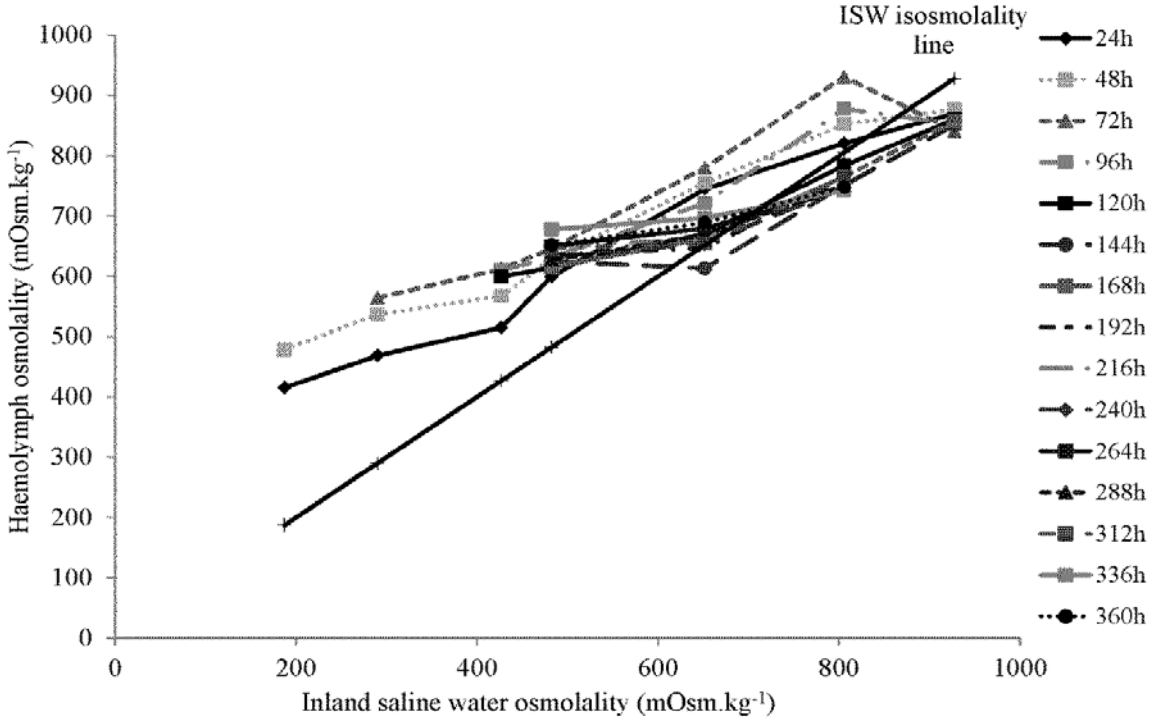


Figure 4.6 Relationship between haemolymph osmolality of blue mussel and ISW osmolality at different salinities.

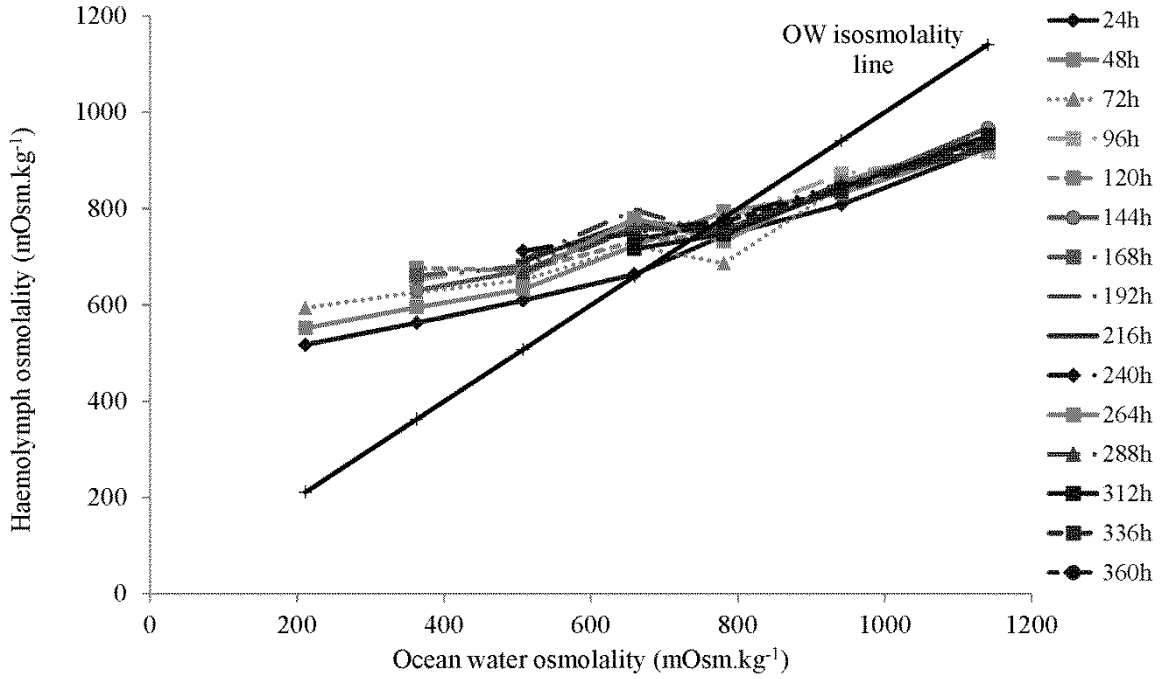


Figure 4.7 Relationship between haemolymph osmolality of blue mussel and OW osmolality at different salinities.

During 360 hpe, the IP of the blue mussel ranged from 658 to 892 mOsm kg^{-1} in ISW and from 716 to 809 mOsm kg^{-1} in OW equivalent to 25 to 34 ppt for ISW and 22 to 25 ppt for OW, respectively (Table 4.3). During the first 72 hpe the IP of the blue mussel in ISW increased from 658 to 892 mOsm kg^{-1}, which equates to salinities from 25 to 34 ppt while during the first 96 hpe the IP of blue mussel in OW increased from 716 to 802 mOsm kg^{-1}, which equates to salinities from 22 to 25 ppt. Thereafter, the IP of the blue mussel in ISW declined and stabilized to around 700 mOsm kg^{-1}, which is equal to 27 ppt. Similarly, the IP of the blue mussel in OW maintained between 770 to 800 mOsm kg^{-1}, which is equal to a salinities of 24 and 25 ppt.

Table 4.3 Relationship between haemolymph osmolality (Y) and medium (X) osmolality of blue mussel in the different types of water and salinities. Data in the same column marked by different letters are significantly different within each water type ($p < 0.05$).

Water type	Time (h)	Equation	R^2	Isosmotic point	
				mOsm kg^{-1}	ppt
OW	24	y = 0.441x + 400.34	0.9802	716.44 ± 5.54^{a}	21.96 ± 0.18^{a}
	48	y = 0.419x + 447.11	0.9847	769.32 ± 5.40^{bcd}	23.71 ± 0.18^{bcd}
	72	y = 0.358x + 487.06	0.8882	759.01 ± 7.15^{bc}	23.37 ± 0.23^{bc}
	96	y = 0.359x + 514.15	0.9555	802.31 ± 3.92^{d}	24.80 ± 0.13^{d}
	120	y = 0.358x + 506.77	0.9199	789.91 ± 6.13^{cd}	24.39 ± 0.20^{cd}
	144	y = 0.416x + 465.64	0.9420	797.37 ± 7.47^{cd}	24.64 ± 0.25^{cd}
	168	y = 0.372x + 505.90	0.9185	805.03 ± 1.97^{d}	24.90 ± 0.07^{d}
	192	y = 0.385x + 497.95	0.8469	809.43 ± 10.54^{d}	25.04 ± 0.35^{d}
	216	y = 0.358x + 507.23	0.8773	790.07 ± 3.77^{cd}	24.40 ± 0.12^{cd}
	240	y = 0.363x + 513.66	0.9701	806.09 ± 4.16^{d}	24.93 ± 0.14^{d}
	264	y = 0.423x + 453.34	0.8898	785.97 ± 7.69^{cd}	24.26 ± 0.25^{cd}
	288	y = 0.425x + 453.96	0.9624	788.97 ± 6.28^{cd}	24.36 ± 0.21^{cd}
	312	y = 0.505x + 369.33	0.9724	744.82 ± 16.81^{ab}	22.90 ± 0.56^{ab}
	336	y = 0.420x + 448.69	0.9550	772.89 ± 2.20^{bcd}	23.83 ± 0.71^{bcd}
	360	y = 0.447x + 430.52	0.9447	776.84 ± 12.92^{bcd}	23.96 ± 0.43^{bcd}
ISW	24	y = 0.658x + 278.54	0.9569	658.03 ± 10.56^{a}	24.80 ± 0.43^{a}
	48	y = 0.579x + 358.48	0.9774	852.53 ± 6.04^{d}	32.63 ± 0.24^{d}
	72	y = 0.554x + 397.79	0.8449	892.53 ± 8.14^{d}	34.24 ± 0.33^{d}
	96	y = 0.559x + 370.48	0.9026	840.70 ± 21.51^{d}	32.16 ± 0.87^{d}
	120	y = 0.525x + 359.61	0.9505	756.21 ± 15.90^{c}	28.75 ± 0.64^{c}

144	y = 0.534x + 326.85	0.7561	722.12 ± 1.59^{ab}	27.38 ± 0.06^{ab}
168	y = 0.549x + 331.33	0.9217	735.50 ± 4.66^{ab}	27.92 ± 0.19^{ab}
192	y = 0.379x + 435.91	0.7712	703.19 ± 11.59^{ab}	26.62 ± 0.47^{ab}
216	y = 0.347x + 468.21	0.7924	717.87 ± 16.66^{ab}	27.21 ± 0.67^{ab}
240	y = 0.351x + 450.94	0.8821	700.54 ± 27.01^{ab}	26.51 ± 1.09^{ab}
264	y = 0.289x + 504.49	0.8996	714.70 ± 22.20^{ab}	27.09 ± 0.89^{ab}
288	y = 0.380x + 436.28	0.8477	704.19 ± 3.48^{ab}	26.66 ± 0.14^{ab}
312	y = 0.355x + 458.74	0.7723	712.66 ± 15.63^{ab}	27.00 ± 0.63^{ab}
336	y = 0.199x + 576.55	0.7477	722.17 ± 12.41^{ab}	27.38 ± 0.50^{ab}
360	y = 0.297x + 504.42	0.9216	720.12 ± 25.00^{ab}	27.30 ± 1.01^{ab}

4.2. Early development of the blue mussel cultured in potassium-fortified ISW

4.2.1. Environmental parameters and haemolymph osmolality

Na^+ concentration and the osmolality did not differ among water types. The addition of K^+ to ISW brought the K^+ concentrations closer to K^+ concentrations in OW without changing the concentrations of other ions. The Na^+/K^+ ratios decreased with the elevated K^+ concentrations. The fortification of K^+ to ISW reduced the Na^+:K^+ ratio from 100.27:1 in ISW27 to 28.58:1 in ISW100K^+, similar to the Na^+:K^+ ratio of OW25. There was no difference in Na^+/K^+ ratio between ISW100K^+ and OW. Mg^{2+}: Ca^{2+} ratios were slightly lower in ISW than in OW. Higher levels of Ca^{2+}, Mg^{2+} were found among ISW compared to those in OW25 while S^{2+} and Mg^{2+}:Ca^{2+} ratios were lower (Table 4.4).

Table 4.4 The ionic composition (mg L^{-1}) of ISW 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^+	6584.00	6824.00	6816.00	6872.00	6943.00	6774.00	6480.00
K^+	65.66	96.25	127.00	152.40	182.30	217.50	226.70
Ca^{2+}	431.10	465.20	462.50	456.90	461.50	451.60	231.20
Mg^{2+}	1145.00	1202.00	1197.00	1189.00	1198.00	1173.00	749.30
S^{2+}	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

4.2.2. Survival

Over 78 per cent of the fertilised eggs transformed successfully to trochophore, and K^+ fortification had no effect ($p > 0.05$) on the hatching success of fertilised eggs. Similarly trochophore larvae were transformed to veliger with 80% of success. Higher K^+ levels significantly ($p < 0.05$) increased the survival of pediveliger from 55% to 68%. Similarly, the number of the newly settling larvae was significantly ($p < 0.05$) higher at higher K^+ (Figure 4.8), wherein, the percentage of settling larvae reached 62% in the highest K^+ levels (ISW100K^+), 24% higher than the ISW control, showing the high sensitivity of pediveliger and settlement stages to the increased K^+ fortification. Stronger linear correlations were shown between survival rate with pediveliger and settling larvae. However, survival of trochophore exhibited stronger ($R^2 = 0.95$) second order relationship with K^+ fortification levels in ISW. The survival of veliger stage of blue mussel was independent of K^+ levels as shown by R^2 value of 0.53 (Table 4.5).

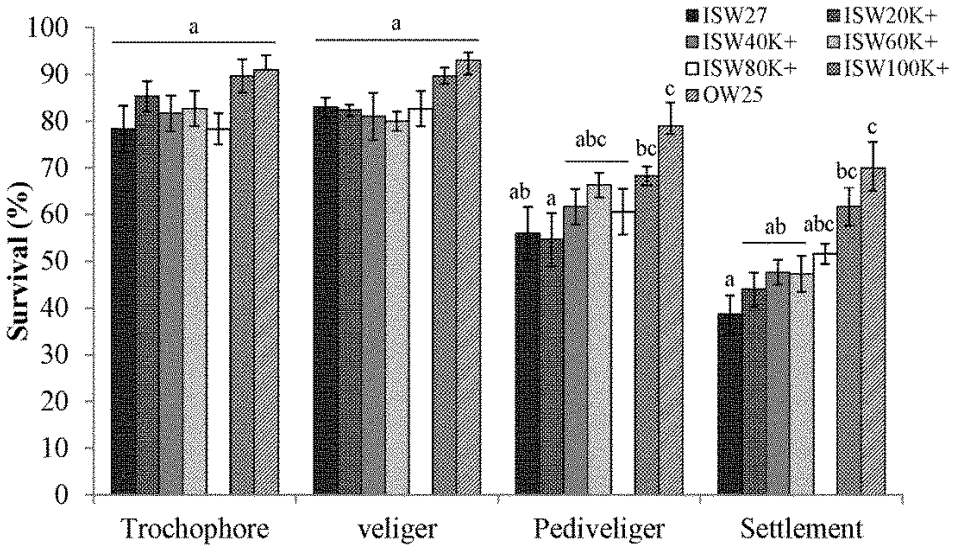


Figure 4.8 Survival of early developmental stages of the blue mussel *Mytilus edulis* in response to K^+ fortification to ISW. Data are presented as mean ± SE. Data with different letters are significantly different ($p < 0.05$).

4.2.3. Size

Size of trochophore (81–84 µm), veliger (120–138 µm) and pediveliger (301–331 µm) were not affected ($p > 0.05$) by K^+ levels (Figure 4.9). Fortification of K^+ significantly ($p < 0.05$) increased the size of settling larvae from 497 µm at the lowest K^+ level to 610 µm at the highest K^+ level (25 % increase in size). This also highlighted the sensitivity of settling larvae to the increase in K^+ fortification levels. There was no difference in the size of settling larvae when exposed to ISW100K^+ than when reared in OW25 (Figure 4.9). Linear regression analysis between K^+ concentrations and the size of early larval blue mussel showed strong correlations in pediveliger (R^2 = 0.89) and settlement stages (R^2 = 0.87). Size of veliger larvae was weakly correlated (R^2 = 0.65) with K^+ concentrations, whereas no correlation (R^2 = 0.01) was observed in trochophore stage (Table 4.5).

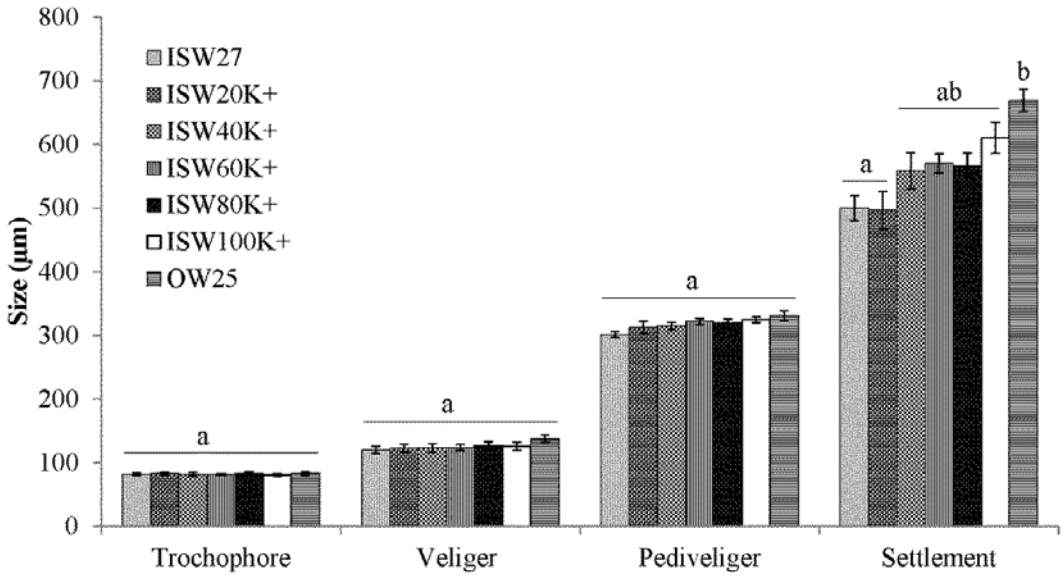


Figure 4.9 Sizes of early developmental stages of the blue mussel *Mytilus edulis* in response to K^+ addition to ISW. Data are presented as mean ± SE. Data with different letters within a stage are significantly different ($p < 0.05$).

4.2.4. Developmental stage interval (DSI)

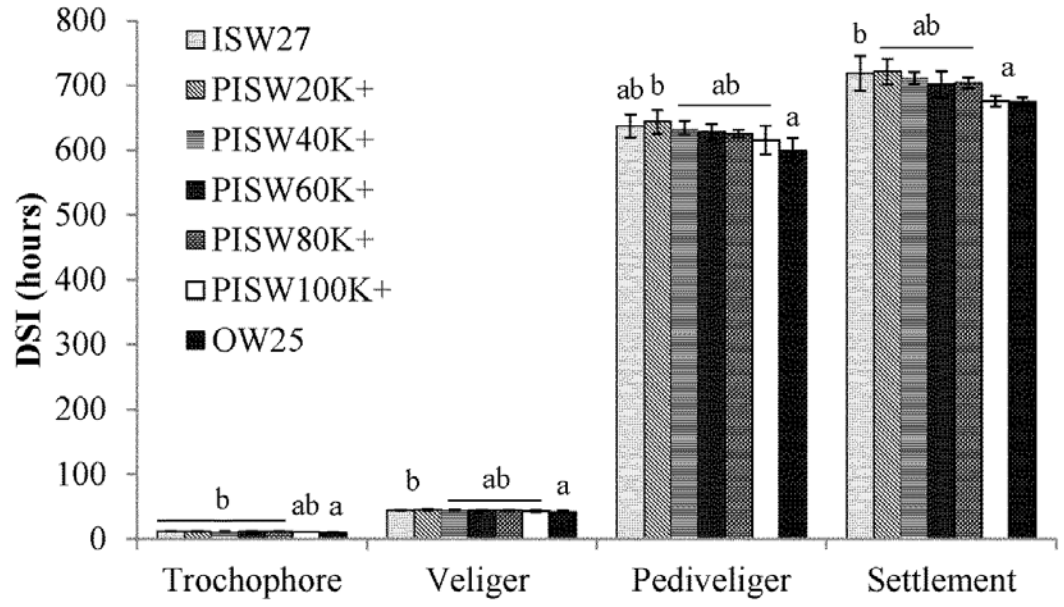


Figure 4.10 Developmental stage interval of early developmental stages of the blue mussel *Mytilus edulis* in response to K^+ addition to ISW. Data are presented as mean ± SE. Data with different letters within a stage are significantly different ($p < 0.05$).

DSI of all larval stages were shorter ($p < 0.05$) under higher K^+ levels. Fertilised eggs lasted 10.33 to 12.5 hours before hatching to trochophore. It took 42.0 to 44.5 hours for trochophore larvae to develop into veliger larvae. DSI for pediveliger varied from 675.3 to 721.7 hours to settle using byssal threads (Figure 4.10). DSI was strongly negatively correlated with K^+ fortification levels in ISW at all studied development stages. However, this negative correlation was linear only in settlement stages (Table 4.5).

4.2.5. Morphological deformity

Normal and abnormal formation of each early stages of blue mussel were shown in Figure 4.11 and Figure 4.13, respectively. Four types of deformities were observed during the larval stages, namely faulty cell cleavage (Figure 4.13 a, b, c), abnormal formation in trochophore larvae (Figure 4.13d), protruding mantle in veliger larvae (Figure 4.13 e), and indented shell margin in veliger (Figure 4.13 f) and in pediveliger (Figure 4.13 g). Deformities occurred in larval stages from trochophore to pediveliger, but were not detected at the settlement stage. Overall, the deformity percentage was low (lower than 5% in all larval stages in any water types). The highest deformity of 4.67% occurred in ISW with no K^+ fortification (Figure 4.12). The K^+ fortification in ISW did not influence ($p > 0.05$) the deformity rate of trochophore and veliger larvae. The deformity rate of pediveliger larvae decreased ($p < 0.05$) with the increase in K^+ levels. K^+ concentrations showed strong negative linear correlations with percentages of deformities in trochophore, veliger and pediveliger larvae but stronger positive second order correlation was observed between K^+ levels and number of deformities in fertilised eggs (Table 4.5).

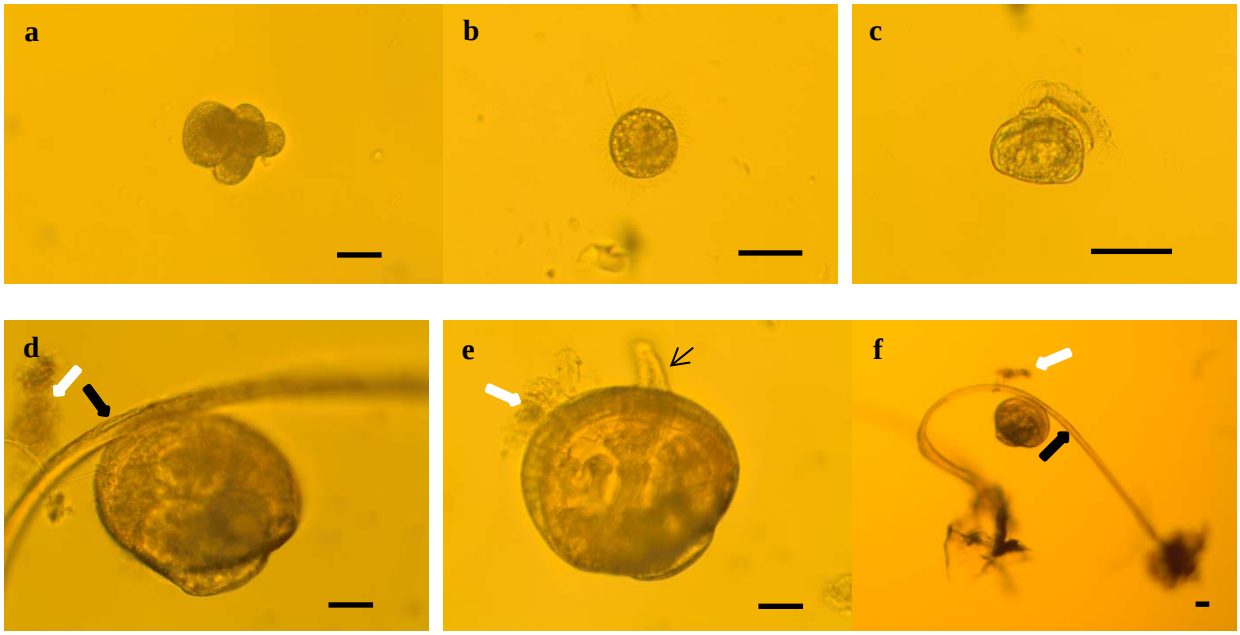


Figure 4.11 Development of early stages of blue mussel *Mytilus edulis* in response to K^+ addition to ISW. (a) eight cell stage; (b) trochophore larva; (c) veliger larva; (d, e, f) settlement larvae; thin arrow: foot; black arrows: byssal thread of adult blue mussel; white arrows: byssal thread of settlement larva of the blue mussel. Scale bar = 100 µm.

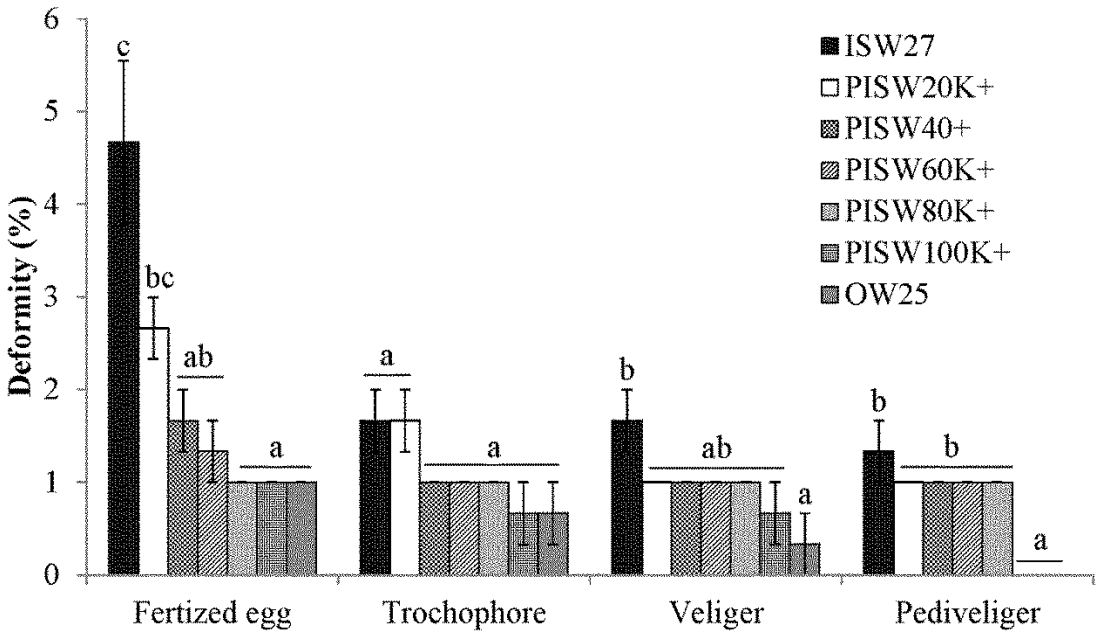


Figure 4.12 Morphological deformity of early developmental stages of the blue mussel *Mytilus edulis* in response to K^+ addition to ISW. Data are presented as mean ± SE. Data with different letters within a stage are significantly different ($p < 0.05$).

