School of Molecular and Life Sciences

Technical Feasibility of Cultivating Local Seaweed Species in Inland Saline Water of Western Australia

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DECLARATION

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PREAMBLE

This study aims to evaluate the technical feasibility of culturing locally available seaweeds in inland saline water (ISW) of Western Australia and in ionically modified ISW. Although, there are lower additional input resources required for existing agricultural farmers to culture seaweed in ISW than other higher order fish, shrimp and molluscan species, there is no comprehensive published information available to culture local seaweed species under ISW environment in Western Australia.

The present thesis is structured so that the information flows easily from one chapter to another within the context of some entire chapters or certain sections of the chapters have been published, therefore there is a possibility of certain duplication, particularly in terms of introduction and methodology.

The thesis is organized into ten (10) chapters.

Chapter 1 is general introduction to the research that includes the background information, justification of the study, the aim, and the objectives of the research.

Chapter 2 reviews the relevant literature on the topic and related research areas including quality of ISW in Australia and Western Australia, and current global aquaculture practice in ISW. Information about the ionic composition and environmental factors influencing characteristics of ISW are also reviewed. This chapter also provides an overview about six studied seaweed species and their potential uses and aquaculture practices.

Chapter 3 presents methodology and summarises the general methods used in the experiments, data collection, and data analysis. This chapter includes a common section of Materials and Methods of subsequent chapters. This attempts to avoid repeating of some of the common protocols followed in rest of the chapters.

Selecting potentially suitable seaweeds species that could be grown in ISW was the first step in order to achieve the aim of this research. Chapter 4 describes the selection procedure for seaweeds to be investigated for their technical feasibility to culture in ISW around Perth region in Western Australia. Five genera of seaweed consist of six species representing green, red and brown seaweeds were shortlisted to be cultured in

ISW and potassium-fortified ISW (K^+ISW). These six species of seaweed are *Cystophora subfarcinata*, *Sargassum linearifolium*, *Sargassum podacanthum*, *Ulva lactuca*, *Glateroupia suspectinata*, *Fushisunagia catenata* (basionym *Lomentaria catenata*) (Appendix 1). As the species of *Fushisunagia catenata* was identified after the publication of the article on *Lomentaria* sp., *Lomentaria catenata* will be used as the name of this species throughout the thesis. Chapter 4 also explores the effects of two sources of K^+ as K_2SO_4 and KCl to fortify ISW. Both sources of K^+ to make 100%K^+ISW result in a similar specific growth rate (SGR) for both *S. linearifolium* and *S. podacanthum* as if cultured in ocean water (OW).

The remaining part of the thesis is divided into three sections. Section 1 focusses on *Sargassum* spp. and comprises of chapters 5 to 7. Section 2 includes Chapter 8 which describes the experiments conducted on *Lomentaria catenata*. Section 3 consists of Chapter 9 which containes two sections: (i) – a preliminary study of culturing *Ulva lactuca* in OW, and (ii) – cultural feasibility of *U. lactuca* in ISW.

Chapter 5 investigates the productivity of *Sargassum* spp. in K^+ISW at 100%, 66% and 33% of [K^+] as in OW. *Sargassum* spp. can sustain a similar growth rate as in OW in K^+ISW at 100% of K^+ concentration. The SGR of *S. linearifolium*'s is higher than *S. podacanthum* at all K^+ISW levels. The section related to the *S. linearifolum* in this chapter, entitled "Productivity of *Sargassum linearifolium* in potassium-fortified inland saline water under laboratory conditions", is published in the journal of *Aquaculture Research* as a research article (Appendix 2.1).

Chapter 6 focuses on the culture of *S. linearifolium* and *S. podacanthum* in 100% K^+ISW and OW, which are enriched weekly by ammonium:phosphate, at the levels of 80:8, 120:12, 160:10, 200:20 and 240:24 µM. The higher nutrient supplementation in OW works better than in K^+ISW in terms of *Sargassum* biomass, however, there is no interaction between water types (OW and K^+ISW) and supplementation of nutrient levels. *S. padocanthum* grows better than *S. linerifolium* in both OW and K^+ISW nutrient-enriched environment. The main segment of this chapter centered on *S. podacanthum*, entitled "Effects of nitrogen and phosphorus enrichment on the growth of *Sargassum podacanthum* cultured in potassium-fortified inland saline water" has been published in *American Journal of Applied Science* as a research article (Appendix 2.2).

Chapter 7 describes the research on effects of temperature and pH on the growth of *S. linearifolium* and *S. podacanthum* cultured in K^+ISW. Temperature of 20–22ºC and ambient water pH levels of 7.0–8.2 of K^+ISW are suitable for cultivating *Sargassum* spp. This chapter has been published in *American Journal of Applied Science* as a research article entitled "Effects of temperature and pH on the growth of *Sargassum linearifolium* and *S. podacanthum* in potassium-fortified inland saline water" (Appendix 2.3).

Chapter 8 analyses the growth feasibility of *Lomentaria catenata* in ISW, K^+ISW at 100%, 66% and 33% of [K^+] as in OW, the effect of ammonium- and phosphate-enriched K^+ISW, and temperature on *L. catenata's* growth. *L. catenata* requires 66% of K^+ISW, ammonium enrichment at 100 µM under temperature of 20–26ºC in ISW. This chapter has been published in *The Journal of Aquaculture and Environment Risk Assessment and Remediation* as a research article entitled "The growth feasibility of *Lomentaria* sp. in laboratory conditions" (Appendix 2.4). This species was identified after the article published as *Lomentaria catenata*, first recorded in Western Australia by the author of this study, and this species has recently been moved to a separate genus as *Fushisunagia catenata*.

Chapter 9 consists of two trials. The first trial is a preliminary trial to culture *Ulva lactuca* in ISW by testing the culture feasibility of *U. lactuca* in OW at three salinities, four stocking densities and three ammonium levels. The most suitable salinity of 30–35 ppt, stocking density of 0.2 kg m^{-2} and ammonium requirement of 56 µM of culturing *U. lactuca* in OW were applied to culture the same species under ISW environment. The second trial investigated the effects of temperature and K^+ISW on the growth of *U. lactuca* under two levels of temperature and three levels of K^+ fortification. *U. lactuca* is able to be cultivated in ISW at 30% K^+ISW under 25–26ºC.

Chapter 10 provides a general and comparative discussion on the culture of four seaweed species in K^+ISW, nutrient-enriched K^+ISW; the effects of different environmental factors, as well as the seasonal effects on the growth of seaweed under laboratory conditions. The environmental conditions to culture each seaweed species and the limitations of this study are then summerised. This chapter also concludes the study by the conclusions and recommendations, highlights the significance sections of the study, and provides suggestions for the further research.

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ABSTRACT

Increasing salinisation has negatively affected agricultural land, wildlife habitats and native vegetation in Australia. Mariculture using inland saline water (ISW) is considered a potential expansion and diversification of aquaculture, including seaweed farming. The seaweed culture can utilise existing agricultural farms where saline water is accessible, as it is less constrained by the requirement for any additional resource(s) and changes in the existing infrastructure. Therefore, cultivating seaweed in ISW can provide an additional source of income and raw seaweed for the aquaculture/-seaweed industry, with a lower capital investment than farming in the sea. This diversification stream of farming income may constitute a convenient choice for farmers, and for environmental protection in Australia. Six naturally distributed seaweed species, representative of green, red and brown seaweeds including *Ulva lactuca, Glateroupia suspectinata, Fushisunagia catenata* (Basionym *Lomentaria catenata*), *Cystophora subfarcinata, Sargassum linearifolium, Sargassum podacanthum,* in Western Australia, were selected to test their cultural feasibility under ISW conditions. These species were identified by Western Australia Herbarium. Four species, *U. lactuca, L. catenata, S. linearifolium, S. podacanthum,* were selected for further studies, to investigate the culture feasibility in potassium-fortified ISW (K^+ISW), nutrient enriched ISW, under different temperature and pH levels of ISW.

As potassium (K^+) deficiency is a major problem for marine species cultivation in ISW, testing the growth feasibility of the target seaweed species in K^+ fortification for ISW is the main preference of this study. Five different experiments were set up for seaweed cultured in K^+ fortification ISW under laboratory conditions consisting of five studied treatments in triplicates or quadruplicates depending on the experimental design and capacity of the culture system. The seaweeds were tested in three levels of K^+ fortification in ISW. These levels were 100, 66 and 33% of K^+ concentration similar as in ocean water (OW) at a similar salinity by the addition of KCl, and were referred as ISW100, ISW66, ISW33, presented three treatments of the experiment, with two controlled treatments of ambient ISW (ISW0) and ambient OW (OW).