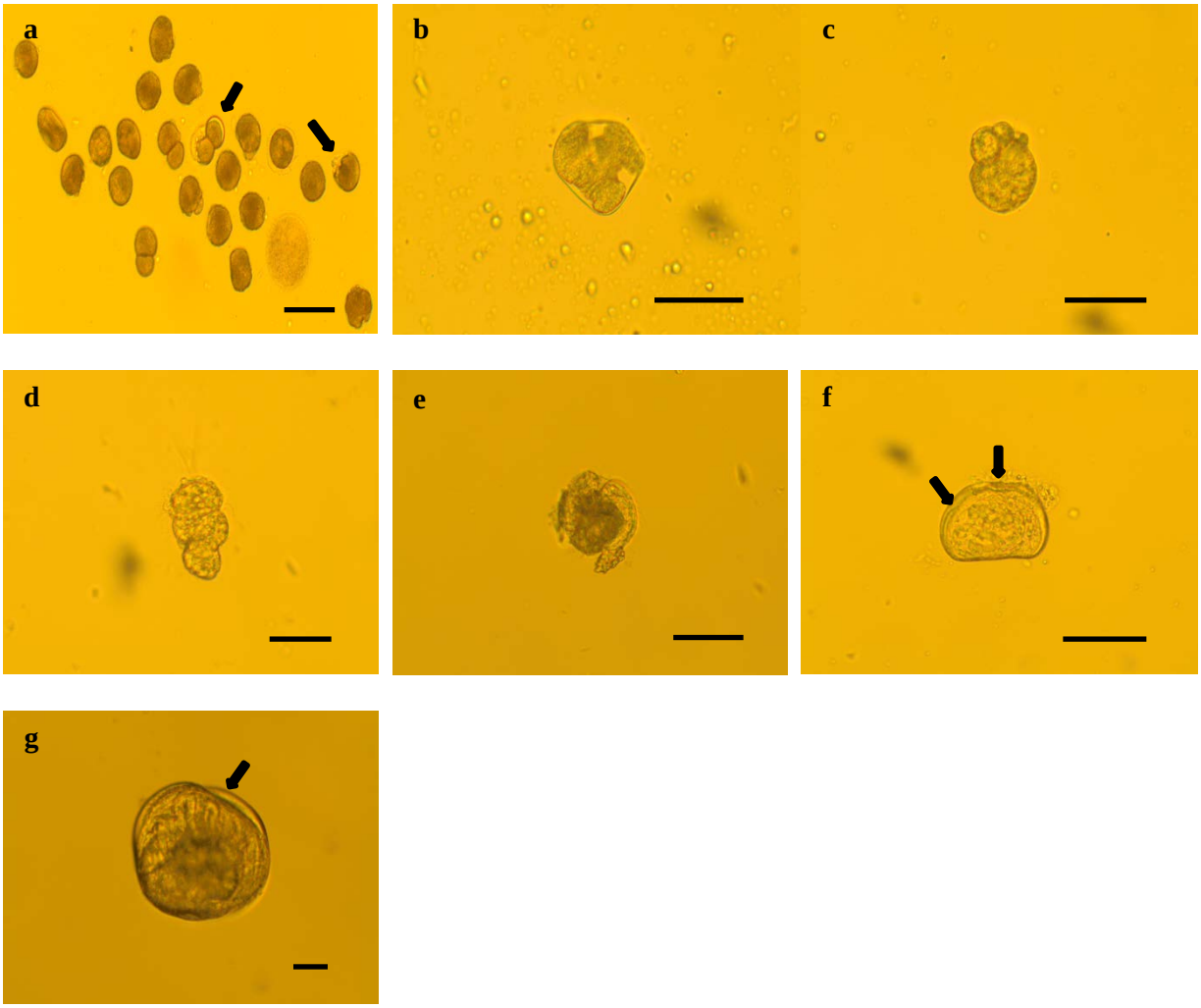


Figure 4.13 Morphological deformity in early larval stages of the blue mussel *Mytilus edulis* in response to K^+ addition to ISW. (a, b, c) deformed cell division; (d) deformed trochophore; (e, f) deformed veliger and (g) deformed pediveliger. Scale bar = 100 µm.

Table 4.5 Linear (shown by *) and second order regressions of the survival, size, DSI and deformity numbers of the blue mussel as a function of K^+ fortification levels in ISW

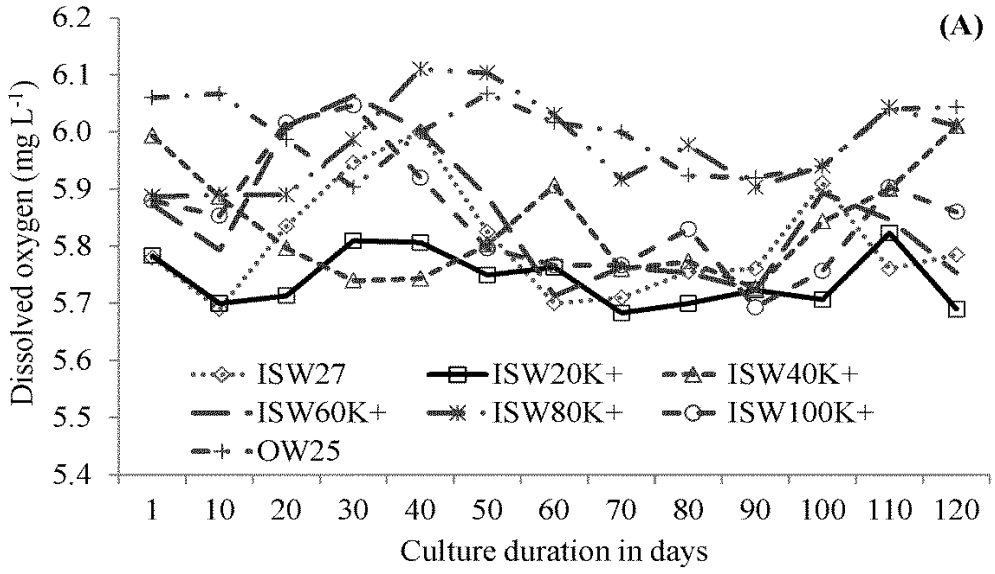
Parameter	Developmental stage	Equation	R^2
Survival (%)	Trochophore	$y = 0.001x^2 - 0.291x + 98.140$	0.95
	Veliger	$y = 0.001x^2 - 0.127x + 87.200$	0.53
	Pediveliger	$y = 0.117x + 45.938$	0.72*
	Settlement	$y = 0.167x + 26.074$	0.88*

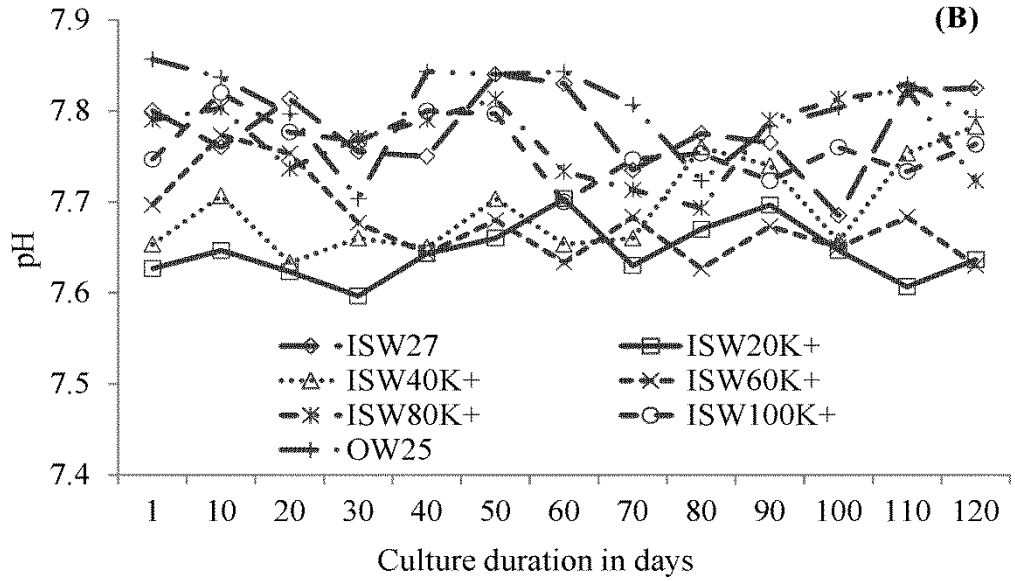
Size (µm)	Trochophore	$y = 0.000x^2 + 0.007x + 81.69$	0.01
	Veliger	$y = 0.001x^2 - 0.073x + 123.590$	0.69
	Pediveliger	$y = 0.149x + 295.070$	0.89*
	Settlement	$y = 0.928x + 425.950$	0.87*
DSI (hours)	Trochophore	$y = -0.000x^2 + 0.028x + 10.960$	0.83
	Veliger	$y = -0.000x^2 + 0.047x + 42.370$	0.83
	Pediveliger	$y = -0.001x^2 + 0.314x + 626.240$	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*

4.3. Survival and growth of the juvenile blue mussel cultured in potassium-fortified ISW

4.3.1. Ionic compositions and environmental parameters

Ionic composition was shown in the Table 4.4. There was no difference in all measured environmental parameters (DO, pH and temperature) among water types. Overall, DO varied from 5.68 – 6.11 mg L^{-1} (Figure 4.14 A), pH from 7.60 – 7.86 (Figure 4.14 B) and temperature from 19.37 - 21.50°C (Figure 4.14 C). These environmental parameters were not significantly ($p > 0.05$) different among water types.


(A)
Dissolved oxygen (mg L-1)
6.2
6.1
6.0
5.9
5.8
5.7
5.6
5.5
5.4
ISW27
ISW20K+
ISW40K+
ISW60K+
ISW80K+
ISW100K+
OW25
1 10 20 30 40 50 60 70 80 90 100 110 120
Culture duration in days


(B)
pH
7.9
7.8
7.7
7.6
7.5
7.4
ISW27
ISW20K+
ISW40K+
ISW60K+
ISW80K+
ISW100K+
OW25
1 10 20 30 40 50 60 70 80 90 100 110 120
Culture duration in days

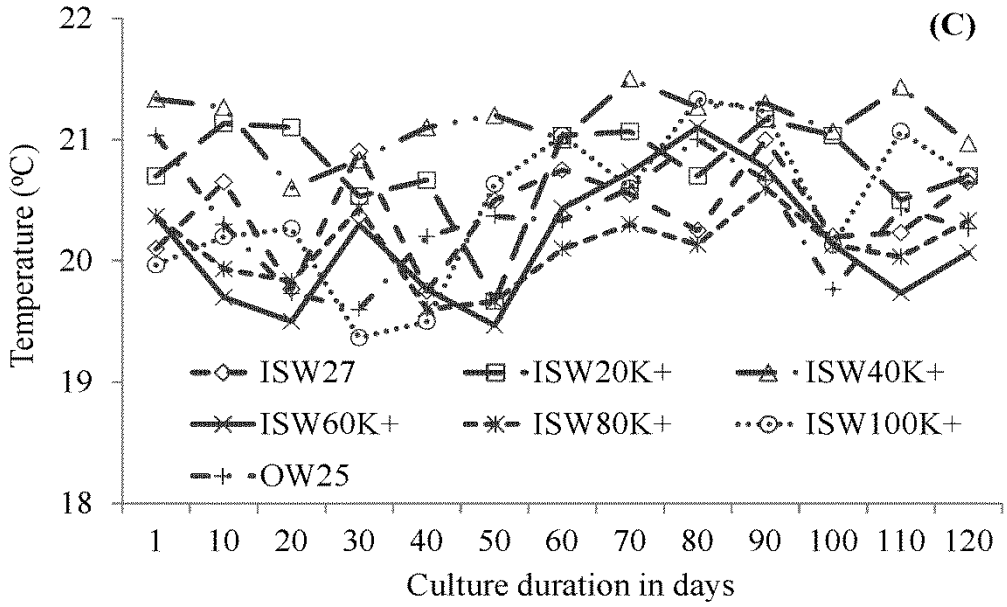


Figure 4.14 Variation of dissolved oxygen (A), pH (B) and temperature (C) in different water types during the cultured period of 120 days

4.3.2. Survival and growth rate

Survival varied from 23 to 85% and was significantly ($p < 0.05$) influenced by the K^+ fortification of ISW (Table 4.6). Survival was improved in ISW100K^+ and was similar to those in OW25. A strong correlation between K^+ fortification levels and survival of the blue mussel ($R^2 = 0.94$) (Table 4.7) was found.

There was no difference in the initial length and weight of blue mussel among water types (Table 4.6). In general, higher K^+-fortified ISW significantly ($p < 0.05$) increased the SGR_L and SGR_W of blue mussel at the end of the experiment. In particular, higher K^+-fortified ISW did not significantly ($p > 0.05$) influence the length and weight of blue mussel at 60 and 120 days post exposure (dpe). Higher K^+-fortified ISW did not improve ($p > 0.05$) the SGR_W in the first and second 60 dpe and SGR_L in the first 60 dpe but significantly ($p < 0.05$) improved the SGRL in the second 60 dpe of blue mussel. There was no significant ($p > 0.05$) difference in the SGR_L of blue mussel among water types of ISW80K^+, ISW100K^+ and OW25. The

SGR_W of blue mussel in this ISW100K^+ were similar to the survival rate of the OW25. K^+ levels showed a strong correlation with SGR_L (R^2 = 0.91) and SGR_W (R^2 = 0.94) (Table 4.7).

Table 4.6 Survival, length and weight, and growth rate in length (SGR_L) and in weight (SGR_W) of blue mussel after 120 days in different water types.

Water types	ISW27	ISW20K^+	ISW40K^+	ISW60K^+	ISW80K^+	ISW100K^+	OW25
Survival (%)	23.33 ± 3.33^{a}	40.00 ± 5.77^{ab}	47.67 ± 6.23^{b}	46.67 ± 6.67^{b}	55.67 ± 5.67^{bc}	83.33 ± 2.03^{cd}	85.33 ± 3.93^{d}
SGR_L (% d^{-1})	0.02 ± 0.01^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.07 ± 0.00^{b}	0.08 ± 0.00^{b}	0.1 ± 0.01^{b}
SGR_W (% d^{-1})	0.08 ± 0.03^{a}	0.06 ± 0.03^{a}	0.12 ± 0.01^{a}	0.11 ± 0.04^{ab}	0.16 ± 0.00^{ab}	0.22 ± 0.02^{b}	0.23 ± 0.02^{b}

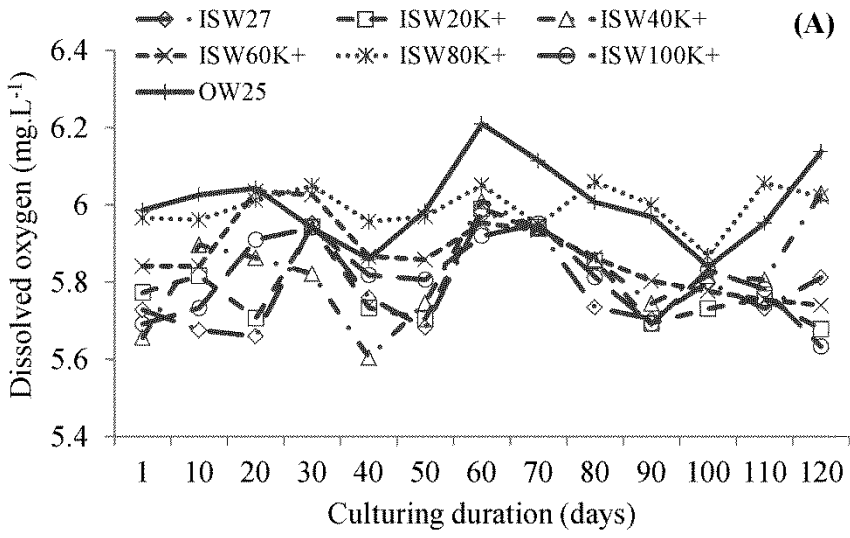
Table 4.7 Second order regression of the survival, SGR_L and SGR_W of the blue mussel as a function of K^+ fortification levels in ISW.

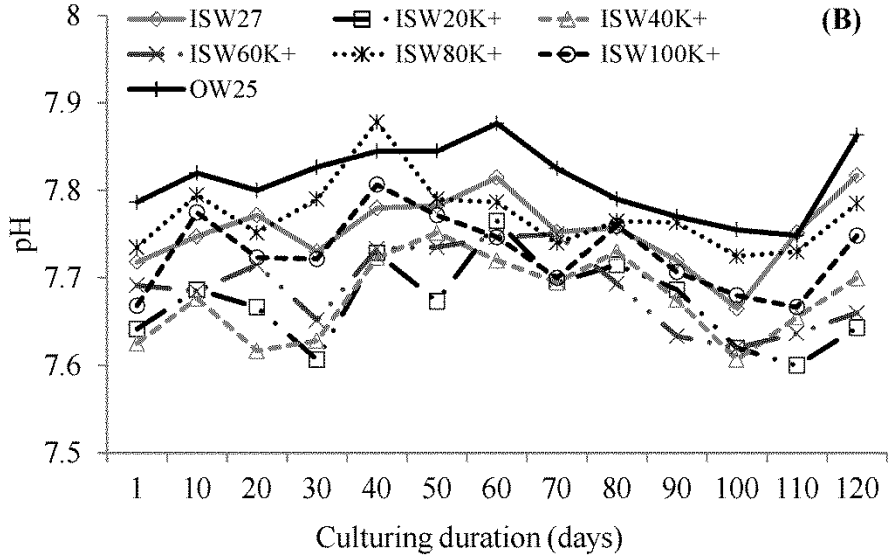
Parameter	Equation	R^2
Survival	$y = 0.001x^2 - 0.003x + 22.997$	0.94
SGR_L (% d^{-1})	$y = 4E\text{-}06x^2 - 0.001x + 0.051$	0.91
SGR_W (% d^{-1})	$y = 6E\text{-}06x^2 - 0.001x + 0.094$	0.94

4.4. Survival, growth, osmoregulatory capacity and proximate composition of blue mussel cultured in potassium-fortified ISW

4.4.1. Ionic composition and environmental parameters

Ionic composition of ISW and OW was shown in Table 4.4. There was no difference in all measured environmental parameters (DO, pH, and temperature) between the two blue mussel groups. Overall, DO varied from 5.44 to 6.30 mg L^{-1} (Figure 4.15 A), pH from 7.56 to 7.91 (Figure 4.15 B), and temperature from 17.77 to 21.87°C (Figure 4.15 C), and these environmental parameters were not significantly ($p > 0.05$) different among all water types.


(A)
ISW27
ISW20K+
ISW40K+
ISW60K+
ISW80K+
ISW100K+
OW25
Dissolved oxygen (mg.L-1)
6.4
6.2
6
5.8
5.6
5.4
1 10 20 30 40 50 60 70 80 90 100 110 120
Culturing duration (days)


(B)
ISW27
ISW20K+
ISW40K+
ISW60K+
ISW80K+
ISW100K+
OW25
pH
8
7.9
7.8
7.7
7.6
7.5
1 10 20 30 40 50 60 70 80 90 100 110 120
Culturing duration (days)

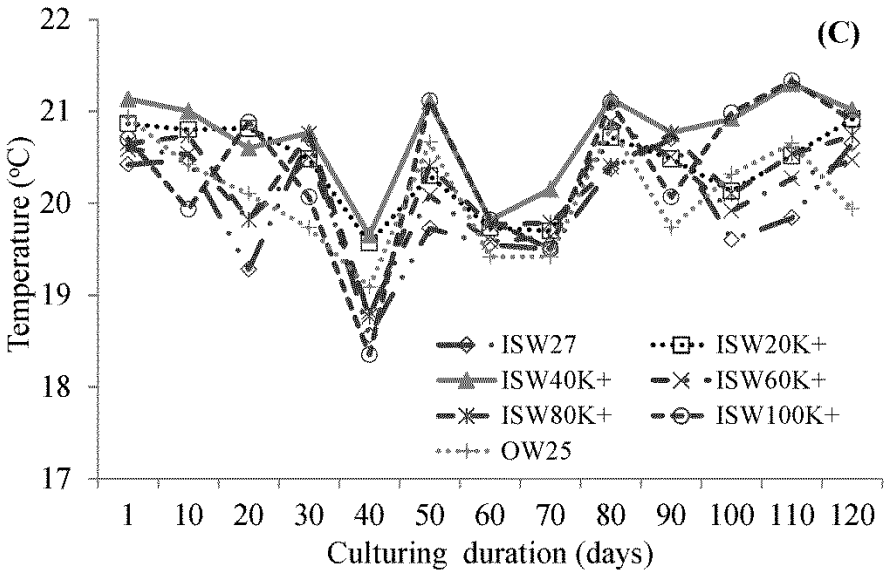


Figure 4.15 Variation of dissolved oxygen (A), pH (B) and temperature (C) in different water types during the cultured period of 120 days.

4.4.2. Survival, growth and OC

Survival varied from 41 to 90% and was similar in two size categories (Table 4.8). Survival significantly ($p < 0.05$) improved in IWS100K$^+$ and was similar to OW25 in both sized blue mussel and was significantly lower than rest of the K$^+$ fortification levels. Second order regression showed a strong correlation existed between K$^+$ levels and survival in both size categories of mussel. Both sizes of mussel maintained their differences in length and weights, however their growth rates (SGR) both in terms of length and weight were similar in all water types, except in OW control (OW25). In OW25 smaller sized mussel showed significantly higher growth. Second order regression showed a strong correlation between K$^+$ levels and SGR (both length and weights) in both smaller and larger mussel (Table 4.10).

At the end of the experiment, the haemolymph osmolality of the blue mussel exposed to different water types ranged from 691 – 740 mOsm kg^{-1} in the smaller group and from 679 – 762 mOsm kg^{-1} in the larger group. The OC of blue mussel was from 23 – 66 mOsm kg^{-1} in smaller group and from 19 – 92 mOsm kg^{-1} in larger group. K$^+$ fortification levels did neither

Table 4.8 Survival, length and weight, and growth rate in length (SGR_L) and in weight (SGR_W) of blue mussel *Mytilus edulis* after 120 days of grow-out in different water types.

Water types	Group	ISW27	$ISW20K^+$	$ISW40K^+$	$ISW60K^+$	$ISW80K^+$	$ISW100K^+$	OW25
Haemolymph osmolality	smaller	$^{1}726.96 \pm 15.54^{b}$	$^{1}736.66 \pm 6.09^{b}$	$^{1}733.26 \pm 5.99^{b}$	$^{1}736.15 \pm 0.46^{b}$	$^{1}739.76 \pm 1.50^{b}$	$^{1}717.33 \pm 5.49^{ab}$	$^{1}690.64 \pm 3.03^{a}$
(mOsm kg^{-1})	larger	$^{1}721.20 \pm 14.72^{ab}$	$^{1}735.02 \pm 4.41^{b}$	$^{1}750.62 \pm 8.31^{b}$	$^{1}761.96 \pm 3.14^{b}$	$^{1}742.17 \pm 9.08^{b}$	$^{1}734.46 \pm 12.50^{b}$	$^{1}679.38 \pm 3.18^{a}$
Osmoregulatory capacity	smaller	$^{1}23.38 \pm 0.31^{a}$	$^{1}65.3\ 3 \pm 6.09^{b}$	$^{1}52.93 \pm 5.99^{b}$	$^{1}66.48 \pm 0.46^{b}$	$^{1}64.09 \pm 1.50^{b}$	$^{1}55.00 \pm 5.49^{b}$	$^{1}30.97 \pm 3.03^{a}$
(mOsm kg^{-1})	larger	$^{1}18.75 \pm 6.58^{a}$	$^{1}63.69 \pm 4.41^{b}$	$^{1}70.29 \pm 8.31^{b}$	$^{2}92.29 \pm 3.14^{b}$	$^{1}66.50 \pm 9.08^{b}$	$^{1}72.13 \pm 12.50^{b}$	$^{1}19.71 \pm 3.18^{a}$
Survival (%)	smaller	$^{1}48.72 \pm 5.13^{a}$	$^{1}41.03 \pm 6.78^{a}$	$^{1}41.03 \pm 6.78^{a}$	$^{1}41.03 \pm 5.13^{a}$	$^{1}58.97 \pm 2.56^{ab}$	$^{1}79.49 \pm 2.56^{b}$	$^{1}84.62 \pm 4.44^{b}$
	larger	$^{1}51.28 \pm 2.56^{a}$	$^{1}48.72 \pm 5.13^{a}$	$^{1}58.97 \pm 2.56^{a}$	$^{1}61.54 \pm 7.69^{a}$	$^{1}64.10 \pm 2.56^{a}$	$^{1}87.18 \pm 2.56^{b}$	$^{1}89.74 \pm 2.56^{b}$
Length (mm)	smaller	$^{1}35.49 \pm 0.17^{abc}$	$^{1}35.32 \pm 0.46^{ab}$	$^{1}34.84 \pm 0.51^{a}$	$^{1}35.22 \pm 0.17^{ab}$	$^{1}36.66 \pm 0.49^{abc}$	$^{1}37.34 \pm 0.35^{bc}$	$^{1}37.52 \pm 0.67^{c}$
	larger	$^{2}43.27 \pm 0.49^{a}$	$^{2}45.49 \pm 0.59^{ab}$	$^{2}44.68 \pm 0.99^{ab}$	$^{2}44.30 \pm 0.89^{ab}$	$^{2}46.14 \pm 0.62^{ab}$	$^{2}47.40 \pm 1.03^{b}$	$^{2}47.83 \pm 0.77^{b}$
SGR_L (% d^{-1})	smaller	$^{1}0.00 \pm 0.00^{a}$	$^{1}0.01 \pm 0.01^{a}$	$^{1}0.00 \pm 0.01^{a}$	$^{1}0.00 \pm 0.00^{a}$	$^{1}0.03 \pm 0.01^{ab}$	$^{1}0.04 \pm 0.0^{b}$	$^{1}0.05 \pm 0.00^{b}$
	larger	$^{1}0.00\ \pm 0.02^{a}$	$^{1}0.02 \pm 0.01^{abc}$	$^{1}0.02 \pm 0.00^{abc}$	$^{1}0.01 \pm 0.01^{ab}$	$^{1}0.04 \pm 0.00^{abc}$	$^{1}0.05 \pm 0.01^{bc}$	$^{1}0.06 \pm 0.00^{c}$
Weight (g)	smaller	$^{1}4.95 \pm 0.00^{ab}$	$^{1}4.80 \pm 0.09^{a}$	$^{1}4.82 \pm 0.15^{a}$	$^{1}4.94 \pm 0.03^{ab}$	$^{1}5.42 \pm 0.09^{abc}$	$^{1}5.61 \pm 0.09^{bc}$	$^{1}5.92 \pm 0.31^{c}$

	larger	$^{2}8.45 \pm 0.28^{a}$	$^{2}9.81 \pm 0.14^{ab}$	$^{2}8.81 \pm 0.62^{ab}$	$^{2}9.13 \pm 0.48^{ab}$	$^{2}9.72 \pm 0.24^{ab}$	$^{2}10.55 \pm 0.66^{ab}$	$^{2}10.97 \pm 0.67^{b}$
SGR_W (% d^{-1})	smaller	$-\ ^{1}0.01 \pm 0.01^{ab}$	$-\ ^{1}0.04 \pm 0.03^{a}$	$^{1}0.04 \pm 0.02^{bc}$	$^{1}0.03 \pm 0.00^{ab}$	$^{1}0.05 \pm 0.02^{bcd}$	$^{1}0.13 \pm 0.02^{cd}$	$^{1}0.12 \pm 0.01^{d}$
	larger	$-\ ^{1}0.03 \pm 0.03^{a}$	$-\ ^{1}0.02 \pm 0.03^{a}$	$-\ ^{1}0.01 \pm 0.03^{a}$	$-\ ^{1}0.01 \pm 0.03^{a}$	$^{1}0.00 \pm 0.03^{a}$	$^{1}0.08 \pm 0.01^{a}$	$^{2}0.08 \pm 0.01^{a}$

Data in the same row with a different alphabet letter are significantly ($p < 0.05$) different. Data in the same column between smaller and larger groups marked by different numerical letters are significantly ($p < 0.05$) different within each water type.

alter haemolymph osmolality nor OC of blue mussel (Table 4.8). There was a correlation between K^+ levels in ISW and OC in smaller and larger mussel (R^2 = 0.72 and 0.75 respectively) with no significant ($p > 0.05$) differences between the two sizes (Table 4.10).

4.4.3. Biochemical composition

Protein accounted for 63 to 68% and 52 to 66% of dried mass of blue mussel meat of the smaller and larger groups, respectively (Table 4.9). Protein content significantly ($p < 0.05$) increased in ISW100K^+ in the smaller-size mussels and in ISW80K^+ and ISW100K^+ in the larger-size mussel. There was no difference between protein content of blue mussel in ISW100K^+ of the smaller group and in ISW80K^+ and ISW100K^+ of the larger group than in OW25. The protein content of blue mussel in other water types was low and did not differ from ISW27. Irrespective of the sizes of mussel (unlike moisture, ash and energy), protein and lipids were strongly correlated to K^+ fortification levels (Table 4.10).

Lipid content varied from 9 to 10% in both mussel groups (Table 4.9). The lipid content remained same in all water types.

ISW80K^+ and ISW100K^+ significantly ($p < 0.05$) lowered the moisture similar to the control both mussel groups (Table 4.9).

Ash content of both groups of mussel gradually increased as the K^+ levels were increased in ISW and finally became similar to the initial values. Mussel cultured in OW and 100 percent K^+ fortified ISW showed significantly higher ash contents.

Energy content was lowest in ISW27 in both sized groups (Table 4.9). In the smaller group, the gross energy of blue mussel in OW25 was significantly ($p < 0.05$) higher than ISW100K^+; however, in the larger group, there was no difference in the gross energy between ISW100K^+ and OW25.

Table 4.9 Protein, lipid, moisture, ash content, and gross energy of blue mussel *M. edulis* after 120 days of culturing in different water types.

Parameter	Group	Initial values	Final values						
			ISW27	ISW20K^{+}	ISW40K^{+}	ISW60K^{+}	ISW80K^{+}	ISW100K^{+}	OW25
Protein (%)	smaller	$^{1}64.82 \pm 0.06^{a}$	$^{1}63.10 \pm 0.40^{a}$	$^{1}64.27 \pm 0.20^{a}$	$^{1}64.52 \pm 0.48^{a}$	$^{1}65.67 \pm 0.25^{ab}$	$^{1}64.88 \pm 0.61^{a}$	$^{1}68.30 \pm 1.40^{b}$	$^{1}68.11 \pm 0.44^{b}$
	larger	$^{1}64.14 \pm 0.54^{bc}$	$^{2}52.27 \pm 0.78^{a}$	$^{2}60.41 \pm 0.52^{b}$	$^{1}63.51 \pm 0.61^{bc}$	$^{1}63.81 \pm 0.51^{bc}$	$^{1}64.97 \pm 0.36^{c}$	$^{1}66.43 \pm 1.22^{c}$	$^{1}65.44 \pm 0.94^{c}$
Lipid (%)	smaller	$^{1}9.58 \pm 0.15^{a}$	9.51^{*}	9.45^{*}	9.71^{*}	9.28^{*}	$^{1}9.95 \pm 0.73^{a}$	$^{1}9.75 \pm 0.50^{a}$	$^{1}10.18 \pm 0.72^{a}$
	larger	$^{1}9.65 \pm 0.42^{a}$	9.95^{*}	9.83^{*}	9.97 ± 0.12^{a}	10.10 ± 0.43^{a}	$^{1}9.75 \pm 0.30^{a}$	$^{2}10.24 \pm 0.01^{a}$	$^{1}10.45 \pm 0.50^{a}$
Moisture (%)	smaller	$^{1}23.40 \pm 0.41^{ab}$	$^{1}25.34 \pm 0.70^{b}$	$^{1}21.63 \pm 0.94^{a}$	$^{1}22.68 \pm 0.67^{ab}$	$^{1}21.41 \pm 1.03^{a}$	$^{1}21.22 \pm 0.80^{a}$	$^{1}20.03 \pm 0.52^{a}$	$^{1}20.22 \pm 0.61^{a}$
	larger	$^{2}19.75 \pm 0.23^{abc}$	$^{2}22.29 \pm 1.56^{c}$	$^{1}21.24 \pm 0.01^{bc}$	$^{2}22.07 \pm 0.23^{c}$	$^{1}20.13 \pm 0.39^{abc}$	$^{2}18.64 \pm 0.83^{ab}$	$^{2}18.03 \pm 0.14^{a}$	$^{2}17.75 \pm 0.59^{a}$
Ash (% DW)	smaller	$^{1}11.63 \pm 0.17^{ab}$	$^{1}10.42 \pm 0.43^{a}$	$^{1}10.18 \pm 0.04^{a}$	$^{1}11.65 \pm 0.27^{ab}$	$^{1}11.14 \pm 0.17^{ab}$	$^{1}12.11 \pm 0.13^{ab}$	$^{1}13.26 \pm 0.43^{b}$	$^{1}12.75 \pm 0.71^{b}$
	larger	$^{2}13.18 \pm 0.47^{cd}$	$^{1}9.99 \pm 0.24^{a}$	$^{1}9.61 \pm 0.15^{a}$	$^{1}11.33 \pm 0.53^{abc}$	$^{1}10.77 \pm 0.57^{ab}$	$^{1}12.85 \pm 0.25^{bcd}$	$^{1}13.94 \pm 0.21^{d}$	$^{1}13.72 \pm 0.59^{d}$
Energy (MJ.kg^{-1})	smaller	$^{1}19.39 \pm 0.12^{ab}$	$^{1}19.17 \pm 0.21^{ab}$	$^{1}19.47 \pm 0.22^{ab}$	$^{1}19.04 \pm 0.15^{a}$	$^{1}19.74 \pm 0.16^{ab}$	$^{1}19.71 \pm 0.12^{ab}$	$^{1}20.00 \pm 0.20^{b}$	$^{1}21.21 \pm 0.22^{c}$
	larger	$^{1}19.58 \pm 0.08^{cde}$	$^{1}18.72 \pm 0.02^{a}$	$^{1}18.76 \pm 0.06^{a}$	$^{1}19.10 \pm 0.00^{abc}$	$^{2}19.06 \pm 0.06^{ab}$	$^{1}19.46 \pm 0.14^{bcd}$	$^{1}19.99 \pm 0.20^{e}$	$^{1}19.90 \pm 0.03^{de}$

* There were only two replicates due to insufficient quantity of sample size, therefore, no statistical differences could be carried out. However, it was assumed that there is no significant differences with the rest of the values in the row, considering mean values are closed to other values in the same row. Data in the same row with different alphabet letters (except *) are significantly ($p < 0.05$) different. Data in the same column between smaller and larger group marked by different numerical letters (except *) are significantly ($p < 0.05$) different within each water type. DW: Dry weight.

Table 4.10 Second order regression of the experimental parameters of the blue mussel as a function of K^+ fortification levels in ISW

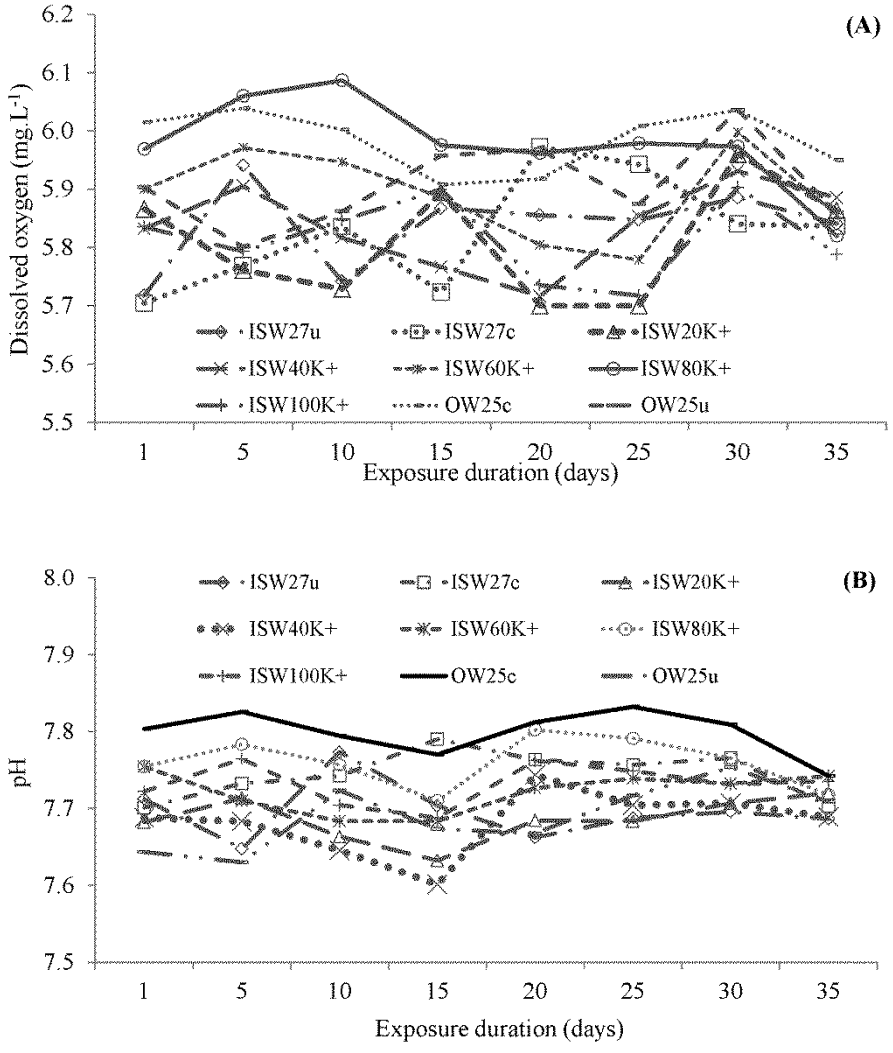
Parameter	Group	Equation	R^2
Osmoregulatory capacity	smaller	$y = -0.006x^2 + 2.037x - 89.866$	0.72
	larger	$y = -0.010x^2 + 3.089x - 152.990$	0.75
Survival	smaller	$y = 0.004x^2 - 0.867x + 89.473$	0.99
	larger	$y = 0.002x^2 - 0.297x + 62.774$	0.96
SGR_L	smaller	$y = 3E\text{-}06x^2 - 0.001x + 0.029$	0.96
	larger	$y = 2E\text{-}06x^2 - 0.000x + 0.017$	0.86
SGR_W	smaller	$y = 5E\text{-}06x^2 - 0.000x - 0.010$	0.86
	larger	$y = 5E\text{-}06x^2 - 0.001x + 0.004$	0.85
Protein	smaller	$y = 0.000x^2 - 0.010x + 63.512$	0.89
	larger	$y = -0.001x^2 + 0.303x + 36.850$	0.95
Lipid	smaller	$y = 0.001x^2 - 0.471x + 57.161$	1
	larger	$y = 0.000x^2 - 0.064x + 14.907$	1
Moisture*	smaller	$y = 0.000x^2 - 0.068x + 28.466$	0.81
	larger	$y = -9E\text{-}05x^2 - 0.004x + 22.914$	0.91
Ash*	smaller	$y = 4E\text{-}05x^2 + 0.005x + 9.756$	0.88
	larger	$y = 0.000x^2 - 0.003x + 9.521$	0.92
Energy*	smaller	$y = 0.094x^2 - 18.713x + 20128.000$	0.76
	larger	$y = 0.034x^2 - 2.109x + 18696.000$	0.97

R^2 data between smaller and larger group in each experimental parameter marked by * is significantly ($p < 0.05$) different otherwise there is no significant differences with the rest of the values

4.5. Size dependent root shedding and byssus thread renewal capacity of the blue mussel exposed to potassium-fortified ISW

4.5.1. Ionic compositions and environmental parameters

Ionic composition in the experimental water types was shown in Table 4.4. There was no difference in all measured environmental parameters (DO, pH and temperature) among three blue mussel size classes. Overall, DO varied from 5.70 to 6.09 mg L^{-1} (Figure 4.16 A), pH (7.60 to 7.83) (Figure 4.16 B) and temperature (19.76 to 21.01°C) (Figure 4.16 C). These environmental parameters were not significantly ($p > 0.05$) different among all water types.



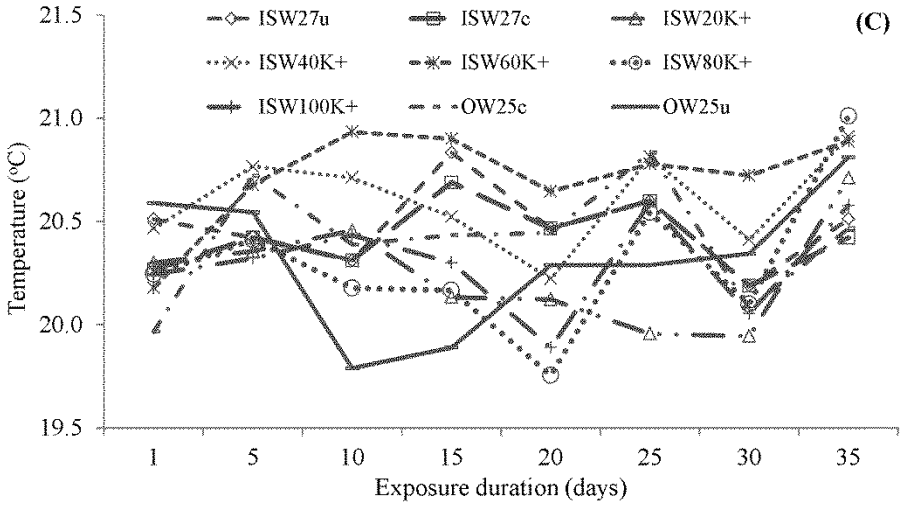


Figure 4.16 Variation of dissolved oxygen (A), pH (B) and temperature (C) in different water types during the exposure duration.

4.5.2. Survival of the blue mussel, byssus thread renewal capacity and root shedding

Survival was 100% in all water types and in all three sized mussels.

The number of the byssus threads increased to the highest number in the first ten days and then decreased during the rest of the exposure duration (Table 4.11). In all three sized blue mussel, the number of renewal byssus thread remained low in ISW27 and ISW20K^{+}.

Table 4.11 Renewal byssus threads (mean number of renewal byssus threads mussel^{-1} day^{-1}) of the blue mussels in the smaller (38.1 ± 0.4 mm), medium (42.5 ± 0.4 mm) and larger size (56.3 ± 0.3 mm) over 5 day periods reared in different K^{+}-fortified inland saline water for 35 days.

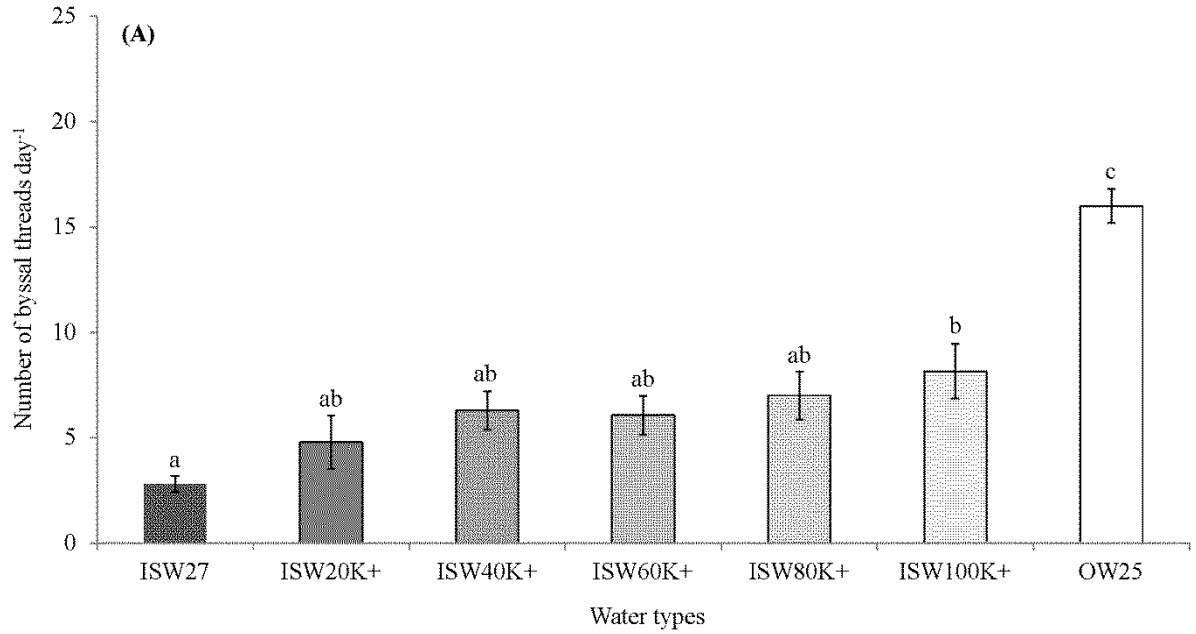
	Day	5	10	15	20	25	30	35
Smaller	ISW27	$_{1}5.5 \pm 0.6^{b}$	$_{1}8.8 \pm 1.3^{b}$	$_{1}1.2 \pm 0.4^{a}$	$_{1}1.1 \pm 0.8^{a}$	$_{1}0.9 \pm 0.2^{a}$	$_{1}1.5 \pm 0.3^{a}$	$_{1}0.7 \pm 0.7^{a}$
	ISW20K^{+}	$_{1}11.7 \pm 2.7^{c}$	$_{1}9.8 \pm 1.9^{bc}$	$_{123}2.6 \pm 0.5^{a}$	$_{1}1.9 \pm 1.1^{a}$	$_{12}2.0 \pm 0.5^{a}$	$_{1}2.6 \pm 1.5^{a}$	$_{12}2.9 \pm 0.7^{ab}$
	ISW40K^{+}	$_{1}11.9 \pm 0.9^{bc}$	$_{12}14.3 \pm 1.3^{c}$	$_{12}1.5 \pm 0.7^{a}$	$_{1}4.5 \pm 3.0^{ab}$	$_{12}2.9 \pm 0.5^{a}$	$_{1}2.7 \pm 1.5^{a}$	$_{12}3.9 \pm 2.0^{a}$
	ISW60K^{+}	$_{1}8.1 \pm 0.2^{a}$	$_{1}9.4 \pm 0.6^{a}$	$_{3}7.5 \pm 2.1^{a}$	$_{1}3.9 \pm 0.7^{a}$	$_{12}5.3 \pm 1.7^{a}$	$_{1}4.7 \pm 0.8^{a}$	$_{12}3.7 \pm 1.2^{a}$
	ISW80K^{+}	$_{1}11.2 \pm 1.67^{ab}$	$_{1}13.6 \pm 4.3^{b}$	$_{123}5.3 \pm 0.8^{ab}$	$_{1}6.8 \pm 0.5^{ab}$	$_{12}4.0 \pm 0.3^{ab}$	$_{1}3.2 \pm 1.5^{a}$	$_{12}4.9 \pm 2.3^{ab}$