The first experiment was to culture all six seaweed species and the results showed that *S. linearifolium, S. podacanthum, L. catenata* and *U. lactuca* were most suitable for

further studies as these four species presented higher specific growth rate (SGR) and longer survival period in K^+ISW. Further four experiments were conducted separately and independently one for each seaweed species at different times and different salinity levels (35 ppt for *Sargassum* spp. and 30–31 ppt for *U. lactuca* and *L. catenata*).

The K^+ deficiency in ISW showed an adverse impact on the growth of seaweeds and all of them died in ISW0 during the first fortnight of culture period. K^+ fortifications at 100%, 66% and 33% of the K^+ concentrations as in OW at a similar salinity were required for a positive biomass growth of *U. lactuca, L. catenata* up to 42 days and up to 56 days for *Sargassum* spp., respectively. The effect of two sources of K^+, KCl and K_2SO_4 at ISW100 on the growth of two *Sargassum* spp., was also investigated under outdoor conditions, which resulted in a similar growth of two *Sargassum* spp. in both K^+ISW and in OW.

The effects of nutrient supplementation on the growth of these four selected seaweed species in K^+ISW were then investigated. The weekly supplementation of two sources of nutrients including ammonium (NH_4-N) and phosphate (PO_4^{3-}-P) supplied by NH_4Cl and NaH_2PO_4, respectively, was investigated on the *Sargassum* spp. growth. Five weekly nutrient enrichment levels of 80:8, 120:12, 160:16, 200:20 and 240:24 µM of NH_4-N:PO_4^{3-}-P in ISW100 and in OW were tested and results, after 84 days, showed that *S. podacanthum* was unable to grow without nutrient enrichment, and a weekly supplementation of 160:16 µM of NH_4-N:PO_4^{3-}-P in ISW100 resulted in a higher but similar standing biomass and SGR as in OW. However, *S. linearifolium* for up to 56 days, did not require any nutrient enrichment for its normal growth in ISW100.

L. catenata was cultured in OW, ISW and ISW66 enriched with 100 µM NH_4-N to test the NH_4-N requirement of *L. catenata*. The results showed that NH_4-N supplementation presented significant effect in ISW66 which resulted in highest biomass than all other waters. NH_4-N:PO_4^{3-}-P requirement for *L. catenata* was tested at three levels of 75:7.5, 150:15, 300:30 in ISW66. However, this combination of NH_4-N:PO_4^{3-}-P had no effect on the growth of *L. catenata* in ISW66. *U. lactuca* growth was tested in weekly enrichment of 0, 28 and 56 µM of NH_4-N in OW and the results indicated that 56 µM of NH_4-N was essential for optimum development.

The effect of temperature on the seaweed growth was also tested independently for each seaweed species. The two *Sargassum* spp. were cultured in ISW100 fortified by K_2SO_4 under two temperature regimes of 20–22ºC and 24–26ºC and two pH levels of ambient pH 7.0–8.2 and low pH at 5.5–6.5. The ambient pH of ISW and temperature 20–22ºC were ideal for *Sargassum* spp. culture as these conditions resulted in higher SGR of *Sargassum* spp. *Sargassum* spp. could not survive under higher temperature of 24–26ºC longer than 14 days. The SGR of *S. linearifolium* was higher than SGR of *S. podacanthum* under both temperature regimes at ambient pH. There was no significant difference in *L. catenata* biomass and length cultured in ISW66 and OW and enriched weekly by 100 µM NH_4-N under the two temperature regimes. The temperature effect on the growth of *U. lactuca* was determined in OW and ISW enriched with 56 µM NH_4-N, under two temperature regimes. The results showed that the temperature of 25–26ºC was preferable for *U. lactuca.*

Hence, this research concluded that it is possible to cultivate certain local species seaweed in ISW of Western Australia, however, its growth is dependent on seasons and K^+ fortification of ISW from 33–100% [K^+] as of OW at a similar salinity is essential. The K^+ fortification source could either be KCl or K_2SO_4. The requirement for weekly nutrient enrichment is species-specific, at 56 and 100 µM NH_4-N for *U. lactuca* in ISW33, *L. catenata* in ISW66, and 160:16 µM of NH_4-N:PO_4^{3-}-P for *S. podacanthum* in ISW100, respectively. Seaweed grow well at the ambient pH of K^+ISW, under temperature from 20–22ºC for *Sargassum* spp., 25–26ºC for *U. lactuca* and 20–26ºC for *L. catenata*. *S. linearifolium* is the best candidate to be cultivated in ISW100 as its SGR is higher and requires no nutrient enrichment.

ABRREVIATION

ABARES	Australian Bureau of Agricultural and Resource Economics and Sciences
ABS	Australian Bureau of Statistics
CARL	Curtin Aquatic Research Laboratory
d	day
K^+ISW	Potassium-fortified inland saline water
ISW	Inland saline water
NLWRA	National Land and Water Resource Audit
OW	Ocean water
ppt	Part per thousand
SGR	Specific growth rate (% d^{-1})
WA	Western Australia

Chemical elements and Units

L	litter
M	mol
C	Carbon
Ca^{2+}	Canxi
Cd^{2+}	Cadmium
Cu^{2+}	Copper
Cr^{6+}	Chromium
K^+	Potassium
$[K^+]$	Potassium concentration
KCl	Anhydrous potassium chloride
K_2SO_4	Potash of sulphate
Mg^{2+}	Magnesium
N	Nitrogen
Na^+	Sodium
NaH_2PO_4	Sodium dihydrogen phosphate
NH_4Cl	Ammonium chloride
NO_3^--N	Nitrate

NO_2^--N	Nitrite
NH_4-N	Ammonium
P	Phosphorus
Pb	Lead
PO_4^{3-}-P	Phosphate
S	Sulfur
TKN	Total Kjeldahl Nitrogen
Zn^{2+}	Zinc

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Appendix 2.1. Article in Journal of Aquaculture Research 261

Bui, H. T. T., Luu, T. Q., Fotedar, R., & Tantulo, U. (2017). 261

Permission form the Journal of Aquaculture Research to reuse this article in this thesis: 262

Appendix 2.2. Article in American Journal of Applied Sciences 263

Bui HTT, Luu TQ, Fotedar R., 2018. 263

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Appendix 2.3. Article in American Journal of Applied Sciences 265

Bui HTT, Luu TQ, Fotedar R., 2018. 265

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Appendix 2.4. Article in Journal of Environmental Risk Assessment and Remediation 267

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CHAPTER 1 INTRODUCTION

1.1 Introduction

The annual growth rate of Australian aquaculture production was 25% between 2004–2005 and 2011–2012 (Australian Bureau of Agricultural and Resource Economics and Sciences [ABARES], 2016), and the aquaculture production contribution increased about 20% in the last decade of the 20th century (Lymbery *et al.*, 2007). The value of Australian aquaculture production in 2015–2016 was $1.3 billion, a rise of 20% from 2012–2013 (ABARES, 2017), to which the Western Australia (WA) contributed 8% in values (Department of Fisheries, 2015) and 19% in production (ABARES, 2017). The aquaculture (fish, crustacean and molluscs) contributed 43% of the total seafood in value and 35% in production in both years 2014–2015 and 2015–2016 (ABARES, 2017). The five core aquaculture species including Southern Bluefin tuna, pearls, Atlantic salmon, prawns and edible oysters in the 20th century (Love & Langenkamp, 2003) have shifted to salmonids, tuna, prawns, edible oysters, pearl oysters in terms of values in 2015–2016 (ABARES, 2017). In this year, seafood consumption was 13.8 kg per capita, which was ranked fourth of the main animal protein sources for Australian consumption, with the acceptance of seaweed (ABARES, 2017). Imported seaweeds are the main supply source of seaweeds for the Australian market. In 2006/07, the import of seaweed reached over 5,000 tonnes with a total value of AU$14 million (Lee, 2008), which shows the high demand for seaweeds in the Australian market. The development of an alternative land-based source of seaweed culture is necessary for both domestic consumption and export (Lee, 2008).

In Australia, 5.7 million ha of the total 770 million ha of land area were salinized in 2000. This figure may increase to 17 million ha in 2050 (Timms, 2005). The inland saline water (ISW) is inexhaustible in natural rivers, lakes and shallow aquifers (Allan *et al.*, 2001). Two main regions of underground ISW in Australia are the wheat-belt in WA, which accounts for 70% of national ISW and covers approximately 18 million hectares (Doupé *et al.*, 2005; Lymbery *et al.*, 2006), and Murray-Darling basin (Allan *et al.*, 2001). About 14% (or 20,000) of national farms in Australia has a sign of salinity (Trewin, 2002).