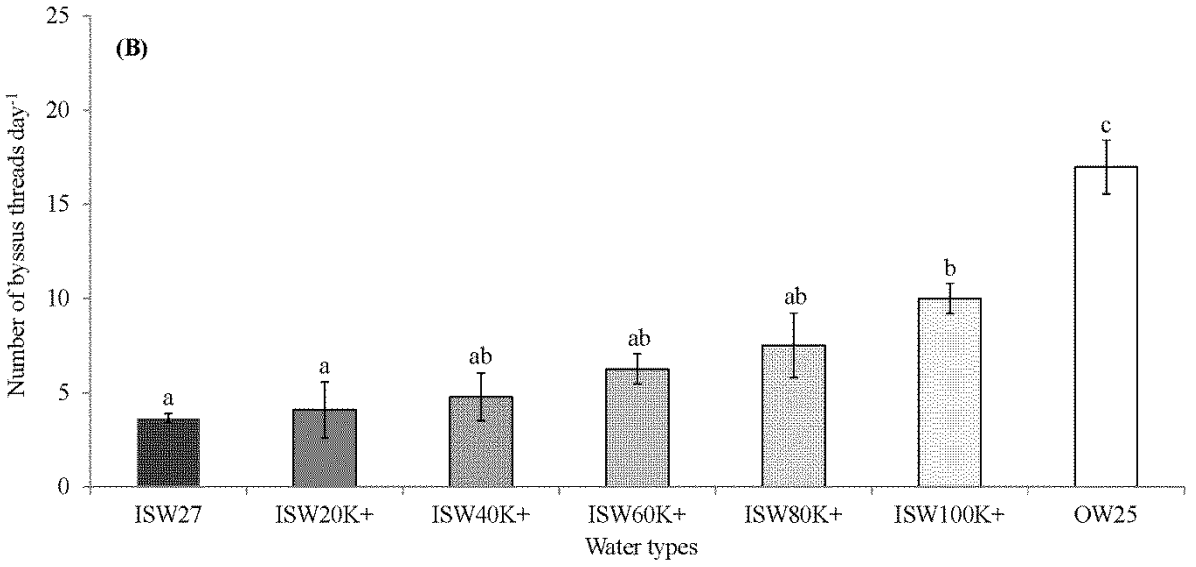
	ISW100K^{+}	$_{1}$11.1 ± 0.7^{ab}	$_{12}$17.9 ± 2.1^{b}	$_{23}$6.3 ± 1.0^{a}	$_{1}$7.0 ± 2.5^{a}	$_{2}$4.1 ± 0.9^{a}	$_{12}$6.1 ± 2.3^{a}	$_{12}$4.5 ± 2.2^{a}
	OW25	$_{2}$20.6 ± 0.7^{bc}	$_{2}$24.1 ± 1.4^{c}	$_{4}$13.1 ± 0.9^{a}	$_{2}$15.7 ± 1.6^{ab}	$_{3}$14.8 ± 0.5^{ab}	$_{2}$13.2 ± 2.3^{a}	$_{2}$9.9 ± 1.7^{a}
Medium	ISW27	$_{12}$9.3 ± 0.9^{b}	$_{12}$11.7 ± 1.2^{b}	$_{1}$0.5 ± 0.3^{a}	$_{1}$1.0 ± 0.8^{a}	$_{1}$0.5 ± 0.3^{a}	$_{1}$1.4 ± 0.3^{a}	$_{1}$1.1 ± 0.3^{a}
	ISW20K^{+}	$_{12}$11.3 ± 1.2^{b}	$_{1}$5.8 ± 0.4^{ab}	$_{12}$1.8 ± 1.4^{a}	$_{1}$1.0 ± 0.9^{a}	$_{1}$3.2 ± 2.5^{ab}	$_{1}$2.5 ± 1.9^{a}	$_{12}$3.1 ± 2.5^{ab}
	ISW40K^{+}	$_{1}$7.7 ± 2.0^{ab}	$_{12}$10.3 ± 0.5^{b}	$_{12}$3.3 ± 0.7^{ab}	$_{12}$6.1 ± 3.2^{ab}	$_{1}$2.4 ± 1.1^{ab}	$_{1}$0.5 ± 0.3^{a}	$_{12}$3.3 ± 2.3^{ab}
	ISW60K^{+}	$_{12}$11.0 ± 2.3^{bc}	$_{2}$14.4 ± 0.5^{c}	$_{12}$5.8 ± 1.9^{ab}	$_{1}$3.5 ± 2.5^{ab}	$_{1}$1.6 ± 0.8^{a}	$_{1}$3.9 ± 0.8^{ab}	$_{12}$3.6 ± 1.9^{ab}
	ISW80K^{+}	$_{3}$19.0 ± 2.0^{c}	$_{12}$12.2 ± 2.5^{bc}	$_{12}$1.8 ± 0.3^{a}	$_{1}$3.5 ± 2.2^{ab}	$_{1}$4.3 ± 2.0^{ab}	$_{1}$7.9 ± 2.9^{ab}	$_{12}$4.1 ± 1.2^{ab}
	ISW100K^{+}	$_{23}$16.3 ± 0.9^{b}	$_{3}$22.4 ± 0.9^{b}	$_{2}$7.1 ± 1.7^{a}	$_{12}$7.5 ± 2.1^{a}	$_{1}$4.6 ± 1.4^{a}	$_{1}$7.6 ± 1.8^{a}	$_{12}$4.4 ± 1.9^{a}
	OW25	$_{3}$22.7 ± 0.7^{b}	$_{3}$23.4 ± 2.4^{b}	$_{1}$14.9 ± 1.2^{ab}	$_{2}$15.6 ± 4.0^{ab}	$_{2}$13.2 ± 2.7^{ab}	$_{2}$17.1 ± 0.8^{ab}	$_{2}$11.1 ± 0.7^{a}
Larger	ISW27	$_{1}$1.7 ± 0.3^{a}	$_{1}$13.7 ± 2.3^{a}	$_{1}$0.3 ± 0.2^{a}	$_{1}$0.8 ± 0.8^{a}	$_{1}$0.6 ± 0.6^{a}	$_{1}$0.8 ± 0.5^{a}	$_{1}$0.6 ± 0.3^{a}
	ISW20K^{+}	$_{2}$11.7 ± 0.7^{cd}	$_{123}$3.4 ± 0.9^{d}	$_{1}$1.5 ± 1.0^{ab}	$_{1}$0.7 ± 0.7^{a}	$_{1}$6.3 ± 2.5^{bc}	$_{1}$4.3 ± 1.3^{ab}	$_{12}$1.8 ± 1.0^{ab}
	ISW40K^{+}	$_{2}$15.0 ± 2.9^{b}	$_{12}$12.2 ± 1.9^{b}	$_{1}$1.5 ± 0.8^{a}	$_{1}$1.3 ± 0.8^{a}	$_{1}$1.6 ± 0.9^{a}	$_{1}$1.5 ± 1.0^{a}	$_{12}$2.8 ± 1.7^{a}
	ISW60K^{+}	$_{2}$13.0 ± 21.5^{b}	$_{12}$11.8 ± 2.7^{ab}	$_{12}$3.9 ± 1.2^{ab}	$_{1}$2.5 ± 1.2^{a}	$_{1}$4.5 ± 1.5^{ab}	$_{1}$3.6 ± 3.2^{ab}	$_{12}$4.9 ± 2.2^{ab}
	ISW80K^{+}	$_{2}$13.7 ± 0.9^{bc}	$_{123}$14.3 ± 2.2^{c}	$_{123}$5.7 ± 3.6^{abc}	$_{1}$8.1 ± 3.0^{abc}	$_{1}$3.3 ± 1.7^{ab}	$_{1}$3.1 ± 1.4^{a}	$_{12}$4.5 ± 0.4^{abc}
	ISW100K^{+}	$_{23}$20.0 ± 2.9^{b}	$_{23}$16.7 ± 3.5^{ab}	$_{23}$13.7 ± 3.1^{ab}	$_{1}$6.2 ± 5.2^{ab}	$_{1}$4.1 ± 1.6^{a}	$_{1}$7.1 ± 3.0^{ab}	$_{2}$7.7 ± 1.6^{ab}
	OW25	$_{3}$29.0 ± 2.5^{c}	$_{3}$23.9 ± 0.4^{bc}	$_{3}$15.3 ± 2.6^{a}	$_{2}$20.3 ± 2.2^{ab}	$_{2}$19.7 ± 1.3^{ab}	$_{2}$18.6 ± 1.5^{ab}	$_{3}$16.1 ± 0.2^{ab}

Data in the same column of each blue mussel size marked by different numerical letters and data in the same row with different alphabet letters are significantly different ($p < 0.05$).

At the end of the experiment, the mean rate of renewed byssus threads varied from 1 to 16 byssus threads day^{-1} in smaller sized (Figure 4.17 A), 1 to 17 byssus threads day^{-1} in medium sized (Figure 4.17 B) and 1 to 20 byssus threads day^{-1} in size larger sized mussel (Figure 4.17 C) when exposed to different water types. Higher levels of K^{+}-fortification to ISW significantly ($p < 0.05$) increased the number of renewed byssus threads of blue mussel in all three sized blue mussel (Figure 4.17 A, B and C). The number of renewed byssus threads of

blue mussel in ISW100K^{+} was significantly higher (5 – 17 times higher) the number of blue mussel in ISW27. The number of renewed byssus threads of blue mussel in OW25 was twice higher than of those in ISW100K^{+}. Also, the blue mussel in OW25 produced significantly ($p < 0.05$) higher number (5 - 7 times) of byssus threads than those of blue mussel in OW25u. There was no significant ($p > 0.05$) difference in the number of byssus threads in uncut blue mussel (OW25u and ISW27u) and ISW27.





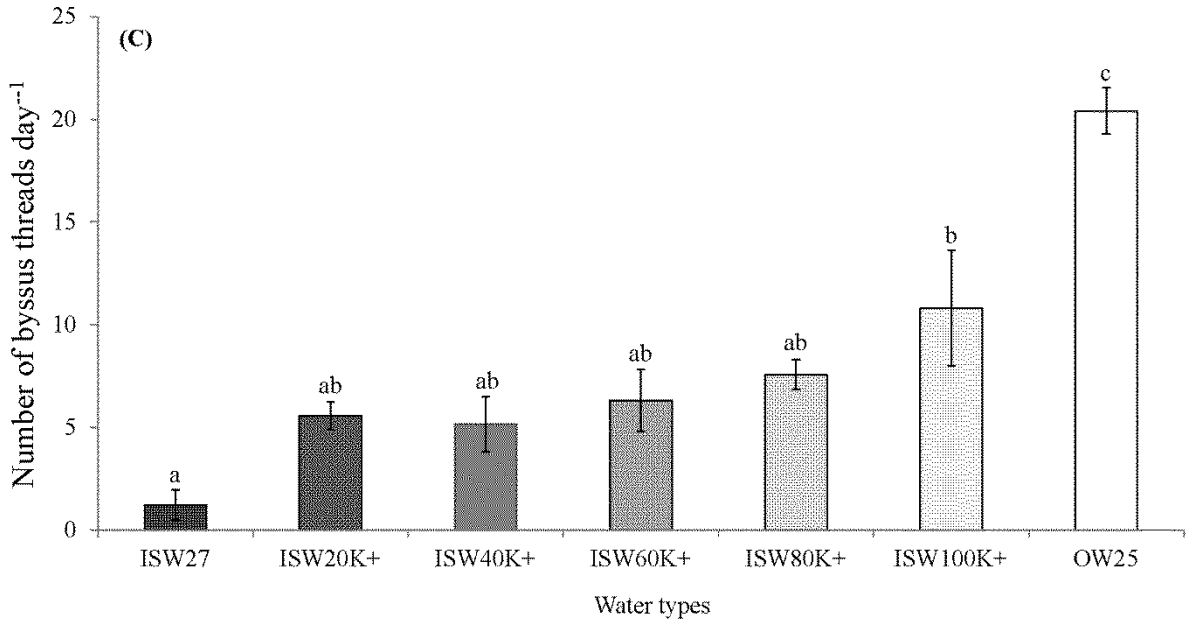


Figure 4.17 Mean number of renewed byssus threads of blue mussel in the smaller (38.1 ± 0.4 mm) (A), medium (42.5 ± 0.4 mm) (B) and larger size (56.3 ± 0.3 mm) (C) reared in different K^+-fortified inland saline waters for 35 days. Data are presented as mean ± SE. Data with different letters are significantly different ($p < 0.05$).

Shedding of the root in the blue mussel was shown in Figure 4.18. The number of shed root varied from 0.01 to 0.07 root day^{-1} in all sized blue mussel (Figure 4.19 A, B and C) and significantly ($p < 0.05$) decreased with the increase in K^+ levels. The blue mussel shedding occurred in ISW27, ISW20K^+, ISW40K^+ and ISW60K^+ in all sized blue mussel. While there was no significant ($p > 0.05$) difference in the number of shed root between OW25c and OW25u, the number of shed root was significantly ($p < 0.05$) different between ISW27c and ISW27u.

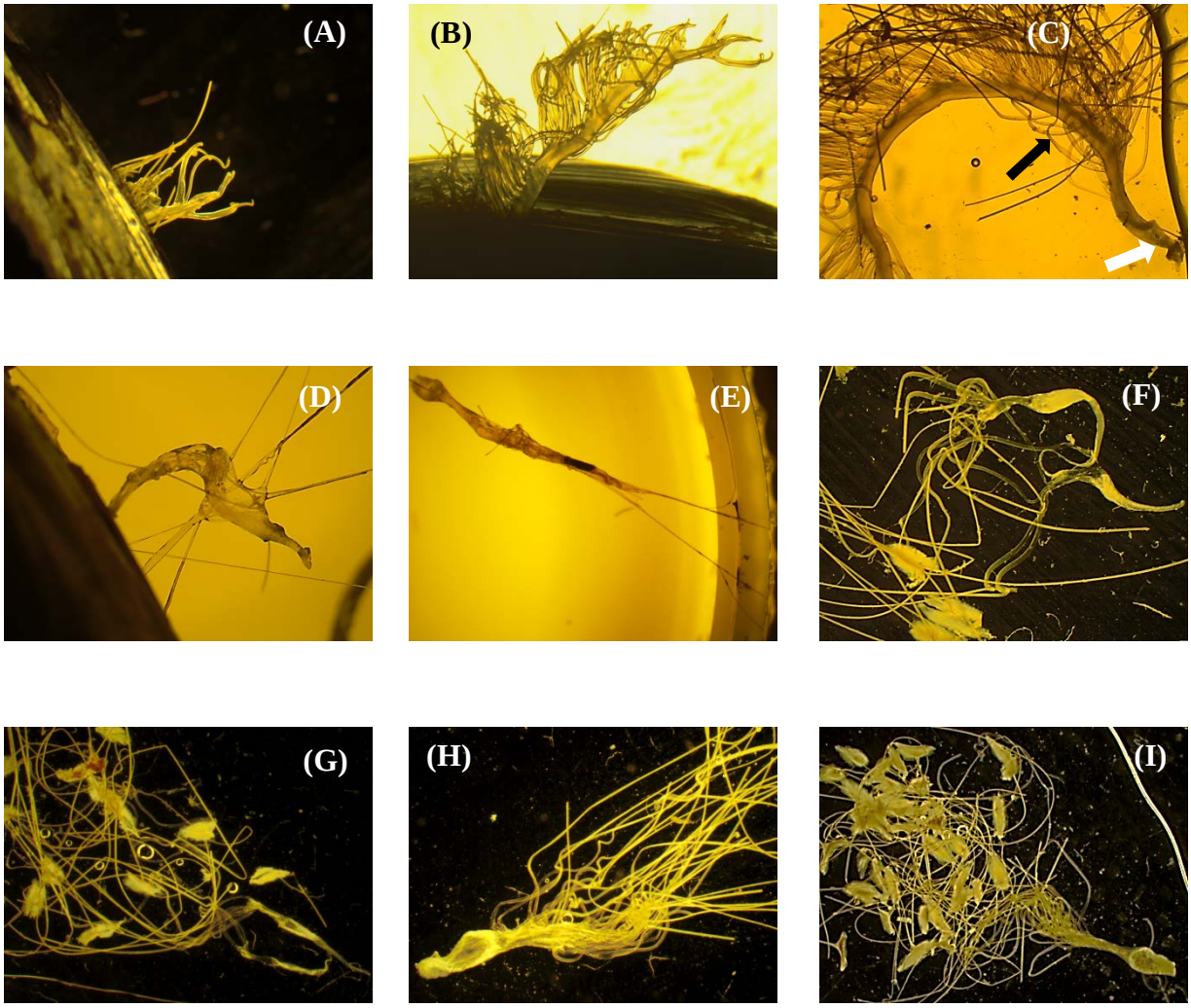
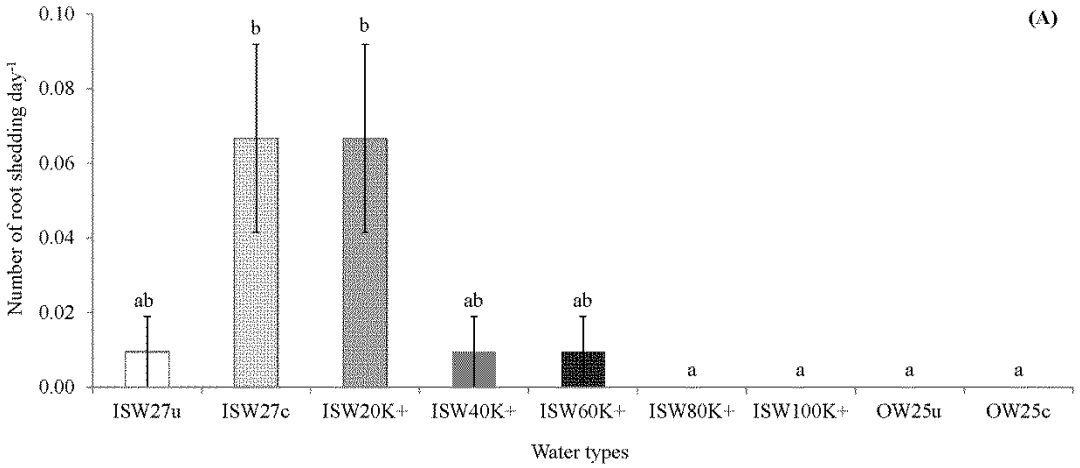


Figure 4.18 Root shedding of the blue mussel when exposed to low K^+ fortified ISW. (A, B) initial signs of root shedding; (C, D, E, F, G, H, I) root shedding; black arrow points towards byssus threads and white arrow towards shed root.



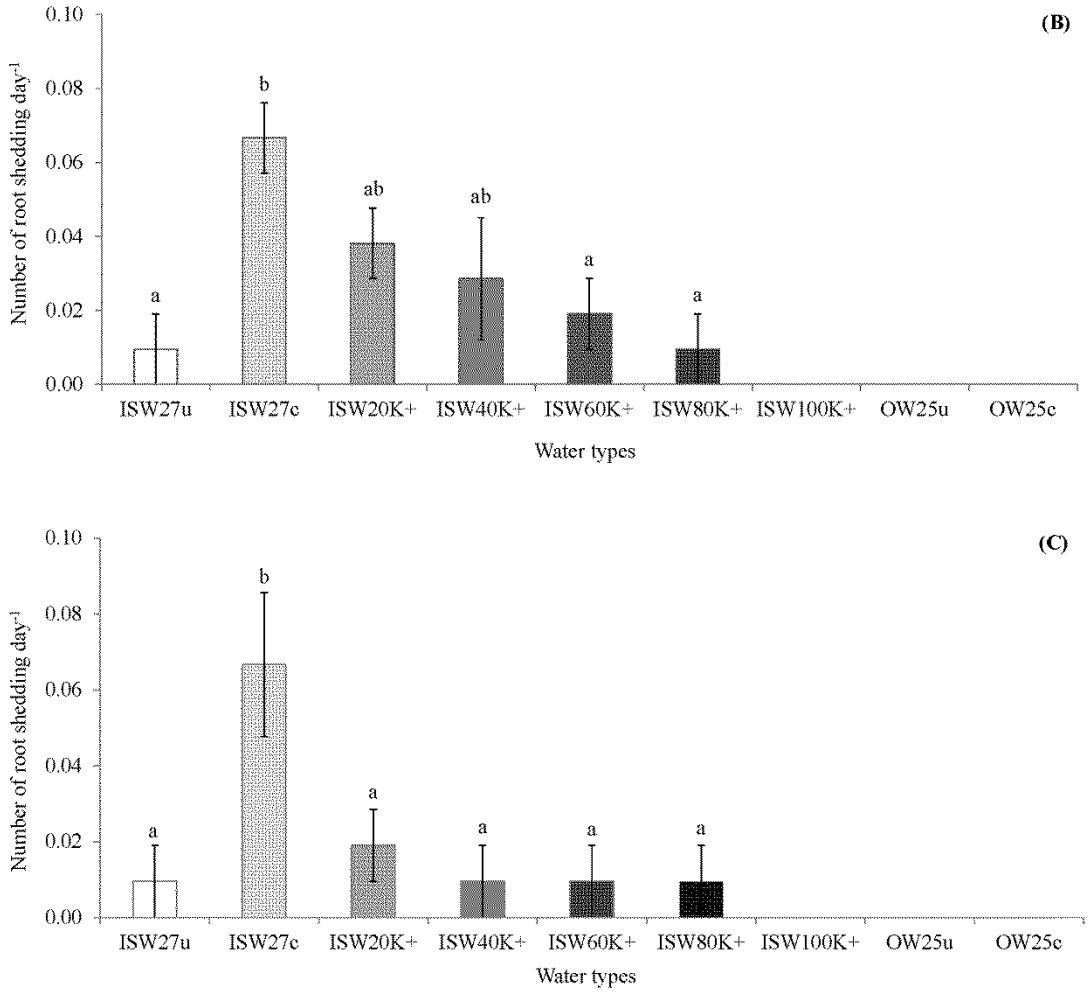


Figure 4.19 Mean number of blue mussel root shedding in the smaller (38.1 ± 0.4 mm) (A), medium (42.5 ± 0.4 mm) (B) and larger mussel (56.3 ± 0.3 mm) (C) reared in different K^+-fortified inland saline waters for 35 days.

Number of byssus threads which were produced by the blue mussel showed correlations (R^2 = 0.67 – 0.74) with K^+ fortification levels in ISW. Linear regression analysis showed that root shedding was strongly negatively correlated with K^+ fortification levels in ISW in the medium mussel while this negative correlation was weak in the larger mussel (Table 4.12).

Table 4.12 Linear regressions of the number of byssus thread renewal capacity and root shedding of the blue mussel as a function of K^+ fortification levels in ISW

Parameter	Size class	Equation	R^2
Byssus thread renewal capacity	smaller	y = 0.057x - 1.385	0.67
	medium	y = 0.067x - 2.560	0.74
	larger	y = 0.086x - 4.984	0.72
Shedding of the root	smaller	y = -0.000x + 0.090	0.76
	medium	y = -0.000x + 0.081	0.93
	larger	y = -0.000x + 0.064	0.66

Chapter 5 DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussions

5.1.1. The survival and osmoregulatory capacities of blue mussel cultured in ISW and OW

This is the first study to document the osmoregulatory mechanisms, a vital physiological adaptation process in changing salinities, of blue mussel in ISW. Organisms unable to adjust their osmoregulation, particularly when they are exposed to extreme salinity fluctuations, eventually die (Costa & Pritchard, 1978; Davenport, 1979b; Gong, Jiang, Lightner, & Collins, 2003; Gong et al., 2004; Prangnell, 2007).

In this study, the dead blue mussel failed to reach these critical osmolality values while the surviving ones showed haemolymph osmolality from ca. 650 to 750 mOsm kg^{-1} in ISW and ca. 730 to 950 mOsm.kg^{-1} in OW. A previous study by Shumway (1977) showed that haemolymph osmolality of surviving blue mussels exposed to 0 to 30 ppt of OW varies from 700 to 880 mOsm kg^{-1}, which equates to a salinity of 25 to 35 ppt, similar to the results of this study.

A strong inverse correlation between mortality and salinity in both ISW and OW indicates the increase in salinity resulted in the vulnerability of blue mussel except in ISW and OW at salinities ranging from 20 to 30 ppt and 20 to 35 ppt, respectively. It is likely that the adjustment of the haemolymph osmolality in blue mussel occurred in the first 120 hpe as there was a general tendency toward increased osmoregulation of mussel in salinities lower than 20 ppt towards the critical values of 600 mOsm kg^{-1} in ISW and 720 mOsm kg^{-1} in OW, the lowest values of haemolymph osmolality of surviving blue mussel in ISW and OW, respectively. All blue mussels in OW that failed to increase the osmolality to 700 mOsm kg^{-1} died. The mussel in ISW died at a lower osmolality of 600 mOsm kg^{-1} and the rate of mortality was faster than for the mussel exposed to OW at a given salinity and exposure time.

The faster mortality rate of blue mussel exposed to low salinities of ISW was intensified by the different ionic composition, especially the K^+ deficiency (Fotedar et al., 2008) in ISW, since K^+ levels in ISW are at least three times lower than in OW. A deficiency in K^+ results in an imbalance in ionic composition, that may for example, change the Na^+/K^+ ratio in a way that restricts the ability of Na^+/K^+ ATPase to function, and consequently results in an osmoregulatory malfunction (Costa & Pritchard, 1978; Gong et al., 2004) causing mortalities. An association between the K^+ deficiency and low survival has been reported for many aquatic species, such as black tiger shrimp (*Penaeus monodon)* (Cawthorne, Beard, Davenport, & Wickins, 1983), western king prawns (*Penaeus latisulcatus)* (Prangnell & Fotedar, 2009), juvenile greenlip abalone (*Haliotis laevigata)* (Fotedar et al., 2008), barramundi (*Lates calcarifer)* (Partridge, Lymbery, & Bourke, 2008), juvenile mulloway (*Argyrosomus japonicas)* (Doroudi et al., 2006), and Australian snapper (*Pagrus auratus)* (Fielder et al., 2001).

The observation that 100% of the mussel died at 35 ppt in ISW but not at 35 ppt in OW indicated a possible negative effect of K^+ deficiency in ISW that may intensify at a higher salinity. The Na^+/K^+ ratio of OW at the same salinity is 29:1, similar to the ratio at lower OW salinities and suitable for the blue mussel to survive. The intensity of the deficiency effect at higher salinities also has been explained by several authors (Fotedar et al., 2008; Gong et al., 2004; Prangnell & Fotedar, 2006a; Tantulo & Fotedar, 2007) in crustaceans. A deficiency in K^+ in ISW results in an imbalance in the ionic composition, and ultimately results in osmoregulatory malfunction (Costa & Pritchard, 1978; Gong et al., 2004) at 35 ppt in ISW.

Slopes from the regression analyses between haemolymph osmolality and medium osmolality have been used to determine the osmoregulatory capacity of various aquatic invertebrates, e.g. *P. latisulcatus* (Sang & Fotedar, 2004), brown tiger shrimp (*Penaeus esculentus)* (Sang & Fotedar, 2005), *P. monodon* (Tantulo & Fotedar, 2007), South American shrimp

(*Macrobrachium amazonicum)* (Charmantier & Anger, 2011), Caledonia blue shrimp (*Litopenaeus stylirostris)* (Pham et al., 2012) and white shrimp (*Litopenaeus vannamei)* (Chong-Robles et al., 2014). The present study confirms the strong correlation (data not shown) between haemolymph and medium osmolality in both ISW and OW.

When the haemolymph osmolality is higher than the medium osmolality (hypo-osmotic condition) and vice versa (hyper-osmotic condition), blue mussel respond to these conditions by using a hyper-osmoregulatory pattern or hypo-osmoregulatory pattern to compensate for ionic loss or influx (Rainbow & Black, 2001), respectively. The results clearly have shown that blue mussel are hyper-hypo osmoregulators in both ISW and OW. A similar osmoregulatory pattern was found in *P. monodon* when exposed to both ISW (Tantulo & Fotedar, 2006) and or OW, or *L. vannamei* when exposed to OW (Chong-Robles et al., 2014) at different salinities.

From the aquaculture point of view, finding suitable salinity close to IP is an important step to minimise the stress caused by maintaining an internal osmolality and may help to optimise the survival and growth of aquatic animals since they spend the least energy on osmoregulation at IP (Burton, 1986; Tantulo & Fotedar, 2006). Therefore, it is expected that blue mussel exposed to salinities where OC values are closer to IP may experience less stress. In OW, the IP of blue mussel varied from 21.96 to 25.04 ppt, which is somewhat lower than the IP of the blue mussel in ISW at 27 ppt. The IP of blue mussel in ISW is closer to the value for *P. monodon*, which shows IPs from 661.7 to 751.6 mOsm kg^{-1} that equate to 23.4 to 25.6 ppt after16 hours of exposure to ISW at a salinity of 5 to 35 ppt (Tantulo & Fotedar, 2007).

In conclusion, this study is the first to document IP of blue mussel in ISW as 700 mOsm kg^{-1}, equal to ca. 27 ppt. One-hundred percent of blue mussel could survive in ISW at 20 to 30 ppt

with the haemolymph osmolality of 650–750 mOsm kg^{-1} and in OW from 20 to 35 ppt with the haemolymph osmolality of 730–950 mOsm kg^{-1}.

5.1.2. Early development of the blue mussel cultured in potassium-fortified ISW

Marine species can be successfully cultured in ISW after ISW is either modified by fortifying it with K^+ salts (KCl or potassium fertilizers) (Fisher et al., 2013; Fotedar et al., 2008; McNevin et al., 2004; Prangnell, 2007; Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006) or formulated feed (Romano & Zeng, 2012; Roy & Davis, 2010; Saoud et al., 2007) for the target species is supplemented with K salts. More studies aiming to culture and improve the feasibility of the hatchery production of marine species in ISW and potassium-fortified ISW are needed. The lack of studies on the hatchery development of molluscs, including blue mussel, in ISW warrants further investigation.