Saline land can be used for aquaculture (Allan *et al.*, 2008; Smith, 1997). Although ISW is seasonal, as most saline water lakes dry in summer (Jenkins, 1997), ISW availability from underground water provides a potential water source for domestic aquaculture (Doupé, Lymbery, Sarre, *et al.*, 2003; Partridge, Lymbery, & George, 2008; Pitman & Läuchli, 2002). ISW aquaculture can help to offset the costs of the salinisation adverse effects such as the costs for ISW treatments (Doupé, Lymbery, Sarre, *et al*., 2003). ISW aquaculture is more cost effective than coastal aquaculture. It is also more effective in reducing the risks of tides, storms and disease expansion than coastal aquaculture (Ogburn, 1997). ISW aquaculture has gradually developed in Australia, focusing primarily on fish and shrimp (Allan *et al*., 2001; Allan *et al*., 2008). However, its economic contribution to national fisheries is still minor (Doupé, Lymbery, Sarre, *et al*., 2003). About 29 shrimp, fish, molluscs and algae species have been researched and commercially cultured in ISW in Australia as summaried by Dinh (2016). It has to be noted that the ambient temperature in most of the salinity-affected areas of Australia is not suitable for shrimp farming (Partridge & Lymbery, 2008; Roy *et al.*, 2010), but could be utilised for cultivating seaweeds.

Marine species cultured in ISW have produced mixed results, due to the fact that most of the ISW is in the temperate area (Partridge, Lymbery, & George, 2008), or does not contain enough potassium (K^+) for marine species (Dinh, 2016; Doroudi *et al.*, 2006; Fielder *et al.*, 2001; Forsberg *et al.*, 1996; Ingram *et al.*, 2002; Partridge & Creeper, 2004; Partridge & Lymbery, 2008; Roy *et al.*, 2007; Shakeeb Ur *et al.*, 2005; Tantulo & Fotedar, 2007). ISW contains lower K^+ concentration than ocean water (OW) at the similar salinity (Boyd *et al.*, 2007; Fielder *et al*., 2001; Fotedar *et al.*, 2011; Ingram *et al*., 2002; Partridge & Lymbery, 2008; Prangnell & Fotedar, 2006a; Saoud *et al.*, 2003). In order to cultivate marine species ISW must be fortified by K^+ of similar concentration in OW (Dinh, 2016; Fielder & Allan, 2003; Fielder *et al*., 2001; Mourad *et al.*, 2012). In addition, the red seaweed *Gracilaria cliftonii* can also develop in ISW, but would be better in K^+ fortification ISW (Kumar *et al.*, 2010).

Up to date, research on seaweed culture in Australian ISW is restricted to only *Gracilaria cliftonii* Withell, Miller and Kraft (Cordover, 2007; Kumar *et al*., 2010), even though there are several studies on seaweed growth, chemical and nutrient uptakes worldwide. Seaweed culture in ISW could be an effective way of conserving

the natural inland resources of water and reducing the risk of typhoons in the open sea. Growing algae, including seaweed in ISW, is one of the choices to initiate their culture in Australia, contributing to natural resource management and expanding the resource base for aquaculture (Borowitzka, 1997). Furthermore, cultivating seaweed in ISW can provide a natural source of seaweed for the industry, which requires lower capital investment compared to building farms in the sea (Borowitzka, 1997).

Seaweeds provide organic carbon for aquatic food chain (Duarte, 1995; Graham *et al.*, 2009). They have been a healthy source of protein for Asian people for centuries. Seaweeds have been gradually introduced to the western markets as nori rolls, salads, agar gels, and provides the sources for the texture industry, plant fertilizers, folders, medicines and biofuels (Huisman, 2000; Lee, 2008). Out of the 221 species (32 chlorophytes, 125 rhodophytes and 64 phaeophytes) of seaweeds that have been used worldwide so far, 145 species (28 chlorophytes, 79 rhodophytes, and 38 phaeophytes) can be used as food and 101 species for phycocolloid production. 25 species have been used in agriculture, and in the manufacturing of paper (Ara *et al.*, 1997; Lindsey Zemke-White & Ohno, 1999). The extracts from seaweeds can be used for cosmetic industry and to produce medicine (Hur *et al.*, 2008; Thuy *et al.*, 2015; B. Wang *et al.*, 2010; Wiltshire *et al.*, 2015; Yende *et al.*, 2014). Seaweeds can clean water discharged from aquaculture by their ability to uptake nutrients and heavy metals (Neori *et al.*, 2004; Partridge, Lymbery, & George, 2008; Troell *et al.*, 1999; Van Khoi & Fotedar, 2011), so they can be cultured with other marine species (Cruz-Suárez *et al.*, 2010; Jin & Dong, 2003; Kitadai & Kadowaki, 2007; Mai *et al.*, 2010; Neori *et al.*, 2004; Neori *et al.*, 1996).