Potassium is a primary intracellular ion in aquatic animals (Roy et al., 2010; Shiau & Hsieh, 2001) and plays a crucial role in acid-base balance, osmoregulation, maintaining membrane potentials (Hadfield et al., 2012) and the Na^+/K^+ ATPase activity (Liu et al., 2014). Na^+/K^+ ATPase, a sodium pump that is present in the gill membrane, transports Na^+ and Cl^- ions between the gill epithelial cells and haemolymph to maintain a stable osmoregulation in invertebrates (Charmantier et al., 1985; Mantel & Farmer, 1983). Na^+/K^+ ATPase activity is dependent on the ratio of Na^+ and K^+ in the surrounding environment (Tantulo & Fotedar, 2007). The optimal ratio of Na^+/K^+ for the normal function of Na^+/K^+ ATPase in marine animals varies from 23.85 to 85.20 in juvenile *H. laevigata* (Fotedar et al., 2008), *P. latisulcatus* (Prangnell & Fotedar, 2005) and *L. vannamei* (Zhu et al., 2004). A deficiency of K^+ can change the Na^+/K^+ ratio in a way that can inhibit the ability of Na^+/K^+ ATPase to function. This may eventually result in the poor survival of marine species (Fisher et al., 2013; Prangnell & Fotedar, 2005, 2006a; Tantulo & Fotedar, 2007; Zhu et al., 2004). In line

with this, early developmental stages of blue mussel showed higher survival rates when exposed to higher K^+ in ISW. The highest survival and growth at Na^+/K^+ ratio of 28.58 in ISW100K^+ was similar to the survival in OW25 that also had the Na^+/K^+ ratio of 28.58. The lowest survival occurred at the Na^+/K^+ ratio of 100.27 in ISW27, suggesting that it is possible to add K^+ to ISW to adjust the optimal Na^+/K^+ ratio for better survival of early larvae of the blue mussel.

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

The effects of K^+ on the DSI of the early larvae of blue mussel 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 mussel 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, Beaumont, & Davenport, 1995) or early larval mussel *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 & Hadfield, 1998; Sánchez-Lazo & Martínez-Pita, 2012; Wassnig & Southgate, 2012; Yang, Glenn Satuito, Bao, & Kitamura, 2008; Yang, Li, Bao, Satuito, & Kitamura, 2011; Young, Alfaro, & Robertson, 2011; Yu, He, Gu, He, & Yan, 2008; Zhao, Zhang, & Qian, 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 mussel.

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 mussel. The 100% K^+ fortification of ISW improves the viability of culturing early stages of blue mussel in ISW. The study shows the feasibility of using ISW fortified K^+ for culturing blue mussel in their early stages.

5.1.3. Survival and growth of the juvenile blue mussel cultured in potassium-fortified ISW

The blue mussels have a worldwide distribution range from temperate northern and southern hemispheres (Buck et al., 2010; Gérard et al., 2008; Gosling, 1992; Hilbish et al., 2000; McDonald et al., 1991; Rawson & Hilbish, 1995; Seed, 1992; Sukhotin et al., 2007). Our previous study (Dinh *et al.*, under review) showed that the blue mussel responded to the changes in OW and ISW at different salinities (5 – 35 ppt at 5 ppt intervals) by adjusting their haemolymph osmolality to reach the isosmotic point, where the haemolymph osmolality is equal to the osmolality of the medium (Burton, 1980), of 700 mOsm kg^{-1} and 800 mOsm kg^{-1} in OW and ISW, respectively which is equivalent to 25 ppt in OW and 27 ppt in ISW (Dinh *et al.*, under review). Therefore, the OW at 25 ppt and ISW at 27 ppt were chosen as controls in this study.

Environmental parameters, such as DO, pH and temperature varied in a suitable range during the experiment compared to those in the previous studies where DO is 6 mg L^{-1}, pH is 7.4 - 8.1 and temperature range is 20 - 29°C (Almada-Villela, Davenport, & Gruffydd, 1982; Berge, Bjerkeng, Pettersen, Schaanning, & Øxnevad, 2006; Fischer, 1986; Lutz, Incze, Porter, & Stotz, 1980; Riisgård, Line, & Pleissner, 2012; Tsuchiya, 1983; Yaroslavtseva & Sergeeva, 2006).

The deficiency of K^+ in ISW is a major problem for the survival and growth of marine organisms cultured in this type of water as evidenced in many marine species such as (Fielder et al., 2001; Fisher et al., 2013; Partridge & Creeper, 2004; Prangnell & Fotedar, 2005, 2006a; Roy et al., 2009; Tantulo & Fotedar, 2007; Zhu et al., 2004). The low K^+-induced reduction in survival has been explained by the imbalance in ionic composition in ISW (Fotedar et al., 2008; Prangnell & Fotedar, 2006a; Tantulo & Fotedar, 2007). This is because K^+ plays important roles in acid-base balance, osmoregulation, maintaining membrane potentials (Hadfield et al., 2012) and the Na^+/K^+ ATPase activity (Liu et al., 2014). A deficiency in K^+ may result in the osmoregulatory malfunction due to the limited activity of Na^+/K^+ ATPase (Costa & Pritchard, 1978; Gong et al., 2004). When the effect is too severe, the deficiency of K^+ in ISW may become lethal to blue mussel (Dinh *et al.*, under review) and also to other marine species (Fotedar et al., 2008; Prangnell & Fotedar, 2009; Tantulo, 2007).

Supplementation of potassium to ISW is required to optimize conditions for the aquaculture of aquatic species in this type of water (Boyd & Thunjai, 2003; Fotedar et al., 2008; Tantulo & Fotedar, 2006). The fortification of K^+ in ISW have shown positive effects on survival and growth of cultured species (Fisher et al., 2013; Fotedar et al., 2008; McNevin et al., 2004; Prangnell, 2007; Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006). Similarly, potassium-fortified ISW improves the survival rate and size, and reduces the developmental

stage interval and deformities, of the early life stages of blue mussel. The 100% K^+ fortified ISW improves the viability of culturing early stages of blue mussel in ISW (Dinh & Fotedar, 2016). Therefore, successful culturing blue mussel at the juvenile stage (15.7 ± 0.29 mm and 0.42 ± 0.02 g) in K^+ fortified ISW is another positive step towards the culture of blue mussel in ISW.

In this study, blue mussel showed lower SGR_L and SGR_W at lower K^+ fortified ISWs. This could be explained by the negative effects caused by the difference in ionic composition of ISW. Deficiency in K^+ concentration at lower K^+ fortified ISWs may result in a severe imbalance of ions between the blue mussel body and the environment (Panikkar, 1968), affecting ion-regulatory mechanisms (Deaton, 2001; Silva & Wright, 1994), and consequently forcing blue mussel to allocate more energy for the osmoregulatory capacity (Zhu et al., 2004). When the energy expenditure is higher than the energy acquisition, it may lessen the energy available for the growth (Zhu et al., 2004) and consequently resulting in the negative growth rate (Panikkar, 1968; Zhu et al., 2004). Low growth rate of marine species, e.g. western king prawn (Prangnell & Fotedar, 2005), pacific white shrimp *Litopenaeus vannamei* (Samocha et al., 2002) and *P. monodon* (Tantulo & Fotedar, 2006), and gulf killifish *Fundulus grandis* (Fisher et al., 2013) when cultured in K^+ deficient ISW is also found. The results on SGR_L and SGR_W of the blue mussel in this study are similar to those of blue mussel at larger sizes (38.1 ± 0.4 mm and 42.5 ± 0.4 mm) where higher potassium fortified ISW increase both survival and growth of the blue mussel (Dinh *et al.*, unpublished).

In conclusion, the ISW with the K^+ fortification of at 80 and 100% increased the survival and SGRs of blue mussel, suggesting the feasibility to culture blue mussel in ISW fortified K^+ at the level of 80% or higher.

5.1.4. Survival, growth, osmoregulatory capacity and proximate composition of blue mussel cultured in potassium-fortified ISW

The DO, pH, and temperature were within the suitable range for the culture of blue mussel (Berge et al., 2006; Riisgård et al., 2012; Yaroslavtseva & Sergeeva, 2006). Previous studies have documented that blue mussels show optimum growth at a DO of 6 mg L^{-1} (Fischer, 1986), at a pH range of 7.4 to 8.1 (Berge et al., 2006), and at a temperature range of 20 to 29°C (Almada-Villela et al., 1982; Lutz et al., 1980; Tsuchiya, 1983).

There are many studies investigating the effects of biological and non-biological factors such as seasonal variation (Dame, 2011; Zandee et al., 1980), tidal regimes (Buschbaum & Saier, 2001), epibionts (Thieltges & Buschbaum, 2007), food quality and quantity (Essink & Bos, 1985; Karayücel & Karayücel, 1999; Lange & Mostad, 1967; Mallet, Carver, Coffen, & Freeman, 1987), air exposure duration (Seed, 1969), stock density (Dickie, Boudreau, & Freeman, 1984; Mallet et al., 1987), and temperature (Mallet et al., 1987) on the survival and growth of blue mussel. However, there is no available data on the effects of K^+-fortified ISW on the survival, growth, stress status and particularly the biochemical composition of blue mussel during the grow-out stages, yet such data are important to assess the feasibility of culturing blue mussel in ISW.

Many studies have also shown that the culture of marine species in ISW results in poor survival (Fielder et al., 2001; Fisher et al., 2013; Partridge & Creeper, 2004; Prangnell & Fotedar, 2005, 2006a; Roy et al., 2009; Tantulo & Fotedar, 2007; Zhu et al., 2004). Similarly the blue mussel cultured in raw ISW (ISW27) showed a low survival at early (Dinh & Fotedar, 2016) and adult stage (Dinh *et al.*, unpublished). This low survival of marine animals in ISW has been explained by the difference in ionic composition between ISW and OW, particularly the extremely low level of K^+ in ISW (Fotedar et al., 2008; Prangnell &

Fotedar, 2006a; Tantulo & Fotedar, 2007), which may impair the normal physiological processes, including osmoregulation (Costa & Pritchard, 1978; Gong et al., 2004). K^+ plays a crucial role in acid–base balance, osmoregulation, maintaining membrane potentials (Hadfield et al., 2012), and the Na^+/K^+ ATPase activity (Liu et al., 2014) of marine aquatic species. A deficiency of K^+ results in an imbalance in Na^+/K^+ ratio in a way that restricts the ability of Na^+/K^+ ATPase to function, and consequently results in osmoregulatory malfunction (Costa & Pritchard, 1978; Gong et al., 2004) that can have lethal consequences.

To improve the survival of marine animals cultured in ISW, previous studies have attempted to add K^+ to ISW to increase the K^+ level close to its level in OW (Fisher et al., 2013; Prangnell & Fotedar, 2005, 2006a; Tantulo & Fotedar, 2007; Zhu et al., 2004) or to mix ISW with OW to change the ionic composition of ISW to be similar to OW. However, the mixture of ISW and OW is not feasible, as most salinised areas are far away from the coastal lines (NLWRA, 2000), and consequently the cost of transporting OW to ISW areas is too high, precluding culturing blue mussel at a commercial scale. Alternatively, the addition of K^+ to ISW has shown improved survival in pacific white shrimp *Litopenaeus vannamei* (Liu et al., 2014), *P. monodon* (Tantulo & Fotedar, 2007), *P. latisulcatus* (Prangnell, 2007), *Pagrus auratus* (Fielder et al., 2001), and the gulf killifish *Fundulus grandis* (Fisher et al., 2013) including early life stages of the blue mussel (Dinh & Fotedar, 2016). Similarly, two adult sized blue mussels showed higher survival in ISW fortified with 100% K^+, indicating that blue mussel can be cultured in ISW fortified with 100% K^+, equivalent to the K^+ level in OW, without affecting the survival rate.

The species such as *P. latisulcatus* (Prangnell & Fotedar, 2005), *L. vannamei* (Samocha et al., 2002), *P. monodon* (Tantulo & Fotedar, 2006), and *H. laevigata* (Fotedar et al., 2008) have also shown a reduced growth rate when cultured in ISW, presumably due to the higher energy expenditure in dealing with osmoregulation, leaving less energy available for growth (Zhu et

al., 2004). The difference in the ionic composition of ISW, particularly the low K^+ level than OW results in a severe imbalance of ions between the blue mussel haemolymph and external media (Panikkar, 1968), affecting ion-regulatory mechanisms (Deaton, 2001; Silva & Wright, 1994) and consequently forcing blue mussel to allocate more energy to adjust the osmoregulation (Zhu et al., 2004). When the energy expenditure is higher than the energy acquisition, it may result in a negative growth rate (Panikkar, 1968; Zhu et al., 2004). This may have occurred in blue mussel in this study, as negative growth rates in terms of weight were observed in blue mussel exposed to a low level of K^+ fortification in both groups. Further, larger mussel potentially could spend more energy in dealing with osmoregulation than the smaller ones, resulting in negative growth rates when cultured in K^+ fortification of 60% (ISWK^+60) or lower. In contrast, the smaller mussel showed a negative growth rate only in ISW with a level of K^+ fortification of 20% or lower.

Marine species show optimal growth when they are exposed to media with a suitable Na^+/K^+ ratio. The fortification of K^+ in ISW increased the K^+ level and Na^+/K^+ ratio closer to OW (28.58:1), the growth rate of blue mussel increased and became similar to OW (Table 5.1).

Table 5.1 Summary of the optimum Na^+/K^+ ratios for the growth and survival of marine species.

Species	Water type	Salinity	Na^+/K^+ ratio	References
Mytilus edulis	ISW100K^+	27	28.58	Dinh and Fotedar (2016)
Haliotis laevigata	ISW	35	23.85	Fotedar et al. (2008)
	ISW + OW	35	28.69 and 23.85	
Penaeus latisulcatus	ISW100K^+	30	31.30	Prangnell and Fotedar (2005)

Litopenaeus vannamei	OW	30	34.1	Zhu et al. (2004)
Penaeus monodon	ISW100K^+	25	23	Tantulo and Fotedar (2006)
	OW	25	22.3	

Besides survival and growth, it is also important to assess the biochemical composition such as protein and lipid contents of the cultured species as the indicators of the flesh quality. However, no information is available on the effects of K^+ addition on the biochemical composition of any cultured species, including blue mussel.

Intracellular K^+ plays a significant role in protein synthesis (Lubin & Ennis, 1964; Weiler & Greenough, 1991). The presence of K^+ can result in the depolarisation of neuronal membranes (Weiler & Greenough, 1991), triggering enzyme reactions for increasing protein synthesis (Lipton & Heimbach, 1977; Verity, Brown, & Cheung, 1980; Wedege, Luqmani, & Bradford, 1977; Weiler & Greenough, 1991). This may explain the increase in protein content in blue mussel in the ISW fortified with 100% K^+ than the ISW with lower levels of K^+ fortification. Importantly, the protein level in ISW100K^+ was similar to the level of blue mussel in OW, an important indication that the protein level of blue mussel cultured in ISW is not lower than when cultured in OW. Lipid content is an important energy source for blue mussel (De Zwaan & Mathieu, 1992; Svensson & Förlin, 2004; Zandee, Holwerda, & De Zwaan, 1980) which accounted for 3 to 18% of the dry weight of blue mussel (Table 5.2) and there was no effect of K^+ fortification on lipid content. Exposure to ISW could also increase the energy expenditure due to osmoregulatory pressures and can be reflected in the observed reduced growth rate. Therefore, it was expected that blue mussel in ISW with low K^+ levels would show lower lipid content, a reflection of low stored energy. However, blue mussel cultured in ISW showed the lipid content was similar to the blue mussel cultured in OW,

indicating no comprise on flesh quality. No significant changes in the lipid contents can be explained as blue mussel can also rely on another energy source such as glycogen (De Zwaan & Mathieu, 1992; Zandee et al., 1980) to generate enough energy to combat stress caused by ISW exposure (Myrand, Guderley, & Himmelman, 2000). Energy closely depends on the variability shown in biochemical composition (Fernández et al., 2015). Our result showed that energy levels of blue mussel in ISW were within the range of the previously documented energy levels of 13.92 to 22.44 MJ kg^{-1} in blue mussel cultured in OW (Fernández et al., 2015). The gross energy was also highest in blue mussel in ISW with 100% K^+ fortification, and this gross energy content was also comparable to blue mussel in OW.

Table 5.2 Biochemical composition (percent of dry weight) of cultured blue mussel *M. edulis*

Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	Gross energy (MJ/kg)	Reference
46–73	4–10		9–17		Dare and Edwards (1975)
30 – >50	6–10				Zandee et al. (1980)
52–82	3–13		4–14	19–23	Okumuş and Stirling (1998)
	13–18				Pleissner, Eriksen, Lundgreen, and Riisgård (2012)
43–63	5–8		7–33	14–22	Fernández et al. (2015)
	9–76*				Gallardi et al. (2014)
54–60	7–8				Pronker, Nevejan, Peene, Geijsen, and Sorgeloos (2008)
45–75	10–13				Pieters et al. (1980)
		5–19			Wyatt et al. (2013)

* denotes the percentage (%) was calculated based on wet weight.

Ash content was evaluated through the amount of inorganic compounds contained in the blue mussel meat. Ash weight varies seasonally among cultured and wild blue mussel (Rodhouse et al., 1984). A previous study (Fotedar et al., 2008) has reported that the ash content of *H. laevigata* was not significantly different among K^+-fortified ISW. The current study showed somewhat different results, as the ash content increased only in blue mussel cultured in ISW with 100% K^+ fortification.

In this study, the OC range of both sized surviving mussel is similar to the OC range of the surviving mussel exposed to raw ISW at salinity range of 5-35 ppt (Dinh *et al.*, unpublished). Higher K^+ fortification in ISW brings OC closer to the isosmotic point and may consequently minimising any potential stress of K^+ fortified ISW on the survival and growth of blue mussel.

In conclusion, the ISW fortified with 100% K^+ increases the survival, growth, protein and ash contents, while it does not alter the lipid but reduces the moisture contents. These results were consistent between the two blue mussel groups and were similar to the blue mussel cultured in OW, suggesting that technically the ISW fortified with 100% K^+ equivalent to the K^+ level in OW is a suitable resource to culture blue mussel.

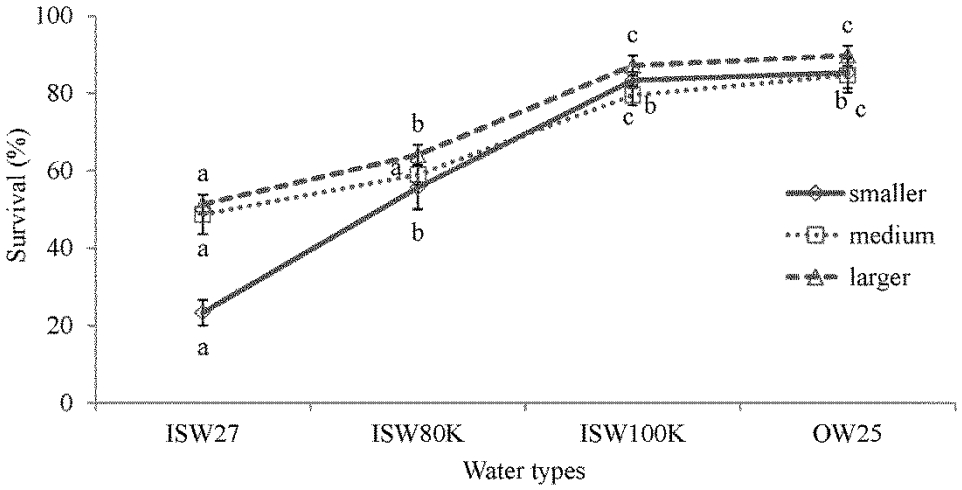


Figure 5.1 Survival of smaller, medium and larger blue mussel cultured in appropriately fortified K^+ ISW and two control water types for 120 days.

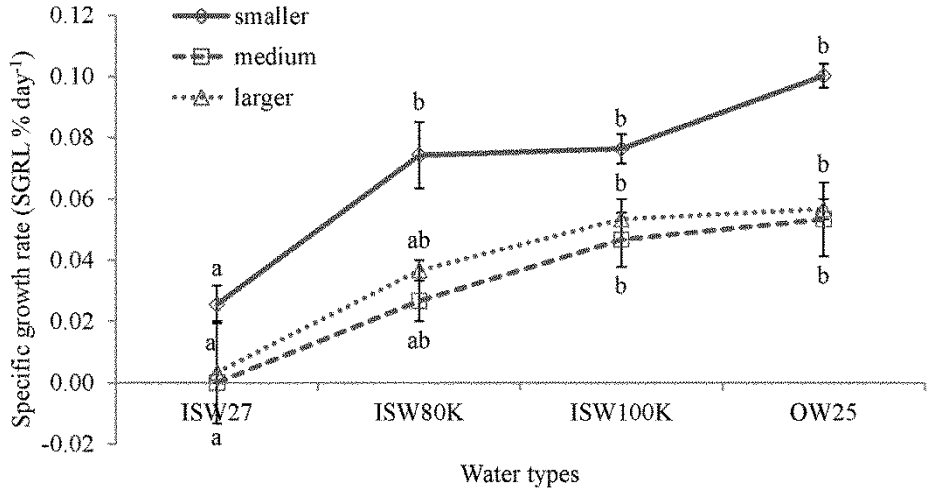


Figure 5.2 Specific growth rate in length of smaller, medium and larger blue mussel cultured in appropriately fortified K^+ ISW and two control water types for 120 days.

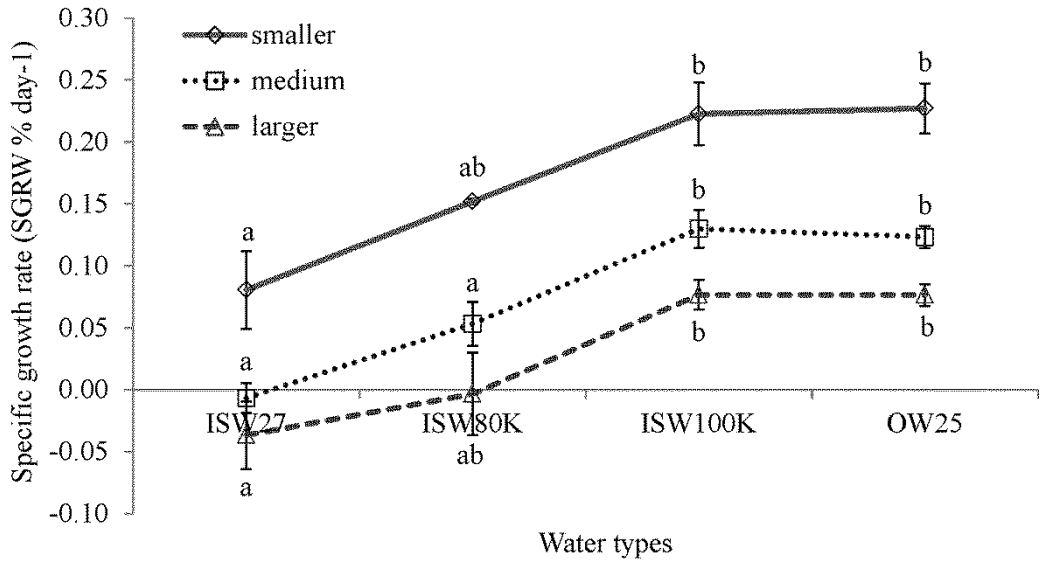


Figure 5.3 Specific growth rate in weight of smaller, medium and larger blue mussel cultured in appropriately fortified K^+ ISW and two control water types for 120 days.

The survival ranged from 23 to 90 % among three sized blue mussel and was lower in raw ISW, which was used as control (Figure 5.1). While the blue mussel showed no difference in the SGR_L and SGR_W between medium and larger sizes, the SGR_L and SGR_W of smaller blue mussel was significantly higher than the other blue mussel sizes suggesting the suitable size to initiate the blue mussel farming in K^+ fortified ISW is at the juvenile stage (Figure 5.2 and Figure 5.3). Furthermore, linear regression showed that survival, SGR_L and SGR_W were strongly correlated with K^+ fortification levels in ISW (Table 5.3) and all these performance indicators of the mussel were independent of their sizes as there was no significant difference in the R^2 values of survival, SGR_L and SGR_W among three size groups of the mussel.

Table 5.3 Linear regression of the SGR_W and SGR_L of the blue mussel as a function of K^+ fortification levels in ISW (80 – 100% K^+ fortification)

Parameter	Size	Regression	R^2
Survival	Smaller	y = 0.382x - 4.200	0.95
	Medium	y = 0.206x + 32.345	0.81
	Larger	y = 0.229x + 33.383	0.84
SGR_L	Smaller	y = 0.000x - 0.001	0.93
	Medium	y = 0.000x - 0.023	0.95
	Larger	y = 0.000x - 0.019	0.99
SGR_W	Smaller	y = 0.001x + 0.015	0.93
	Medium	y = 0.001x - 0.068	0.90
	Larger	y = 0.001x - 0.092	0.80

There was also no significant difference in the condition indices of the mussel among water types within each sized blue mussel, however, the condition indices of the medium and larger blue mussels were significantly higher than smaller blue mussel sizes (Table 5.4). This shows that the mussels do not grow uniformly in terms of length and weight and are not influenced by the K^+ fortification levels.

Table 5.4 Condition index (length/weight) of three sized blue mussel groups: smaller (15.7 ± 0.29 mm), medium (34.25 ± 0.59 mm) and larger (45.12 ± 0.47 mm) cultured in K^+ fortified ISW for 120 days

Group	ISW27	ISW20K^+	ISW40K^+	ISW60K^+	ISW80K^+	ISW100K^+	OW25
Smaller	^{1}0.036 ± 0.002	^{1}0.036 ± 0.002	^{1}0.034 ± 0.004	^{1}0.034 ± 0.002	^{1}0.034 ± 0.001	^{1}0.032 ± 0.001	^{1}0.033 ± 0.002
Medium	^{2}0.007 ± 0.000	^{2}0.007 ± 0.000	^{2}0.007 ± 0.000	^{2}0.007 ± 0.000	^{2}0.007 ± 0.000	^{2}0.007 ± 0.000	^{2}0.006 ± 0.000
Larger	^{2}0.005 ± 0.000	^{2}0.005 ± 0.000	^{2}0.005 ± 0.000	^{2}0.005 ± 0.000	^{3}0.005 ± 0.000	^{2}0.005 ± 0.000	^{2}0.004 ± 0.000

There was no significant ($p > 0.05$) difference in the data in the same row. Data in the same column within each sized blue mussel group marked by different numerical letters are significantly ($p < 0.05$) different within each water type.