Apart from salinity, temperature and pH of water are also important factors that affect the maturation, reproduction and growth of seaweeds (Bird *et al.*, 1978; Cui *et al.*, 2014; Ding *et al.*, 2013; Uchida, 1993). The salinity and temperature can influence the growth of seaweed by affecting the nutrient uptake (Jie *et al.*, 2008), the photosynthesis (Scherner *et al.*, 2013; Simon *et al.*, 1999), chlorophyll-a content, spore biomass and the heavy metal absorption of seaweeds (Kamer & Fong, 2001; Mamboya *et al.*, 2009; Scherner *et al.*, 2013; Sousa *et al.*, 2007). Seaweeds' requirement of salinity varies widely depending on species. For example, *Ulva* requires only 5–40 ppt (Choi *et al.*,

2010; Kamer & Fong, 2001), whereas *Sargassum* requires from 24–42 ppt for its growth (Hanisak & Samuel, 1987).

Temperature needs also vary depending on species. For instance, in the same genus of Ulva, the *U. rigida* develops well from 7 to 25^0C and reaches the optimum growth at 17^0C (de Casabianca *et al.*, 2002). On the other hand, *U. curvata* growth is inferior at low temperature and grows best at 25°C. The optimal temperature for growth of *U. lactuca, U. rigida* and *U. scandinavica* is 10°C (Malta *et al.*, 1999), while *U. scandinavica* can grow at -5°C for 2 weeks in the darkness while in contact with anoxia and sulphide, and can live under the freezing winter condition (Kamermans *et al.*, 1998; Vermaat & Sand-Jensen, 1987).

The pH also affects the photosynthesis, growth of seaweed and seaweeds' ability to absorb heavy metals (Davis *et al.*, 2000; Drechsler & Beer, 1991; Figueira *et al.*, 1997; Hidayat *et al.*, 2015; Maberly, 1992).

Seaweeds need 18 nutritional elements to grow, of which some ions (potassium, calcium, sodium, magnesium, hydrogen, carbon, oxygen, and sulphur) are available in water. However, the natural level of nitrogen and phosphorus found in the water is not enough to meet the demand required by algae (Robards *et al.*, 1994). The two common types of nitrogen found in OW are ammonium (NH_4-N) and nitrate (NO_3^--N) (Burgess *et al.*, 2003). Seaweeds prefer NH_4-N to NO_3^--N (Ahmad *et al.*, 2011) and require high concentration of NH_4-N (Campbell, 2001).

Studies on seaweed growth and nutrient uptakes have been widely researched over the last few decades (Ahmad *et al*., 2011; Bird *et al*., 1978; Campbell, 1999; Coffaro & Sfriso, 1997; Coutinho & Zingmark, 1993; Dailer *et al.*, 2012; de Casabianca *et al*., 2002; Flindt *et al.*, 1997; Gordillo *et al.*, 2001; Kitadai & Kadowaki, 2007; Larned, 1998; Pérez-Mayorga *et al.*, 2011; Perini & Bracken, 2014). The effect of ecological factors, light intensity, salinity, and temperature on the growth and chemical compositions of some seaweed species in OW have been well studied (Andrew & Viejo, 1998; Chen & Zou, 2014; Choi *et al*., 2010; Cui *et al*., 2014; Gao & Hua, 1997; Hanisak & Samuel, 1987; Rao & Rao, 2002; Yuan *et al.*, 2014). In addition, research on the effect of nutrients on the growth of some seaweed species has also been conducted (Lapointe, 1986, 1995; Lapointe *et al.*, 2014). However, no research has

been done on the nutrient requirement of seaweeds and K^+ fortification in ISW except *Gracilaria cliftonii* (Kumar *et al*., 2010). Whereas, there are 1,300 species of red seaweeds, 2,000 species of green seaweeds and 350 species of brown seaweeds recorded in Australia (Huisman, 2000; Womersley, 1987). Of these, *Grateloupia subpectinata, Lomentaria catenata, Ulva lactuca*, *Cystophora subfacinata*, *Sargassum linearifolium* and *Sargassum podacanthum* are either native or naturally distributed in WA (Huisman, 2000; Womersley, 1987).

To date, studies on the ISW aquaculture in Australia have been centred on fish, mollusc, shrimp and crustacean (Allan *et al*., 2001; Allan *et al.*, 2008; Dinh, 2016; Ingram *et al*., 2002; Kumar, 2008; Partridge, Lymbery, & Bourke, 2008; Partridge *et al*., 2006; Prangnell & Fotedar, 2006b), and are limited to only one species of red seaweed *Gracilaria cliftonii* in ISW by mixing with OW (Kumar *et al*., 2010). No study has been conducted on cultivating the local distributed seaweeds in K^+-fortified ISW (K^+ISW) yet opening a direction for this current study.

1.2 Aims and Scope of the Study

The aim of the present study was to examine the feasibility of cultivating local seaweeds of WA in ISW and to find the suitable candidates for seaweed culture in Australian ISW. To do this, the study examined the growth of candidate seaweeds in K^+ISW, and the effects of pH and temperature on the growth of candidate seaweeds under the laboratory conditions, as well as to identify their nutrient requirements in K^+ISW.

Aim

The aim of this study is to investigate the growth feasibility of local seaweed species of WA in ISW, which will contribute the technical knowledge of seaweed aquaculture using ISW by:

- Providing the understanding of the need of K^+ fortification for culturing *Sargassum linearifolium*, *Sargassum podacanthum*, *Lomentaria catenata* and *Ulva lactuca* in ISW.

- Providing the suitable temperature and pH range for culturing the above species in K^+ISW
- Providing information about the nutrient requirement for culturing the above seaweed species in K^+ISW.

Objectives of the Study

1. To investigate the suitable candidates of seaweed species for culturing in WA ISW
2. To investigate the relationship between the growth of chosen seaweed species and K^+-fortification in ISW in WA.
3. To examine the effects of ammonium and phosphate enrichment on the growth of chosen seaweed species in K^+ISW in WA.
4. To determine the effect of pH and temperature of K^+ISW on the growth of chosen seaweed species in WA.

CHAPTER 2 LITERATURE REVIEWS

2.1 Inland Saline Water

Inland saline water (ISW), where the salinity of water in the inland areas is above 3 ppt, is found all over the world including Australia (Waiser & Robarts, 2009) (Table 2-1). In Australia, salination expansion is considered one of the biggest environmental problems, and one possible way of reducing the negative effect of ISW is to pump the ISW into large reservoirs (Allan *et al*., 2008). In the last five years, the inland water storage of Australia gradually decreased from 80% of its capacity in 2012 to 65% in 2016 (Argent, 2017).

Table 2-1. Distribution of salinised areas in the three largest affected countries

Country	Total area (km^2)	Cultivated land (km^2)	Arable land (%)	Irrigated land (km^2)	Percentage of salinised affected land (%)
Australia	7,617,930	471,550	6.15	237.8	8.70
India	3,287,240	1,535,063	42.1	6526.3	16.60
USA	9,826,675	1,669,302	18.1	2259.0	23.00

(Ghassemi *et al.*, 1995)

Salinity-affected land areas were over 2.5 million hectares in 1995 (Nulsen, 1997), 5.7 million hectares in 2000 and is forecasted to be more than 17 million hectares in 2050, at about 80% of lakes and wetlands salinised (Timms, 2005) (Figure 2-1) which has negatively affected agriculture and ecology (Department of Agriculture and Food, 2013; Nulsen, 1997). However, it opens up an opportunity for inland aquaculture (Doupé, Lymbery, & Starcevich, 2003; Kolkovski, 2010). In WA, the development of an aquaculture industry has huge potential, based upon the availability of a large ISW resource, suitable soil and water salinity, and adequate existing farm and road infrastructure (Doupé, Lymbery, Sarre, *et al*., 2003). The salinization of agricultural and public land has expanded since 1998 (Robertson *et al.*, 2010), covering more than one million hectares in the south-west of WA (Furby *et al.*, 2010) and can potentially be up to 2.8–4.5 million hectares (George *et al.*, 2005).