Length-weight relationship is an important parameter to identify the condition (Maligaiah & Solai, 2015) and estimate the biomass (Park & Oh, 2002) of the bivalve. The parameter is isometric in most bivalve and different among species (Park & Oh, 2002). A previous study

showed that length-weight relationship of blue mussel is $1.708\text{length}^{2.850}$ which is close to the parameter in larger blue mussel of this study (Table 5.5).

Table 5.5 Length-weight relationships of three sized blue mussel groups: smaller (15.7 ± 0.29 mm), medium (34.25 ± 0.59 mm) and larger (45.12 ± 0.47 mm) cultured in K^+ fortified ISW for 120 days

Group	Relationship
Smaller	Weight = $1.953\text{length}^{1.964}$
Medium	Weight = $1.391\text{length}^{2.294}$
Larger	Weight = $0.668\text{length}^{2.506}$

5.1.5. Size-dependent root shedding and byssus thread renewal capacity of the blue mussel exposed to potassium-fortified ISW

Byssus threads play an important role in securing blue mussel to their substrates (Aldred et al., 2006; Allen et al., 1976; Kishore et al., 2014) and reducing the risk of being captured by predators (Cheung et al., 2006; Côté, 1995; Garner & Litvaitis, 2013). Also, the formation of byssus threads is also important to assess their ability to secure blue mussel to artificial substrates and to select the most suitable substrate. Yet, most of these studies investigating the byssus formation of blue mussel are conducted in OW. However, little is known about the blue mussel byssus thread renewal capacity and root shedding which are important for their attachment ability to substrate in K^+ fortified ISW. Blue mussel reach marketable sizes at 40-60 mm (FAO, 2004-2015). Therefore, three different marketable sized size classes were exposed to ISW with different levels of K^+ addition

The DO, pH and temperature were in the suitable range for the development of blue mussel (Berge et al., 2006; Fischer, 1986; Riisgård et al., 2012; Yaroslavtseva & Sergeeva, 2006). Past studies documented the suitable range of environmental factors for the development of

blue mussel are 6 mg L^{-1} for DO (Fischer, 1986), pH of 7.4 to 8.1 (Berge et al., 2006) and at temperature of 20 to 29°C (Almada-Villela et al., 1982; Lutz et al., 1980; Tsuchiya, 1983).

The survival of blue mussel depends on various factors such as the epibionts (Thieltges & Buschbaum, 2007), food quality and quantity (Essink & Bos, 1985; Karayücel & Karayücel, 1999; Lange & Mostad, 1967; Mallet et al., 1987), seasonal variation (Dame, 2011; Zandee et al., 1980), tidal regimes (Buschbaum & Saier, 2001), air exposure duration (Seed, 1969), temperature (Mallet et al., 1987) and stock density (Dickie et al., 1984; Mallet et al., 1987). There was some evidence that deficient K^+ levels in ISW may result in poor survival of marine species cultured in ISW, such as *H. laevigata* (Fotedar et al., 2008), *P. latisulcatus* (Prangnell & Fotedar, 2005), *L. vannamei* (Zhu et al., 2004), *P. monodon* (Tantulo & Fotedar, 2006), *P. auratus* (Fielder et al., 2001) and the gulf killifish *Fundulus grandis* (Fisher et al., 2013). However, ISW, which was fortified with K^+, improved survival of cultured species (Fisher et al., 2013; Fotedar et al., 2008; Partridge, Lymbery, & Bourke, 2008; Prangnell & Fotedar, 2006b; Roy et al., 2010; Tantulo & Fotedar, 2006). The K^+ fortified ISW improved survival of blue mussel early life stages (Dinh & Fotedar, 2016) and adult blue mussel (Dinh *et al*., unpublished).

The renewal of byssus threads of bivalvia is affected by various environmental factors as summarized in Table 5.6. Overall, there is no consistent pattern of the influence of environmental factors on the formation of byssus threads of bivalvia species. Some species, for example, *Geukensia demissa* and *Mytella charruana* produce less byssus threads at low temperature than at high temperature (Brodsky et al., 2011). The number of byssus threads increases with the increasing salinity from 8 to 17 ppt and decreases at the salinity from 17 to 23 ppt (Young, 1985). Glaus (1968) and Price (1980) reported that blue mussel respond to the increased water velocity by increasing rates of byssus thread formation while Moeser et al. (2006) found the opposite pattern that blue mussel reduce the numbers of the byssus thread

when water velocity increases. Martella (1974) reported that tidal fluctuation does not influence the formation of byssus threads of blue mussel.

The important finding of this study is that higher levels of K^+ addition to ISW significantly increased the formation of byssus threads in the blue mussel. A previous study has shown that blue mussel exposed to low levels of Ca^{2+} and Mg^{2+} may result in low osmotic pressure (Winkle, 1970) and reduce the formation of byssus threads (Winkle, 1970). However, in this study the Ca^{2+} and Mg^{2+} levels in ISW were higher than those in OW; therefore these ions were unlikely the cause of the low formation of byssus threads. The results from this study have shown that the number of byssus threads produced by the blue mussel decreased during a 35-day experiment. In this study, lower renewal capacity of byssus thread at lower K^+ fortification levels suggests the negative effects of the deficiency of K^+ in ISW. Previous studies (Petrone et al., 2015; Sagert & Waite, 2009; Tamarin, Lewis, & Askey, 1976; Yu et al., 2011) have shown that the injection of K^+ at the concentration of 0.56 into the base of mussel foot induce the formation of byssus threads. In line with this, the previous study (Dinh & Fotedar, 2016) has shown that the fortification of K^+ in ISW significantly increase the number of settling larvae of the blue mussel. Deficiency in K^+ level has been known to impair the osmoregulatory ability (Panikkar, 1968) and compelled the blue mussel to allocate the energy to deal with the imbalance of osmoregulatory process (Deaton, 2001; Silva & Wright, 1994). As a consequence it may be a trade off with the energy allocation to the formation of byssus threads (equal to 7.5% (Hawkins & Bayne, 1985)) and reduced the number of byssus threads produced by the blue mussel at the lower K^+ fortified ISW.

Table 5.6 Factors influence the byssus thread renewal capacity of molluscs

Factor	Findings	Byssus thread mussel^{-1} day^{-1}	Reference
Temperature increase	Increase in byssus thread renewal capacity	1 - 14	Allen et al. (1976)
		4 - 7	Young (1985)
Salinities (8.5 – 17 ppt): Salinity increase	Decrease in decrease in byssus thread renewal capacity	1 - 4	Allen et al. (1976); Winkle (1970); Young (1985)
Pesticides: Endofulfan (organochlorine) and Trichlorphon (organophosphate) increase	Decrease in byssus thread renewal capacity with the increase in the pesticide concentration from 0.1 to 0.8 mg L^{-1}	2 - 10	Roberts (1975)
Circadian rhythm	byssus thread renewal capacity at night time > day time	1 - 7	Martella (1974)
Water velocity	No effect		Martella (1974)
	Increased velocity with the decrease in	3 - 23	Moeser et al. (2006)

	byssus thread renewal capacity		
Tidal regime	Vary	2 - 6	Young (1985)
Toxic dinoflagellate	byssus thread renewal capacity decrease	2 - 7	Shumway, Pierce, and Knowlton (1987)
Predator	More, shorter and thicker byssus thread renewal capacity with the present of predator (crab)	8 with urchin 10 with crab	Côté (1995)
Substrate: wettability decrease	Increase in byssus thread renewal capacity	2 -5	Aldred et al. (2006)
Epibiont		- 11 (Epibiont-attached mussels) - 8 (Epibiont-removed mussels) - 5 (Unattached mussels)	Thieltges and Buschbaum (2007)
Seasonal variation	Reduction to lowest number in winter and increase in summer to reach peak at June and July		Moeser et al. (2006)

Based on the previous studies (Denny & Gaylord, 2010; Iwasaki, 2015; Young, 1985), it is unclear whether the byssus root is completely separated from of the mussel or not. So far, this study is the first report on the root shedding of the blue mussel when exposed to K^+ fortified ISW. The shedding of blue mussel is affected by several factors, for example epizoism (Witman & Suchanek, 1984), light conditions (Duchini, Boltovskoy, & Sylvester, 2015), wave (Paine & Levin, 1981; Witman, 1987) and water velocity (Schneider, Wethey, Helmuth, & Hilbish, 2005). The shedding rate in the mussel *M. edulis* and *M. galloprovincialis* and the hybrid genotypes at the sizes of 25 to 35 mm were 48%, 34% and 24% at the water velocity of 8 m s^{-1} while 82% of shedding was reported in the blue mussel at the water velocity of 10 m s^{-1} (Schneider et al., 2005). In this study, the formation of byssus threads of blue mussel in K^+-fortified ISW, particularly in ISW100K^+ indicates that blue mussel can reduce the shedding risk. Although the shedding results in natural (Hunt & Scheibling, 2001; Paine & Levin, 1981) and economic loss (Bourque & Myrand, 2006), the underlying mechanism is poorly understood. Possibly, under the deficiency in K^+, the energy, which is allocated to the byssus formation, is traded off to solve the imbalance in the osmoregulatory capacities (Deaton, 2001; Silva & Wright, 1994), and may resulting in the byssus root malfunction, for example low tenacity and consequently causing the mussel dislodgement (Denny & Gaylord, 2010).

In conclusion, potassium-fortified ISW did not affect the survival of blue mussel while the higher level of potassium fortification to ISW significantly increases the byssus thread renewal capacity and reducing the root shedding in the blue mussel.

5.2. Conclusions

ISW, which is deficient in K^+, is lethal to the blue mussel at salinities of lower than 20 ppt and higher than 30 ppt. The lethal effect is associated with the osmoregulatory capacities. A

deficiency in K^+ results in an imbalance in ionic composition, that may change the Na^+/K^+ ratio in a way that restricts the ability of Na^+/K^+ ATPase to function, and consequently results in an osmoregulatory malfunction causing mortalities in blue mussel. The isosmotic point of blue mussel was 700 mOsm kg^{-1} in ISW, equal to a salinity of 27 ppt and stabilized between 770 to 800 mOsm kg^{-1} in OW, equal to salinities of 24 to 25 ppt.

K^+ fortification in ISW shows invariable effects on the blue mussel at different stages.

The later stages of early development are more sensitive to K^+ deficiency, which might is associated with the development of osmoregulatory organs. 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.

Higher K^+ significantly improved the survival of the juvenile blue mussel. ISW with 80 and 100% K^+ fortification ($ISW100K^+$) significantly increased the SGR in length while only $ISW100K^+$ significantly increased SGR in weight of the juvenile blue mussel. These results highlight the feasibility of culturing the juvenile blue mussel in ISW with 80 or/and 100% K^+ fortification.

ISW with 100% K^+ fortification ($ISW100K^+$) significantly increased the survival, SGR (both length and weight) protein, ash, and energy contents of the cultured blue mussel and brought

it similar to OW. The osmoregulatory capacity was similar among K^+ fortified ISW and between sized blue mussel. Lipid and moisture contents of both sized mussel were similar to initial values in all water types, however, moisture alone was influenced by different K^+ fortification levels. The survival, growth, and protein and lipid contents of blue mussel cultured in ISW100K^+ were similar to those cultured in OW and were independent of the cultured sizes. These results highlight the potential of culturing blue mussels in 100% K^+-fortified ISW.

Higher K^+-fortification of ISW significantly increased the byssus thread renewal capacity and decreased the root shedding in all three size classes. The 100 percent fortification of K^+ in ISW is recommended for culturing blue mussel in order to increase their attachment to a substrate.

5.3. Recommendations

Further studies aiming at other physiological responses of blue mussel when exposed to ISW are needed, for example the activity of Na^+/K^+ ATPase and underlying mechanisms affecting the survival of the blue mussel.

The investigation of the supplement of K^+ to the diets, for example, K^+ enriched microalgae as the another source of K^+ for the blue mussel aquaculture is needed.

Understanding of the effect of ISW on the broodstock conditioning of the blue mussel, when cultured in ISW, may close the whole life cycle of the blue mussel and enable the future successful hatchery in ISW.

Long term implication of 100% K^+ fortified ISW for the blue mussel aquaculture should be carried out to validate the interaction of other factors on the performance of this species.

REFERENCES

Aarset, A. V., & Zachariassen, K. E. (1982). Effects of oil pollution on the freezing tolerance and solute concentration of the blue mussel *Mytilus edulis*. *Marine Biology, 72*(1), 45-51.

ABARES. (2014). *Australian fisheries and aquaculture statistics 2013*: Australian Bureau of Agricultural and Resource Economics and Sciences.

ABARES. (2014). Profiling Australian fisheries and aquaculture production: Department of Agriculture and Water Resources.

Ackerman, J. D., Sim, B., Nichols, S. J., & Claudi, R. (1994). A review of the early life history of zebra mussels (*Dreissena polymorpha*): comparisons with marine bivalves. *Canadian Journal of Zoology, 72*(7), 1169-1179.

Aldred, N., Ista, L. K., Callow, M. E., Callow, J. A., Lopez, G. P., & Clare, A. S. (2006). Mussel (*Mytilus edulis*) byssus deposition in response to variations in surface wettability. *Journal of The Royal Society Interface, 3*(6), 37-43.

Allan, G. L., Banens, B., & Fielder, S. (2001). Developing commercial inland saline aquaculture in Australia: Part 2. Resource inventory and assessment. *NSW fisheries final report series*(31), 116 p.

Allan, G. L., Dignam, A., & Fielder, S. (2001). Developing commercial inland saline aquaculture in Australia: Part 1. National R & D plan. *Fisheries Research and Development Corporation. Canberra. FRDC Project*(98/335), 33 p.

Allan, G. L., & Fielder, D. S. (1998). *Inland saline aquaculture activities in New South Wales.*

Allan, G. L., Fielder, D. S., Fitzsimmons, K. M., Applebaum, S. L., Raizada, S., Burnell, G., & Allan, G. (2009). Inland saline aquaculture. *In G. Burnell & G.L. Allan, eds. New technologies in aquaculture: improving production efficiency, quality and environmental management. Woodhead Publishing limited,* 1118-1147.

Allan, G. L., Heasman, H., & Bennison, S. (2008). *Development of industrial-scale inland saline aquaculture: Coordination and communication of R&D in Australia*: NSW Department of Primary Industries.

Allen, J. A., Cook, M., Jackson, D. J., Preston, S., & Worth, E. M. (1976). Observations on the rate of production and mechanical properties of the byssus threads of *Mytilus edulis* L. *Journal of Molluscan Studies, 42*(2), 279-289.

Almada-Villela, P. C., Davenport, J., & Gruffydd, L. D. (1982). The effects of temperature on the shell growth of young *Mytilus edulis* L. *Journal of Experimental Marine Biology and Ecology*, *59*(2), 275-288.

Andersen, S., Grefsrud, E. S., & Harboe, T. (2013). Effect of increased pCO2 level on early shell development in great scallop (*Pecten maximus* Lamarck) larvae. *Biogeosciences, 10*, 6161–6184.

Babarro, J. M. F., & Comeau, L. A. (2014). Byssus attachment strength of two mytilids in mono-specific and mixed-species mussel beds. *Biofouling, 30*(8), 975-985.

Babarro, J. M. F., & Fernández Reiriz, M. J. (2006). Variability of taurine concentrations in *Mytilus galloprovincialis* as a function of body size and specific tissue. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 145*(1), 94-100.

Babarro, J. M. F., Reiriz, M. J. F., Garrido, J. L., & Labarta, U. (2006). Free amino acid composition in juveniles of *Mytilus galloprovincialis*: Spatial variability after Prestige oil spill. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *145*(2), 204-213.

Barman, U. K., Jana, S. N., Garg, S. K., Bhatnagar, A., & Arasu, A. R. T. (2005). Effect of inland water salinity on growth, feed conversion efficiency and intestinal enzyme activity in growing grey mullet, *Mugil cephalus* (Linn.): Field and laboratory studies. *Aquaculture International, 13*(3), 241-256.

Bayne, B. L. (1964a). Primary and secondary settlement in *Mytilus edulis* L. (Mollusca). *The Journal of Animal Ecology*, *33*(3), 513-523.

Bayne, B. L. (1964b). The responses of the larvae of *Mytilus edulis* L. to light and to gravity. *Oikos*, *15*(1), 162-174.

Bayne, B. L. (1965). Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia, 2*(1), 1-47.

Bayne, B. L. (1971). *Some morphological changes that occur at the metamorphosis of the larvae of Mytilus edulis.* Paper presented at the Fourth European Marine Biology Symposium.

Bayne, B. L., Holland, D. L., Moore, M. N., Lowe, D. M., & Widdows, J. (1978). Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom, 58*(04), 825-841.

Bayne, B. L., & Thompson, R. J. (1970). Some physiological consequences of keeping *Mytilus edulis* in the laboratory. *Helgoländer wissenschaftliche Meeresuntersuchungen, 20*(1-4), 526-552.

Bennetts, D. A., Webb, J. A., Stone, D. J. M., & Hill, D. M. (2006). Understanding the salinisation process for groundwater in an area of south-eastern Australia, using hydrochemical and isotopic evidence. *Journal of Hydrology, 323*(1), 178-192.

Beresford, Q., Bekle, H. B. Q., Mulcock, J., & Phillips, H. (2001). The salinity crisis: landscapes, communities and politics. *University of Western Australia Press, Claremont, Perth, Western Australia, Australia*, 324 p.

Berge, J. A., Bjerkeng, B., Pettersen, O., Schaanning, M. T., & Øxnevad, S. (2006). Effects of increased sea water concentrations of CO_2 on growth of the bivalve *Mytilus edulis* L. *Chemosphere, 62*(4), 681-687.

Borsa, P., Rolland, V., & Daguin-Thiébaut, C. (2012). Genetics and taxonomy of Chilean smooth-shelled mussels, *Mytilus* spp.(Bivalvia: Mytilidae). *Comptes rendus biologies, 335*(1), 51-61.

Bourque, C. W. (2008). Central mechanisms of osmosensation and systemic osmoregulation. *Nature Reviews Neuroscience, 9*(7), 519-531.

Bourque, F., & Myrand, B. (2006). *Étude descriptive du dégrappage en milieu lagunaire aux Îles-de-la-Madeleine*: Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, Direction de l'innovation et des technologies.

Boyd, C. A., Boyd, C. E., & Rouse, D. B. (2007). Potassium budget for inland, saline water shrimp ponds in Alabama. *Aquacultural engineering, 36*(1), 45-50.

Boyd, C. E., & Thunjai, T. (2003). Concentrations of major ions in waters of inland shrimp farms in China, Ecuador, Thailand, and the United States. *Journal of the World Aquaculture Society, 34*(4), 524-532.

Brinckerhoff, P. (2004). Coal seam gas water management study. *Report NRO0011. Brisbane, Department of Natural Resources, Mines and Energy*, 6 p.

Brodsky, S., Walters, L., Schneider, K., & Hoffman, E. (2011). Cold temperature effects on byssal thread production by the native mussel *Geukensia demissa* versus the non-native mussel *Mytella charruana*. *University of Central Florida Undergraduate Research Journal, 5*(1), 1-10.

Brune, D. E., Reach, C. D., & O'Connor, J. T. (1981). *Inland saltwater as a medium for the production of biomass.* Paper presented at the Biotechnology and bioengineering symposium 11.

Buck, B. H., Ebeling, M. W., & Michler-Cieluch, T. (2010). Mussel cultivation as a co-use in offshore wind farms: potential and economic feasibility. *Aquaculture Economics & Management, 14*(4), 255-281.

Burnham, B. L., & Peterka, J. J. (1975). Effects of saline water from North Dakota lakes on survival of fathead minnow (*Pimephales promelas*) embryos and sac fry. *Journal of the Fisheries Board of Canada, 32*(6), 809-812.

Burton, R. F. (1980). Osmoregulation in aquatic animals: The interpretation of regulation curves. *Comparative Biochemistry and Physiology Part A: Physiology, 66*(2), 157-160.

Burton, R. F. (1986). Ionic regulation in crustacea: the influence of temperature on apparent set points. *Comparative Biochemistry and Physiology Part A: Physiology, 84*(1), 135-139.

Buschbaum, C., & Saier, B. (2001). Growth of the mussel *Mytilus edulis* L. in the Wadden Sea affected by tidal emergence and barnacle epibionts. *Journal of Sea Research, 45*(1), 27-36.

Butler, A. (1998). *Key environmental constraints for research.* Paper presented at the ACIAR Proceedings.

Caceres-Martinez, J., Robledo, J. A. F., & Figueras, A. (1994). Settlement and post-larvae behaviour of *Mytitus galtoprovincialis*: field and laboratory experiments. *Marine Ecology Progress Series, 112*, 107-117.

Carpizo-Ituarte, E., & Hadfield, M. G. (1998). Stimulation of metamorphosis in the polychaete *Hydroides elegans* Haswell (Serpulidae). *The Biological Bulletin, 194*(1), 14-24.

Carrington, E. (2002). Seasonal variation in the attachment strength of blue mussels: causes and consequences. *Limnology and Oceanography, 47*(6), 1723-1733.

Carver, S., Storey, A., Spafford, H., Lynas, J., Chandler, L., & Weinstein, P. (2009). Salinity as a driver of aquatic invertebrate colonisation behaviour and distribution in the wheatbelt of Western Australia. *Hydrobiologia, 617*(1), 75-90.

Cawthorne, D. F., Beard, T., Davenport, J., & Wickins, J. F. (1983). Responses of juvenile *Penaeus monodon* Fabricius to natural and artificial sea waters of low salinity. *Aquaculture, 32*(1), 165-174.

Charmantier, G., & Anger, K. (2011). Ontogeny of osmoregulatory patterns in the South American shrimp *Macrobrachium amazonicum*: loss of hypo-regulation in a land-

locked population indicates phylogenetic separation from estuarine ancestors. *Journal of Experimental Marine Biology and Ecology, 396*(2), 89-98.

Charmantier, G., Charmantier-Daures, M., & Young-Lai, W. W. (1985). *Lethal and sublethal effects of potash brine on different stages of the lobster, Homarus americanus*: Canadian technical report of fisheries and aquatic sciences No. 1344. Fisheries and Environmental Sciences, Fisheries Research Branch, Department of Fisheries and Oceans, Biological Station.

Cheung, S. G., Luk, K. C., & Shin, P. K. S. (2006). Predator-labeling effect on byssus production in marine mussels *Perna viridis* (L.) and *Brachidontes variabilis* (Krauss). *Journal of chemical ecology, 32*(7), 1501-1512.

Chipperfield, P. N. J. (1953). Observations on the breeding and settlement of *Mytilus edulis* (L.) in British waters. *Journal of the Marine Biological Association of the United Kingdom, 32*(02), 449-476.

Chong-Robles, J., Charmantier, G., Boulo, V., Lizárraga-Valdéz, J., Enríquez-Paredes, L. M., & Giffard-Mena, I. (2014). Osmoregulation pattern and salinity tolerance of the white shrimp *Litopenaeus vannamei* (Boone, 1931) during post-embryonic development. *Aquaculture, 422*, 261-267.

Christensen, H. T., Dolmer, P., Petersen, J. K., & Tørring, D. (2012). Comparative study of predatory responses in blue mussels (*Mytilus edulis* L.) produced in suspended long line cultures or collected from natural bottom mussel beds. *Helgoland Marine Research, 66*(1), 1-9.

Collins, A., Russell, B., Walls, A., & Hoang, T. (2005). Inland prawn farming studies into the potential for inland marine prawn farming in Queensland. *Queensland Department of Primary Industries and Fisheries.*

Costa, C. J., & Pritchard, A. W. (1978). The response of *Mytilus edulis* to short duration hypoosmotic stress. *Comparative Biochemistry and Physiology Part A: Physiology, 61*(1), 149-155.

Côté, I. M. (1995). Effects of predatory crab effluent on byssus production in mussels. *Journal of Experimental Marine Biology and Ecology, 188*(2), 233-241.

Côté, I. M., & Jelnikar, E. (1999). Predator-induced clumping behaviour in mussels (*Mytilus edulis* Linnaeus). *Journal of Experimental Marine Biology and Ecology, 235*(2), 201-211.

Dame, R. F. (2011). *Ecology of marine bivalves: an ecosystem approach*: CRC Press, London.

Dare, P. J., & Edwards, D. B. (1975). Seasonal changes in flesh weight and biochemical composition of mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales. *Journal of Experimental Marine Biology and Ecology, 18*(2), 89-97.

Dare, P. J., Edwards, D. B., & Davies, G. (1983). Experimental collection and handling of spat mussels (*Mytilus edulis* L.) on ropes for intertidal cultivation. *Issue 74 of Fisheries research technical report. Ministry of Agriculture Fisheries and Food, Directorate of Fisheries Research*, 23 p.

Davenport, J. (1979a). The isolation response of mussels (*Mytilus edulis* L.) exposed to falling sea-water concentrations. *Journal of the Marine Biological Association of the United Kingdom, 59*(01), 123-132.

Davenport, J. (1979b). Is *Mytilus edulis* a short term osmoregulator? *Comparative Biochemistry and Physiology Part A: Physiology, 64*(1), 91-95.

Davenport, J. (1981). The opening response of mussels (*Mytilus edulis*) exposed to rising sea-water concentrations. *Journal of the Marine Biological Association of the United Kingdom, 61*(03), 667-678.

De Zwaan, A., & Mathieu, M. (1992). Cellular energy metabolism in the Mytilidae: an overview. *The Mussel Mytilus: ecology, physiology, genetics and culture. Elsevier, Amsterdam*, 223-307.

Deaton, L. E. (1992). Osmoregulation and epithelial permeability in two euryhaline bivalve molluscs: *Mya arenaria* and *Geukensia demissa*. *Journal of Experimental Marine Biology and Ecology, 158*(2), 167-177.

Deaton, L. E. (2001). Hyperosmotic volume regulation in the gills of the ribbed mussel, *Geukensia demissa*: rapid accumulation of betaine and alanine. *Journal of Experimental Marine Biology and Ecology, 260*(2), 185-197.

Deaton, L. E. (2008). *Osmotic and ionic regulation in molluscs. In "Osmotic and ionic regulation: cells and animals" (Ed: Evans, D.H.)*: CRC Press.

Denny, M. W., & Gaylord, B. (2010). Marine ecomechanics. *Annual Review of Marine Science, 2*, 89-114.

Dickie, L. M., Boudreau, P. R., & Freeman, K. R. (1984). Influences of stock and site on growth and mortality in the blue mussel (*Mytilus edulis*). *Canadian Journal of Fisheries and Aquatic Sciences, 41*(1), 134-140.

Dinh, Q. H., & Fotedar, R. (2016). Early development of the blue mussel *Mytilus edulis* (Linnaeus, 1758) cultured in potassium-fortified inland saline water. *Aquaculture, 452*, 373-379. doi: doi:10.1016/j.aquaculture.2015.11.025

Dobretsov, S., & Wahl, M. (2008). Larval recruitment of the blue mussel *Mytilus edulis*: The effect of flow and algae. *Journal of Experimental Marine Biology and Ecology, 355*(2), 137-144.

Dobretsov, S. V. (1999). Effects of macroalgae and biofilm on settlement of blue mussel (*Mytilus edulis* L.) larvae. *Biofouling, 14*(2), 153-165.

Doroudi, M., Webster, G. K., Allan, G. L., & Fielder, D. S. (2007). Survival and Growth of Silver Perch, *Bidyanus bidyanus*, a Salt-tolerant Freshwater Species, in Inland Saline Groundwater from Southwestern New South Wales, Australia. *Journal of the World Aquaculture Society, 38*(2), 314-317.

Doroudi, M. S., Fielder, D. S., Allan, G. L., & Webster, G. K. (2006). Combined effects of salinity and potassium concentration on juvenile mulloway (*Argyrosomus japonicus*, Temminck and Schlegel) in inland saline groundwater. *Aquaculture Research, 37*(10), 1034-1039.

Doupé, R. G., Lymbery, A. J., & Starcevich, M. R. (2003). Rethinking the land: the development of inland saline aquaculture in Western Australia. *International Journal of Agricultural Sustainability, 1*(1), 30-37.

Doupe, R. G., Sarre, G. A., Partridge, G. J., Lymbery, A. J., & Jenkins, G. I. (2005). What are the prospects for black bream Acanthopagrus butcheri (Munro) aquaculture in salt-affected inland Australia? *Aquaculture Research, 36*(14), 1345-1355.

Dowd, W. W., & Somero, G. N. (2013). Behavior and survival of *Mytilus* congeners following episodes of elevated body temperature in air and seawater. *The Journal of experimental biology, 216*(3), 502-514.

Duchini, D., Boltovskoy, D., & Sylvester, F. (2015). Detachment, displacement and reattachment activity in a freshwater byssate mussel (*Limnoperna fortunei*): the effects of light, temperature and substratum orientation. *Biofouling, 31*(7), 599-611.

Dwivedi, J., & Trombetta, L. D. (2006). Acute toxicity and bioaccumulation of tributyltin in tissues of *Urolophus jamaicensis* (yellow stingray). *Journal of Toxicology and Environmental Health, Part A, 69*(14), 1311-1323.

Essink, K., & Bos, A. H. (1985). Growth of three bivalve molluscs transplanted along the axis of the Ems estuary. *Netherlands Journal of Sea Research, 19*(1), 45-51.

Evans, D. H., Piermarini, P. M., & Choe, K. P. (2005). The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological reviews, 85*(1), 97-177.

Eyster, L. S., & Pechenik, J. A. (1987). Attachment of *Mytilus edulis* L. larvae on algal and byssal filaments is enhanced by water agitation. *Journal of Experimental Marine Biology and Ecology, 114*(2), 99-110.

FAO. (2004-2015). *Cultured Aquatic Species Information Programme. Mytilus edulis. Cultured Aquatic Species Information Programme. The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department (online). Food and Agriculture Organization of the United Nations, Rome. Updated 1 January 2004.*: Aquaculture Council of Western Australia.

FAO. (2014). The State of World Fisheries and Aquaculture 2014. Rome. 223 pp.

Ferdowsian, R., Pannell, D. J., McCarron, C., Ryder, A., & Crossing, L. (2001). Explaining groundwater hydrographs: separating atypical rainfall events from time trends. *Soil Research, 39*(4), 861-876.

Fernández, A., Grienke, U., Soler-Vila, A., Guihéneuf, F., Stengel, D. B., & Tasdemir, D. (2015). Seasonal and geographical variations in the biochemical composition of the blue mussel (*Mytilus edulis* L.) from Ireland. *Food chemistry, 177*, 43-52.

Fielder, D. S., Bardsley, W. J., & Allan, G. L. (2001). Survival and growth of Australian snapper, *Pagrus auratus*, in saline groundwater from inland New South Wales, Australia. *Aquaculture, 201*(1), 73-90.

Fischer, H. (1986). Influence of temperature, salinity, and oxygen on the cadmium balance of mussels *Mytilus edulis*. *Marine Ecology Progress Series MESEDT, 32*(2/3), 265-278.

Fisher, C., Bodinier, C., Kuhl, A., & Green, C. (2013). Effects of potassium ion supplementation on survival and ion regulation in Gulf Killifish *Fundulus grandis* larvae reared in ion deficient saline waters. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 164*(4), 572-578.

Fitzpatrick, R. W., Baker, A. K. M., Raven, M., Rogers, S., Degens, B., George, R., & Kirby, J. (2005). *Mineralogy, biogeochemistry, hydro-pedology and risks of sediments, salt efflorescences and soils in open drains in the wheatbelt of Western Australia.* Paper presented at the Regolith.

Flood, M., Stobutzki, I., Andrews, J., Ashby, C., Begg, G., Fletcher, R., . . . Gray, C. (2012). Status of key Australian fish stocks reports 2012, 420 p.

Forsberg, J. A., Dorsett, P. W., & Neill, W. H. (1996). Survival and growth of red drum *Sciaenops ocellatus* in saline groundwaters of West Texas, USA. *Journal of the World Aquaculture Society, 27*(4), 462-474.

Fotedar, R., Harries, S., & Savage, S. (2008). Survival, growth and osmolality of greenlip abalone *Haliotis laevigata* (Donovan 1808) when exposed to different ionic profiles of inland saline water. *Aquaculture Research, 39*(5), 441-448.

Fourtanier, E., & Kociolek, J. P. (1999). Catalogue of the diatom genera. *Diatom Research, 14*(1), 1-190.

Gallardi, D., Hobbs, K., Mills, T., Couturier, C., Parrish, C. C., & Murray, H. M. (2014). Effects of extended ambient live holding on cultured blue mussels (*Mytilus edulis* L.) with reference to condition index, lipid profile, glycogen content and organoleptic testing. *Aquaculture, 430*, 149-158.

Gardner, J., & Thompson, R. J. (2001). The effects of coastal and estuarine conditions on the physiology and survivorship of the mussels *Mytilus edulis*, *M. trossulus* and their hybrids. *Journal of Experimental Marine Biology and Ecology, 265*(2), 119-140.

Garner, Y. L., & Litvaitis, M. K. (2013). Effects of injured conspecifics and predators on byssogenesis, attachment strength and movement in the blue mussel, *Mytilus edulis*. *Journal of Experimental Marine Biology and Ecology, 448*, 136-140.

Gazeau, F., Gattuso, J. P., Dawber, C., Pronker, A. E., Peene, F., Peene, J., . . . Middelburg, J. J. (2010). Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. *Biogeosciences, 7*(7), 2051-2060.

Genzano, G. N., Excoffon, A. C., Acuña, F. H., & Zamponi, M. O. (2003). Hydroid colonies as primary substrata for recruits of the mussel *Mytilus edulis platensis* front off Mar del Plata, Argentina. *Ophelia, 57*(2), 53-61.

George, R., K., R., Hill-T., J., & N., B. (2005). *Salinity investment framework: agricultural land and infrastructure. Resource management technical report 270*: Department of Agriculture, Government of Western Australia.

George, R. J., & Frantom, P. W. C. (1988). *Preliminary groundwater and salinity investigations in the eastern wheatbelt: 2. Merredin catchment*: Department of Agriculture and Food, Western Australia, Report 89.

Gérard, K., Bierne, N., Borsa, P., Chenuil, A., & Féral, J.-P. (2008). Pleistocene separation of mitochondrial lineages of *Mytilus* spp. mussels from Northern and Southern Hemispheres and strong genetic differentiation among southern populations. *Molecular Phylogenetics and Evolution, 49*(1), 84-91.

Ghassemi, F., Jakeman, A. J., & Nix, H. A. (1995). *Salinisation of land and water resources: human causes, extent, management and case studies*: CAB international.

Glaus, K. J. (1968). *Factors influencing production of byssus threads in Mytilus edulis.* Paper presented at the Biological Bulletin.

Gong, H., Jiang, D. H., Lightner, D. V., & Collins, C. (2003). Dietary modification for desert shrimp farming. *Global aquaculture advocate, 6*(4), 50-51.

Gong, H., Jiang, D. H., Lightner, D. V., Collins, C., & Brock, D. (2004). A dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. *Aquaculture Nutrition, 10*(4), 227-236.

Gooley, G., Ingram, B., & McKinnon, L. (1998). *Inland saline aquaculture-a Victorian perspective*.

Gosling, E. (1992). Systematics and geographic distribution of *Mytilus. The Mussel Mytilus: Ecology, physiology, genetic and culture. Development in Aquacultue and Fisheries Science, 25*.

Gosling, E. (2003). *Bivalve molluscs: biology, ecology and culture*: John Wiley & Sons.

Grizzle, R. E., Short, F. T., Newell, C. R., Hoven, H., & Kindblom, L. (1996). Hydrodynamically induced synchronous waving of seagrasses:'monami'and its possible effects on larval mussel settlement. *Journal of Experimental Marine Biology and Ecology, 206*(1), 165-177.

Hadfield, C. A., Clayton, L. A., Cohrs, D. K., & Murphy, D. S. (2012). Acute morbidity and mortality in invertebrates and fish following exposure to potassium-deficient saltwater. *Journal of fish diseases, 35*(7), 549-553.

Halse, S. A., Ruprecht, J. K., & Pinder, A. M. (2003). Salinisation and prospects for biodiversity in rivers and wetlands of south-west Western Australia. *Australian Journal of Botany, 51*(6), 673-688.

Hammer, U. T. (1986). *Saline lake ecosystems of the world* (Vol. 59): Dr W. Junk Publishers.

Hawkins, A. J. S., & Bayne, B. L. (1985). Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies and maintenance requirements. *Marine Ecology Progress Series, 25*, 181-188.

Heath, D. D., Rawson, P. D., & Hilbish, T. J. (1995). PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada. *Canadian Journal of Fisheries and Aquatic Sciences, 52*(12), 2621-2627.

Helrich, K. C. (1990). *Official methods of Analysis of the AOAC.*: Association of Official Analytical Chemists Inc. Arlington, Virginia, USA (1298 pp.).

Hickman, R. W. (1992). Mussel cultivation. *The mussel Mytilus: Ecology, physiology, genetics and culture. Elsevier, New York*, 465-510.

Hilbish, T. J., Mullinax, A., Dolven, S. I., Meyer, A., Koehn, R. K., & Rawson, P. D. (2000). Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): routes and timing of transequatorial migration. *Marine Biology, 136*(1), 69-77.

His, E., Seaman, M. N. L., & Beiras, R. (1997). A simplification the bivalve embryogenesis and larval development bioassay method for water quality assessment. *Water Research, 31*(2), 351-355.

Hiscock, I. D. (1953). Osmoregulation in Australian freshwater mussels (Lamellibranchiata). I. Water and chloride ion exchange in Hyridella australis (Lam.). *Marine and Freshwater Research, 4*(2), 317-329.

Hoare, K., Beaumont, A. R., & Davenport, J. (1995). Variation among populations in the resistance of *Mytilus edulis* embryos to copper: adaptation to pollution? *Marine ecology progress series. Oldendorf, 120*(1), 155-161.

Hummel, H., Amiard-Triquet, C., Bachelet, G., Desprez, M., Marchand, J., Sylvand, B., . . . Sinke, J. (1996). Free amino acids as a biochemical indicator of stress in the estuarine bivalve *Macoma balthica*. *Science of the total environment, 188*(2), 233-241.

Hummel, H., Bogaards, R. H., de Wolf, L., Sinke, J., & Poortvliet, T. (1994). Evaluation of free amino acids as a biochemical indicator of metal pollution. *Marine Environmental Research, 38*(4), 303-312.

Hunt, H. L., & Scheibling, R. E. (1996). Physical and biological factors influencing mussel (*Mytilus trossulus, M. edulis*) settlement on a wave-exposed rocky shore. *Marine ecology progress series. Oldendorf, 142*(1), 135-145.

Hunt, H. L., & Scheibling, R. E. (2001). Predicting wave dislodgment of mussels: variation in attachment strength with body size, habitat, and season. *Marine Ecology Progress Series, 213*, 157-164.

Hutchinson, W. (1998). *Inland saline aquaculture in South Australia*.

Hutchinson, W. G., & Flowers, T. J. (2008). *Research to foster investor attraction and establishment of commercial aquaculture parks aligned to major saline groundwater interception schemes in South Australia*: SARDI Aquatic Sciences Report.

Ingram, B. A., McKinnon, L. J., & Gooley, G. J. (2002). Growth and survival of selected aquatic animals in two saline groundwater evaporation basins: an Australian case study. *Aquaculture Research, 33*(6), 425-436.

Iwasaki, K. (2015). Behavior and Taxis of Young and Adult *Limnoperna fortunei Limnoperna Fortunei* (pp. 249-260): Springer.

Jain, A. K., Kumar, G., & Mukherjee, S. C. (2006). Survival and growth of early juveniles of barramundi, *Lates calcarifer* (Bloch, 1790) in inland saline groundwater. *Journal of Biological Research, 5*, 93-97.

Jain, A. K., Raju, K. D., Kumar, G., Ojha, P. K., & Reddy, A. K. (2007). Strategic manipulation of inland saline groundwater to produce *Macrobrachium rosenbergii* (De Man) postlarvae. *Journal of Biological Research, 8*, 151-157.

John, J. (2000). *A guide to diatoms as indicators of urban stream health*: Land and Water Resources Research and Development Corporation.

Johnson, S. L., & Wright, A. H. (2003). Mine void water resource issues in Western Australia. *Water and Rivers Commission, Perth, Australia*.

Karayücel, S., & Karayücel, İ. (1999). Growth and mortality of mussels (*Mytilus edulis* L.) reared in Lantern Nets in Loch Kishorn, Scotland. *Turkish Journal of Veterinary and Animal Sciences, 23*(5), 397-402.

Kay, W. R., Halse, S. A., Scanlon, M. D., & Smith, M. J. (2001). Distribution and environmental tolerances of aquatic macroinvertebrate families in the agricultural zone of southwestern Australia. *Journal of the North American Benthological Society, 20*(2), 182-199.

Keighery, G. (2000). Wheatbelt wonders under threat. *Landscope, summer, 2001*.

King, P. A., McGrath, D., & Gosling, E. M. (1989). Reproduction and settlement of *Mytilus edulis* on an exposed rocky shore in Galway Bay, west coast of Ireland. *Journal of the Marine Biological Association of the United Kingdom, 69*(02), 355-365.

Kishore, P., Hunter, J. R., Zeng, C., & Southgate, P. C. (2014). The effects of different culture apparatuses and current velocities on byssus production by the black-lip pearl oyster, *Pinctada margaritifera*. *Aquaculture, 434*, 74-77.

Kolkovski, S. (2010). *An overview on desert aquaculture in Australia*. Paper presented at the Aquaculture in desert and arid lands: development constraints and opportunities. FAO Technical Workshop.

Kolkovski, S., Curnow, J., & King, J. A. (2010). commercialization of marine fish larvae feeds–Microdiets.

Kube, S., Gerber, A., Jansen, J. M., & Schiedek, D. (2006). Patterns of organic osmolytes in two marine bivalves, *Macoma balthica*, and *Mytilus* spp., along their European distribution. *Marine Biology, 149*(6), 1387-1396.

Kube, S., Sokolowski, A., Jansen, J. M., & Schiedek, D. (2007). Seasonal variability of free amino acids in two marine bivalves, *Macoma balthica* and *Mytilus* spp., in relation to

environmental and physiological factors. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147*(4), 1015-1027.

Kumar, R. N., McCullough, C. D., & Lund, M. A. (2009). Water resources in Australian mine pit lakes. *Mining Technology, 118*(3-4), 205-211.

Kumar, V., Fotedar, R., & Dods, K. (2010). Effect of inland saline water ionic profiles on growth, chemical composition and agar characteristics of *Gracilaria cliftonii* (Withell, Miller and Kraft 1994) under laboratory conditions. *Aquaculture International, 18*(5), 869-881.

Kurihara, H. (2008). Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates.

Lachance, A. A., Myrand, B., Tremblay, R., Koutitonsky, V., & Carrington, E. (2008). Biotic and abiotic factors influencing attachment strength of blue mussels *Mytilus edulis* in suspended culture. *Aquatic Biology, 2*(2), 119-129.

Ladoukakis, E. D., Saavedra, C., Magoulas, A., & Zouros, E. (2002). Mitochondrial DNA variation in a species with two mitochondrial genomes: the case of *Mytilus galloprovincialis* from the Atlantic, the Mediterranean and the Black Sea. *Molecular Ecology, 11*(4), 755-769.

Lane, D. J. W., Beaumont, A. R., & Hunter, J. R. (1985). Byssus drifting and the drifting threads of the young post-larval mussel *Mytilus edulis*. *Marine Biology, 84*(3), 301-308.

Lange-Bertalot, H., Cavancini, P., Tagliaventi, N., & Alfinito, S. (2003). Diatoms of Sardinia. Rare and 76 new species in rock pools and other ephemeral waters.– Iconographia Diatomologica 12. *Gantner, Ruggell.*

Lange, R., & Mostad, A. (1967). Cell volume regulation in osmotically adjusting marine animals. *Journal of Experimental Marine Biology and Ecology, 1*(2), 209-219.

Lee, C. L. (1997). *Design and operation of a land-based closed recirculating hatchery system for the topshell, Trochus niloticus, using treated bore water.* Paper presented at the Trochus: status, hatchery practice and nutrition: proceedings of a workshop held at Northern Territory University, 6-7 June 1996.

Lee, C. L. (1998). *Potential of inland saline water for aquaculture of molluscs.* Paper presented at the ACIAR PROCEEDINGS.

Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.-O., Olrog, L., . . . Syversen, U. (2005). Improving marine water quality by mussel farming: a profitable solution for Swedish society. *AMBIO: A Journal of the Human Environment, 34*(2), 131-138.

Lipton, P., & Heimbach, C. J. (1977). The effect of extracellular potassium concentration on protein synthesis in guinea-pig hippocampal slices[1,2]. *Journal of neurochemistry, 28*(6), 1347-1354.

Liu, H., Tan, B., Yang, J., Lin, Y., Chi, S., Dong, X., & Yang, Q. (2014). Effect of various Na/K ratios in low-salinity well water on growth performance and physiological response of Pacific white shrimp *Litopenaeus vannamei*. *Chinese Journal of Oceanology and Limnology, 32*, 991-999.

Liu, J. M. (2001). Experiment on raising the survival of Chinese shrimp *Fenneropenaeus chinensis* in saline groundwater. *Shandong Fisheries, 18*(1), 13-14.

Livingstone, D. R. (1985). Biochemical measurements *The effects of stress and pollution on marine animals* (pp. 81-132): Praeger Publishers New York.

Livingstone, D. R., Widdows, J., & Fieth, P. (1979). Aspects of nitrogen metabolism of the common mussel *Mytilus edulis*: adaptation to abrupt and fluctuating changes in salinity. *Marine Biology, 53*(1), 41-55.

Love, G., & Langenkamp, D. (2003). Australian aquaculture-industry profiles for selected species. *ABARE EReport*(03.8).

Lowe, D. M., Moore, M. N., & Bayne, B. L. (1982). Aspects of gametogenesis in the marine mussel *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom, 62*(01), 133-145.

Lowen, J. B., Innes, D. J., & Thompson, R. J. (2013). Predator-induced defenses differ between sympatric *Mytilus edulis* and *M. trossulus*. *Marine ecology. Progress series, 475*, 135-143.

Lubin, M., & Ennis, H. L. (1964). On the role of intracellular potassium in protein synthesis. *Biochimica et Biophysica Acta (BBA)-Specialized Section on Nucleic Acids and Related Subjects, 80*(4), 614-631.

Lutz, R. A., Chalermwat, K., Figueras, A. J., Gustafson, R. G., & Newell, C. (1991). Mussel aquaculture in marine and estuarine environments throughout the world. *W. Menzel. Culture of estuarine and marine bivalve mollusks in temperate and tropical regions. CRC Press, Boca Raton, Florida*, 57-97.

Lutz, R. A., Incze, L. S., Porter, B., & Stotz, J. K. (1980). Seasonal variation in the condition raft-cultured mussels (*Mytilus edulis* L.). *Proceedings of the World Mariculture Society, 11*, 262-268.

Maligaiah, R. M., & Solai, S. (2015). Morphometry, length-weight relationships and condition index of *Parreysia favidens* (Benson, 1862)(Bivalvia: Unionidae) from river Seeta in the Western Ghats, India. *Indian Journal of Fisheries, 62*(1), 18-24.

Mallet, A. L., Carver, C. E. A., Coffen, S. S., & Freeman, K. R. (1987). Winter growth of the blue mussel *Mytilus edulis* L.: importance of stock and site. *Journal of Experimental Marine Biology and Ecology, 108*(3), 217-228.

Mantel, L. H., & Farmer, L. L. (1983). Osmotic and ionic regulation. *The biology of Crustacea, 5*, 53-161.

Martella, T. (1974). Some factors influencing byssus thread production in *Mytilus edulis* (mollusca: Bivalvia) Linnaeus, 1758. *Water, Air, and Soil Pollution, 3*(2), 171-177.

Martínez-Lage, A., Rodríguez, F., González-Tizón, A., Prats, E., Cornudella, L., & Méndez, J. (2002). Comparative analysis of different satellite DNAs in four *Mytilus* species. *Genome, 45*(5), 922-929.

Mateeva, T. A. (1948). The biology of *Mytilus edulis* L. in eastern Murman. *Trudy murmansk, biol. Inst, 1*, 215-241.

McDonald, J. H., Seed, R., & Koehn, R. K. (1991). Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. *Marine Biology, 111*(3), 323-333.

McGrath, D., King, P. A., & Gosling, E. M. (1988). Evidence for the direct settlement of *Mytilus edulis* larvae on adult mussel beds. *Marine ecology progress series. Oldendorf, 47*(1), 103-106.

McKinnon, L. J., Ingram, B. A., & Gooley, G. J. (1998). Fish production from salt affected land: profit potential from a persistent problem. *Trees and Natural Resources, 40*(2), 29.

McNevin, A. A., Boyd, C. E., Silapajarn, O., & Silapajarn, K. (2004). Ionic supplementation of pond waters for inland culture of marine shrimp. *Journal of the World Aquaculture Society, 35*(4), 460-467.

Moeser, G. M., & Carrington, E. (2006). Seasonal variation in mussel byssal thread mechanics. *Journal of Experimental Biology, 209*(10), 1996-2003.

Moeser, G. M., Leba, H., & Carrington, E. (2006). Seasonal influence of wave action on thread production in *Mytilus edulis*. *Journal of Experimental Biology, 209*(5), 881-890.

Myrand, B., Guderley, H., & Himmelman, J. H. (2000). Reproduction and summer mortality of blue mussels *Mytilus edulis* in the Magdalen Islands, southern Gulf of St. Lawrence. *Marine ecology. Progress series, 197*, 193-207.

NLWRA. (2000). *Dryland Salinity in Australia – Key Findings, Fast Facts 21*: Department of Treasury and Finance.

Nott, R., Pridham, M., Roe, J., Ibbott, J., & Leeson, A. (2004). Merredin townsite groundwater pumping and desalination pilot project.

Nulsen, R. (1997). *Inland saline waters in Australia.* Paper presented at the Inland saline aquaculture. Proceedings of a workshop held in Perth, Western Australia.

Ogburn, D. M. (1998). *Environmental considerations in the use and management of inland saline water bodies for aquaculture.* Paper presented at the ACIAR PROCEEDINGS.

Okumuş, I., & Stirling, H. P. (1998). Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. *Aquaculture, 159*(3), 249-261.

Ompi, M. (2011). Settlement behaviour and size of mussel larvae from the family Mytilidae (*Brachidontes erosus* (Lamarck, 1819), *Brachidontes rostratus* (Dunker, 1857), *Trichomya hirsutus* (Lamarck, 1819), and *Mytilus galloprovincialis* Lamarck, 1819. *Journal of Coastal Development, 13*(3), 215-227.

Paine, R. T., & Levin, S. A. (1981). Intertidal landscapes: disturbance and the dynamics of pattern. *Ecological monographs, 51*(2), 145-178.

Panikkar, N. K. (1968). Osmotic behaviour of shrimps and prawns in relation to their biology and culture. *FAO Fisheries Report, 2*(57), 527-538.

Park, K. Y., & Oh, C. W. (2002). Length-weight relationships of bivalves from coastal waters of Korea. *Naga, The ICLARM Quarterly, 25*(1), 21-22.

Partridge, G. J. (2008). *Inland saline aquaculture: overcoming biological and technical constraints towards the development of an industry.* Murdoch University.

Partridge, G. J., & Creeper, J. (2004). Skeletal myopathy in juvenile barramundi, *Lates calcarifer* (Bloch), cultured in potassium-deficient saline groundwater. *Journal of fish diseases, 27*(9), 523-530.

Partridge, G. J., Lymbery, A. J., & Bourke, D. K. (2008). Larval rearing of barramundi (*Lates calcarifer*) in saline groundwater. *Aquaculture, 278*(1), 171-174.

Partridge, G. J., Lymbery, A. J., & George, R. J. (2008). Finfish mariculture in inland Australia: a review of potential water sources, species, and production systems. *Journal of the World Aquaculture Society, 39*(3), 291-310.

Partridge, G. J., Sarre, G. A., Ginbey, B. M., Kay, G. D., & Jenkins, G. I. (2006). Finfish production in a static, inland saline water body using a Semi-Intensive Floating Tank System (SIFTS). *Aquacultural engineering, 35*(2), 109-121.

Pechenik, J. A., Eyster, L. S., Widdows, J., & Bayne, B. L. (1990). The influence of food concentration and temperature on growth and morphological differentiation of blue mussel *Mytilus edulis* L. larvae. *Journal of Experimental Marine Biology and Ecology, 136*(1), 47-64.

Pequeux, A. (1995). Osmotic regulation in crustaceans. *Journal of Crustacean Biology*, 1-60.

Pernet, F., Tremblay, R., & Bourget, E. (2003). Settlement success, spatial pattern and behavior of mussel larvae Mytilus spp. in experimental downwelling' systems of varying velocity and turbulence. *Marine Ecology Progress Series, 260*, 125-140.

Petrone, L., Kumar, A., Sutanto, C. N., Patil, N. J., Kannan, S., Palaniappan, A., . . . Miserez, A. (2015). Mussel adhesion is dictated by time-regulated secretion and molecular conformation of mussel adhesive proteins. *Nature communications, 6*.

Pham, D., Charmantier, G., Wabete, N., Boulo, V., Broutoi, F., Mailliez, J.-R., . . . Charmantier-Daures, M. (2012). Salinity tolerance, ontogeny of osmoregulation and zootechnical improvement in the larval rearing of the Caledonian Blue Shrimp, *Litopenaeus stylirostris* (Decapoda, Penaeidae). *Aquaculture, 362*, 10-17.

Piedrahita, R. H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture, 226*(1), 35-44.

Pierce, S. K. (1971). A source of solute for volume regulation in marine mussels. *Comparative Biochemistry and Physiology Part A: Physiology, 38*(3), 619-635.

Pieters, H., Kluytmans, J. H., Zandee, D. I., & Cadee, G. C. (1980). Tissue composition and reproduction of *Mytilus edulis* in relation to food availability. *Netherlands Journal of Sea Research, 14*(3), 349-361.

Pieters, H., Kluytmans, J. H., Zurburg, W., & Zandee, D. I. (1979). The influence of seasonal changes on energy metabolism in *Mytilus edulis* (L.). I. Growth rate and biochemical composition in relation to environmental parameters and spawning *Cyclic phenomena in marine plants and animals* (pp. 285-292): Pergamon Press Oxford.

Pitman, M. G., & Läuchli, A. (2002). Global impact of salinity and agricultural ecosystems *Salinity: environment-plants-molecules* (pp. 3-20): Springer.

Pleissner, D., Eriksen, N. T., Lundgreen, K., & Riisgård, H. U. (2012). Biomass composition of blue mussels, *Mytilus edulis*, is affected by living site and species of ingested microalgae. *International Scholarly Research Notices, 2012*, 1-12.

Prangnell, D. I. (2007). *Physiological responses of western king prawns, Penaeus latisulcatus, in inland saline water with different potassium concentrations.* (PhD thesis), Ph.D. thesis, Curtin University of Technology.

Prangnell, D. I., & Fotedar, R. (2005). The effect of potassium concentration in inland saline water on the growth and survival of the western king shrimp, *Penaeus latisulcatus* Kishinouye, 1896. *Journal of Applied Aquaculture, 17*(2), 19-34.

Prangnell, D. I., & Fotedar, R. (2006a). Effect of sudden salinity change on *Penaeus latisulcatus* Kishinouye osmoregulation, ionoregulation and condition in inland saline water and potassium-fortified inland saline water. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 145*(4), 449-457.

Prangnell, D. I., & Fotedar, R. (2006b). The growth and survival of western king prawns, *Penaeus latisulcatus* Kishinouye, in potassium-fortified inland saline water. *Aquaculture, 259*(1), 234-242.

Prangnell, D. I., & Fotedar, R. (2009). Effect of sudden change in potassium concentration on *Penaeus latisulcatus* Kishinouye survival, osmolality and health in inland saline water cultures. *Hydrobiologia, 626*(1), 145-153.

Price, H. A. (1980). Seasonal variation in the strength of byssal attachment of the common mussel *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom, 60*(04), 1035-1037.

Price, H. A. (1981). Byssus thread strength in the mussel, *Mytilus edulis*. *Journal of Zoology, 194*(2), 245-255.

Price, H. A. (1982). An analysis of factors determining seasonal variation in the byssal attachment strength of *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom, 62*(01), 147-155.

Pronker, A. E., Nevejan, N. M., Peene, F., Geijsen, P., & Sorgeloos, P. (2008). Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus 1758). Part I. Impact of different micro-algae mixtures on broodstock performance. *Aquaculture International, 16*(4), 297-307.

Pulfrich, A. (1996). Attachment and settlement of post-larval mussels (*Mytilus edulis* L.) in the Schleswig-Holstein Wadden Sea. *Journal of Sea Research, 36*(3), 239-250.

Rahman, S. U., Jain, A. K., Reddy, A. K., Kumar, G., & Raju, K. D. (2005). Ionic manipulation of inland saline groundwater for enhancing survival and growth of *Penaeus monodon* (Fabricius). *Aquaculture Research, 36*(12), 1149-1156.

Rainbow, P. S., & Black, W. H. (2001). Effects of changes in salinity on the apparent water permeability of three crab species: *Carcinus maenas*, *Eriocheir sinensis* and *Necora puber*. *Journal of Experimental Marine Biology and Ecology, 264*(1), 1-13.

Rajagopal, S., Van der Velde, G., Jenner, H. A., Van der Gaag, M., & Kempers, A. J. (1996). Effects of temperature, salinity and agitation on byssus thread formation of zebra mussel *Dreissena polymorpha*. *Netherland Journal of Aquatic Ecology, 30*(2-3), 187-195.

Rawson, P. D., & Hilbish, T. J. (1995). Distribution of male and female mtDNA lineages in populations of blue mussels, *Mytilus trossulus* and *M. galloprovincialis*, along the Pacific coast of North America. *Marine Biology, 124*(2), 245-250.

Riisgård, H. U., Line, B., & Pleissner, D. (2012). Effect of salinity on growth of mussels, *Mytilus edulis*, with special reference to Great Belt (Denmark). *Open Journal of Marine Science, 2*(04), 167-176.

Roberts, D. (1975). The effect of pesticides on byssus formation in the common mussel, *Mytilus edulis*. *Environmental Pollution (1970), 8*(4), 241-254.

Rodhouse, P. G., Roden, C. M., Burnell, G. M., Hensey, M. P., McMahon, T., Ottway, B., & Ryan, T. H. (1984). Food resource, gametogenesis and growth of *Mytilus edulis* on the shore and in suspended culture: Killary Harbour, Ireland. *Journal of the Marine Biological Association of the United Kingdom, 64*(03), 513-529.

Rodriguez, S. R., Ojeda, F. P., & Inestrosa, N. C. (1993). Settlement of benthic marine invertebrates. *Marine ecology progress series. Oldendorf, 97*(2), 193-207.

Romano, N., & Zeng, C. (2012). Osmoregulation in decapod crustaceans: implications to aquaculture productivity, methods for potential improvement and interactions with elevated ammonia exposure. *Aquaculture, 334*, 12-23.

Roy, L. A., & Davis, D. A. (2010). *Requirements for the culture of the Pacific white shrimp, Litopenaeus vannamei, reared in low salinity waters: water modification and nutritional strategies for improving production.* Paper presented at the Avances en Nutrición Acuicola X-Memorias del X Simposio Internacional de Nutrición Acuicola.

Roy, L. A., Davis, D. A., Saoud, I. P., Boyd, C. A., Pine, H. J., & Boyd, C. E. (2010). Shrimp culture in inland low salinity waters. *Reviews in Aquaculture, 2*(4), 191-208.

Roy, L. A., Davis, D. A., & Whitis, G. N. (2009). Pond-to-pond variability in post-larval shrimp, *Litopenaeus vannamei*, survival and growth in inland low-salinity waters of west Alabama. *Aquaculture Research, 40*(16), 1823-1829.

Sagert, J., & Waite, J. H. (2009). Hyperunstable matrix proteins in the byssus of *Mytilus galloprovincialis*. *Journal of Experimental Biology, 212*(14), 2224-2236.

Salama, R. B., Farrington, P., Bartle, G. A., & Watson, G. D. (1993a). The chemical evolution of groundwater in a first-order catchment and the process of salt accumulation in the soil profile. *Journal of Hydrology, 143*(3), 233-258.

Salama, R. B., Farrington, P., Bartle, G. A., & Watson, G. D. (1993b). Salinity trends in the wheatbelt of Western Australia: results of water and salt balance studies from Cuballing catchment. *Journal of Hydrology, 145*(1), 41-63.

Samocha, T. M., Hamper, L., Emberson, C. R., Davis, A. D., McIntosh, D., Lawrence, A. L., & Van Wyk, P. M. (2002). Review of some recent developments in sustainable shrimp farming practices in Texas, Arizona, and Florida. *Journal of Applied Aquaculture, 12*(1), 1-42.

Sánchez-Lazo, C., & Martínez-Pita, I. (2012). Induction of settlement in larvae of the mussel *Mytilus galloprovincialis* using neuroactive compounds. *Aquaculture, 344*, 210-215.

Sang, H. M., & Fotedar, R. (2004). Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) reared at different salinities. *Aquaculture, 234*(1), 601-614.

Sang, H. M., & Fotedar, R. (2005). The effects of air exposure on hemolymph osmoregulatory capacity of brown tiger shrimp, *Penaeus esculentus*, and western king shrimp, *Penaeus latisulcatus*, reared at different salinities. *Journal of Applied Aquaculture, 16*(3-4), 79-94.

Saoud, I. P., Kreydiyyeh, S., Chalfoun, A., & Fakih, M. (2007). Influence of salinity on survival, growth, plasma osmolality and gill Na+–K+–ATPase activity in the rabbitfish *Siganus rivulatus*. *Journal of Experimental Marine Biology and Ecology, 348*(1), 183-190.

Saoud, I. P., Roy, L. A., & Davis, D. A. (2007). Chelated potassium and arginine supplementation in diets of Pacific white shrimp reared in low-salinity waters of west Alabama. *North American journal of aquaculture, 69*(3), 265-270.

Saranchova, O. L., & Flyachinskaya, L. P. (2001). The influence of salinity on early ontogeny of the mussel *Mytilus edulis* and the starfish *Asterias rubens* from the White Sea. *Russian Journal of Marine Biology, 27*(2), 87-93.

Satuito, C. G., Natoyama, K., Yamazaki, M., & Fusetani, N. (1995). Induction of attachment and metamorphosis of laboratory cultured mussel *Mytilus edulis galloprovincialis* larvae by microbial film. *Fisheries science, 61*(2), 223-227.

Schneider, K. R., Wethey, D. S., Helmuth, B. S. T., & Hilbish, T. J. (2005). Implications of movement behavior on mussel dislodgement: exogenous selection in a *Mytilus* spp. hybrid zone. *Marine Biology, 146*(2), 333-343.

Seed, R. (1969). The Ecology of *Mytilus edulis* L. (Lamellibranchiata) on Exposed Rocky Shores. I. Breeding and Settlement. *Oecologia, 3*(3-4), 277-316.

Seed, R. (1992). Systematics evolution and distribution of mussels belonging to the genus Mytilus: an overview. *American Malacological Bulletin, 9*(2), 123-137.

Seed, R. T. H. S., & Suchanek, T. H. (1992). Population and community ecology of Mytilus. *Developments in aquaculture and fisheries science, 25*, 87-169.

Selin, N. I., & Vekhova, E. E. (2004). Effects of environmental factors on byssal thread formation in some members of the family Mytilidae from the Sea of Japan. *Russian Journal of Marine Biology, 30*(5), 306-313.

Shiau, S., & Hsieh, J. (2001). Dietary potassium requirement of juvenile grass shrimp *Penaeus monodon*. *Fisheries science, 67*(4), 592-595.

Shumway, S. E., Pierce, F. C., & Knowlton, K. (1987). The effect of Protogonyaulax tamarensis on byssus production in *Mytilus edulis* L., *Modiolus modiolus* Linnaeus, 1758 and *Geukensia demissa* Dillwyn. *Comparative Biochemistry and Physiology Part A: Physiology, 87*(4), 1021-1023.

Silva, A. L., & Wright, S. H. (1994). Short-term cell volume regulation in *Mytilus californianus* gill. *The Journal of experimental biology, 194*(1), 47-68.

Smaal, A. C. (2002). European mussel cultivation along the Atlantic coast: production status, problems and perspectives. *Hydrobiologia, 484*(1-3), 89-98.

Śmietanka, B., Burzyński, A., & Wenne, R. (2009). Molecular population genetics of male and female mitochondrial genomes in European mussels *Mytilus*. *Marine Biology, 156*(5), 913-925.

Śmietanka, B., Zbawicka, M., Wołowicz, M., & Wenne, R. (2004). Mitochondrial DNA lineages in the European populations of mussels (*Mytilus* spp.). *Marine Biology, 146*(1), 79-92.

Smith, B., & Barlow, C. (1999). *Inland saline aquaculture. Proceedings of a workshop held in Perth, Western Australia, 6-7 August 1997*. Paper presented at the Inland saline aquaculture. Proceedings of a workshop held in Perth, Western Australia, 6-7 August 1997.

Sokolowski, A., Wolowicz, M., & Hummel, H. (2003). Free amino acids in the clam *Macoma balthica* L. (Bivalvia, Mollusca) from brackish waters of the southern Baltic

Sea. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 134*(3), 579-592.

Sprung, M. (1983). Reproduction and fecundity of the mussel *Mytilus edulis* at Helgoland (North Sea). *Helgoländer Meeresuntersuchungen, 36*(3), 243-255.

Sprung, M. (1984). Physiological energetics of mussel larvae (*Mytilus edulis*). I. Shell growth and biomass. *Marine Ecology Progress Series*.

Stephan, M., & Hobsbawn, P. (2014). Australian fisheries and aquaculture statistics 2013, Fisheries Research and Development Corporation project 2010/208. ABARES, Canberra, November. CC BY 3.0.

Sukhotin, A. A., Strelkov, P. P., Maximovich, N. V., & Hummel, H. (2007). Growth and longevity of *Mytilus edulis* (L.) from northeast Europe. *Marine Biology Research, 3*(3), 155-167.

Svane, I., & Ompi, M. (1993). Patch dynamics in beds of the blue mussel *Mytilus edulis* L.: effects of site, patch size, and position within a patch. *Ophelia, 37*(3), 187-202.

Svensson, S., & Förlin, L. (2004). Analysis of the importance of lipid breakdown for elimination of okadaic acid (diarrhetic shellfish toxin) in mussels, *Mytilus edulis*: results from a field study and a laboratory experiment. *Aquatic Toxicology, 66*(4), 405-418.

Swivedi, S. N., & Lingaraju, G. M. (1986). Strategy of prawn and fish culture in saline sub-soil waters of semiarid zone in Haryana. *Mahasagar, 19*(2), 97.

Tamarin, A., Lewis, P., & Askey, J. (1976). The structure and formation of the byssus attachment plaque in *Mytilus*. *Journal of morphology, 149*(2), 199-221.

Tantulo, U. (2007). *Physiological responses of black tiger prawn (Penaeus monodon Fabricius, 1798) reared in inland saline water*: Curtin University of Technology.

Tantulo, U., & Fotedar, R. (2006). Comparison of growth, osmoregulatory capacity, ionic regulation and organosomatic indices of black tiger prawn (*Penaeus monodon* Fabricius, 1798) juveniles reared in potassium fortified inland saline water and ocean water at different salinities. *Aquaculture, 258*(1), 594-605.

Tantulo, U., & Fotedar, R. (2007). Osmo and ionic regulation of black tiger prawn (*Penaeus monodon* Fabricius 1798) juveniles exposed to K+ deficient inland saline water at different salinities. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 146*(2), 208-214.

Taukulis, F. E., & John, J. (2009). Development of a diatom-based transfer function for lakes and streams severely impacted by secondary salinity in the south-west region of Western Australia. *Hydrobiologia, 626*(1), 129-143.

Thieltges, D. W., & Buschbaum, C. (2007). Mechanism of an epibiont burden: Crepidula fornicata increases byssus thread production by *Mytilus edulis*. *Journal of Molluscan Studies, 73*(1), 75-77.

Thomas, R. P., Morini, S., CISAU., IPTRID., AGLL., & FAO. (2005). Management of irrigation-induced salt-affected soils. 4 p.

Thompson, R. J. (1979). Fecundity and reproductive effort in the blue mussel (*Mytilus edulis*), the sea urchin (*Strongylocentrotus droebachiensis*), and the snow crab (*Chionoecetes opilio*) from populations in Nova Scotia and Newfoundland. *Journal of the Fisheries Board of Canada, 36*(8), 955-964.

Thorarinsdóttir, G. G., & Gunnarsson, K. (2003). Reproductive cycles of *Mytilus edulis* L. on the west and east coasts of Iceland. *Polar Research, 22*(2), 217-223.

Timms, B. V. (2005). Salt lakes in Australia: present problems and prognosis for the future. *Hydrobiologia, 552*(1), 1-15.

Timms, B. V. (2009). Study of the saline lakes of the Esperance Hinterland, Western Australia, with special reference to the roles of acidity and episodicity. *Natural Resources and Environmental Issues, 15*(1), 44.

Toro, J. E., Thompson, R. J., & Innes, D. J. (2002). Reproductive isolation and reproductive output in two sympatric mussel species (*Mytilus edulis, M. trossulus*) and their hybrids from Newfoundland. *Marine Biology, 141*(5), 897-909.

Trendall, J., Aldert, J., & Lymberyt, A. (1997). A national environmental management policy for land-based fish farming. *Inland Saline Aquaculture*, 30.

Tsuchiya, M. (1983). Mass mortality in a population of the mussel *Mytilus edulis* L. caused by high temperature on rocky shores. *Journal of Experimental Marine Biology and Ecology, 66*(2), 101-111.

Van der Hiele, T., Rijstenbil, J. W., Creemers, J., & Heringa, J. (2014). Composition, Treatment and Use of Saline Groundwater for Aquaculture in the Netherlands. *WORLD AQUACULTURE*, 23.

Verity, M. A., Brown, W. J., & Cheung, M. (1980). Isolation of ribosome containing synaptosome subpopulation with active in vitro protein synthesis. *Journal of neuroscience research, 5*(2), 143-153.

Waite, J. H. (1992). The formation of mussel byssus: anatomy of a natural manufacturing process *Structure, cellular synthesis and assembly of biopolymers* (pp. 27-54): Springer.

Waite, J. H., Qin, X.-X., & Coyne, K. J. (1998). The peculiar collagens of mussel byssus. *Matrix Biology, 17*(2), 93-106.

Wassnig, M., & Southgate, P. C. (2012). Effects of settlement cues on behaviour and substrate attachment of hatchery reared winged pearl oyster (*Pteria penguin*) larvae. *Aquaculture, 344*, 216-222.

Wedege, E., Luqmani, Y., & Bradford, H. F. (1977). Stimulated incorporation of amino acids into proteins of synaptosomal fractions induced by depolarizing treatments. *Journal of neurochemistry, 29*(3), 527-537.

Weiler, I. J., & Greenough, W. T. (1991). Potassium ion stimulation triggers protein translation in synaptoneurosomal polyribosomes. *Molecular and Cellular Neuroscience, 2*(4), 305-314.

Wiegemann, M. (2005). Adhesion in blue mussels (*Mytilus edulis*) and barnacles (genus Balanus): mechanisms and technical applications. *Aquatic Sciences-Research Across Boundaries, 67*(2), 166-176.

Wild, A. (2003). *Soils, land and food: managing the land during the twenty-first century*: Cambridge University Press.

Willmer, P. (2006). Osmoregulation in Invertebrates. *ENCYCLOPEDIA OF LIFE SCIENCES. John Wiley & Sons, Ltd. www.els.net, 11p*.

Winkle, J. W. V. (1970). Effect of environmental factors on byssal thread formation. *Marine Biology, 7*(2), 143-148.

Witkowski, A. (2000). Diatom flora of marine coasts I. Iconographia Diatomologica, Annoted Diatom Micrographs, Lange-Bertalot, H: ARG Gantner Verlag KG Chapitre III Variabilité hydro-climatique de la lagune de Padre.

Witman, J. D. (1987). Subtidal coexistence: storms, grazing, mutualism, and the zonation of kelps and mussels. *Ecological monographs, 57*(2), 167-187.

Witman, J. D., & Suchanek, T. H. (1984). Mussels in flow: drag and dislodgement by epizoans. *Marine Ecology Progress Series, 16*, 259-268.

Wonham, M. J. (2004). Mini-review: distribution of the Mediterranean mussel, *Mytilus galloprovincialis* (Bivalvia: Mytilidae), and hybrids in the northeast Pacific. *Journal of Shellfish Research, 23*(2), 535-544.

Wyatt, J., Kenny, S., Hobbs, K. D., Mills, T., Marshall, H. D., & Murray, H. M. (2013). The effect of extended wet-storage on the condition, physiology and stress response of cultured blue mussels (*Mytilus edulis* L. 1758) during summer and fall in northeastern Newfoundland. *Aquaculture, 372*, 111-118.

Yang, J., Glenn Satuito, C., Bao, W., & Kitamura, H. (2008). Induction of metamorphosis of pediveliger larvae of the mussel *Mytilus galloprovincialis* Lamarck, 1819 using neuroactive compounds, KCl, NH4Cl and organic solvents. *Biofouling, 24*(6), 461-470.

Yang, J., Li, Y., Bao, W., Satuito, C. G., & Kitamura, H. (2011). Larval metamorphosis of the mussel *Mytilus galloprovincialis* Lamarck, 1819 in response to neurotransmitter blockers and tetraethylammonium. *Biofouling, 27*(2), 193-199.

Yaroslavtseva, L. M., & Sergeeva, E. P. (2006). Adaptivity of the bivalve *Mytilus trossulus* larvae to short-and long-term changes in water temperature and salinity. *Russian Journal of Marine Biology, 32*(2), 82-87.

Yonge, C. M. (1962). On the primitive significance of the byssus in the Bivalvia and its effects in evolution. *Journal of the Marine Biological Association of the United Kingdom, 42*(01), 113-125.

Yool, A. J., Grau, S. M., Hadfield, M. G., Jensen, R. A., Markell, D. A., & Morse, D. E. (1986). Excess potassium induces larval metamorphosis in four marine invertebrate species. *The Biological Bulletin, 170*(2), 255-266.

Young, G. (1985). Byssus-thread formation by the mussel *Mytilus edulis*: effects of environmental factors. *Marine ecology progress series. Oldendorf, 24*(3), 261-271.

Young, T., Alfaro, A. C., & Robertson, J. (2011). Effect of neuroactive compounds on the settlement of mussel (*Perna canaliculus*) larvae. *Aquaculture, 319*(1), 277-283.

Yu, J., Wei, W., Danner, E., Ashley, R. K., Israelachvili, J. N., & Waite, J. H. (2011). Mussel protein adhesion depends on interprotein thiol-mediated redox modulation. *Nature chemical biology, 7*(9), 588-590.

Yu, X., He, W., Gu, J., He, M., & Yan, Y. (2008). The effect of chemical cues on settlement of pearl oyster *Pinctada fucata martensii* (Dunker) larvae. *Aquaculture, 277*(1), 83-91.

Zandee, D. I., Holwerda, D. A., & De Zwaan, A. (1980). Energy metabolism in bivalves and cephalopods. *Animals and environmental fitness, 1*, 185-206.

Zandee, D. I., Kluytmans, J. H., Zurburg, W., & Pieters, H. (1980). Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. *Netherlands Journal of Sea Research, 14*(1), 1-29.

Zhao, B., Zhang, S., & Qian, P. (2003). Larval settlement of the silver-or goldlip pearl oyster *Pinctada maxima* (Jameson) in response to natural biofilms and chemical cues. *Aquaculture, 220*(1), 883-901.

Zhu, C., Dong, S., Wang, F., & Huang, G. (2004). Effects of Na/K ratio in seawater on growth and energy budget of juvenile *Litopenaeus vannamei*. *Aquaculture, 234*(1), 485-496.

Zurburg, W., Hummel, H., Bogaards, R. H., De Wolf, L., & Ravestein, H. (1989). Free amino acid concentrations in *Mytilus edulis* L. from different locations in the southwestern part of the Netherlands: their possible significance as a biochemical stress indicator. *Comparative Biochemistry and Physiology Part A: Physiology, 93*(2), 413-417.

APPENDIX

LIST OF PUBLICATIONS:

1. Dinh, Q. H. & Fotedar, R., 2016. Early development of the blue mussel *Mytilus edulis* (Linnaeus, 1758) cultured in potassium-fortified inland saline water. Aquaculture 452, 373 - 379.

2. Dinh, Q. H., Fotedar, R. & Dinh, K. V., submitted. The survival and osmoregulatory capacities of blue mussels *Mytilus edulis* (Linnaeus, 1758) cultured in inland saline water and ocean water: a comparison.

3. Dinh, Q. H., Fotedar, R. & Dinh, K. V., submitted. Survival and growth of the juvenile blue mussel *Mytilus edulis* (Linnaeus, 1758) cultured in potassium-fortified inland saline water.

4. Dinh, Q. H., Fotedar, R. & Dinh, K. V., submitted. Growth, survival, osmoregulatory capacity and proximate composition of blue mussels *Mytilus edulis* (Linnaeus, 1758) cultured in potassium-fortified inland saline water.

5. Dinh, Q. H. & Fotedar, R., submitted. Size dependent root shedding and byssus thread renewal capacity of the blue mussel *Mytilus edulis* (Linnaeus, 1758) exposed to potassium-fortified inland saline water.