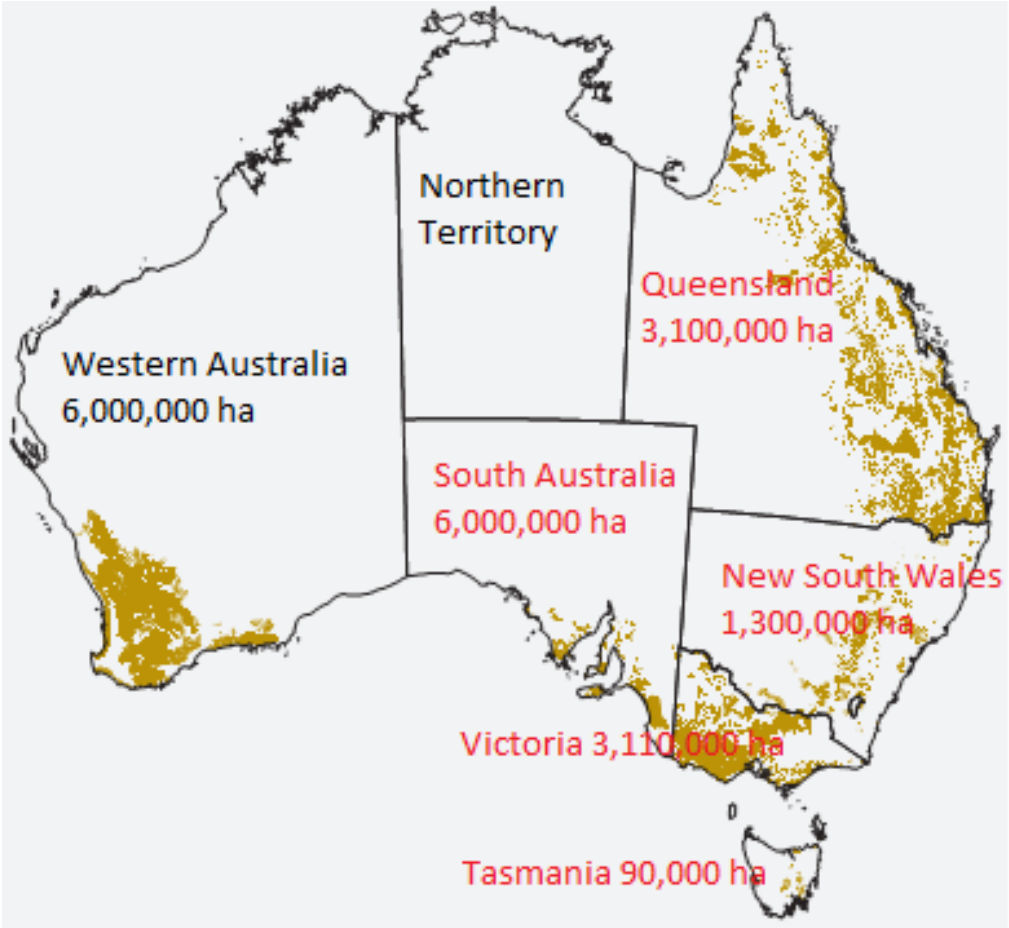


Figure 2-1. Distribution of forecast salinised areas in Australia in 2050

Source: (National Land and Water Resources Audit [NLWRA], 2001)

The quality of ISW varies extensively and depends on location and depth. Generally, pH, salinity and ionic concentration increase along the depth of the groundwater (Nulsen, 1997). For instance, the pH of the ISW in the Merredin catchment (WA) is from 3.9 at 6 metres depth to 4.7 at 17 metres depth, and 6.3 at 33 and 45 metres depth (Nulsen, 1997). ISW in Outokumpu, Kerimaki, is slightly alkaline and this gradually increases with depth (Nurmi *et al.*, 1988).

ISW salinity differs widely. Generally, salinity is lower than 35 ppt (Nulsen, 1997), in a range of 0–320 ppt, and the salinity of two-thirds of the ISW that is suitable for marine aquaculture in WA is 5–40 ppt (Mazor & George, 1992). In particular, the ISW salinity in the East Belka catchment (WA) is 3–13 ppt (George, 1992).

Ionic concentration changes along the depth of the groundwater, undergoing extreme fluctuation. In WA, calcium concentration is more than 300 mg L^{-1} at less than 45 metres depth, or in some places, it is only 28 mg L^{-1} (Nulsen, 1997). However, it is more than 3000 mg L^{-1} at deeper than 1000 metres in Fennoscandian Shield (Nurmi *et al*., 1988). The concentration of sulphate (SO_4) in ISW is lower in WA (Partridge, Lymbery, & Bourke, 2008; Prangnell & Fotedar, 2006b) but is significantly higher than ocean water (OW) in Texas (Forsberg *et al*., 1996; Saoud *et al*., 2003). Nitrogen in ISW in WA is quite low, from zero to 3 mg L^{-1} in terms of nitrate concentration in the depth of shallower than 45 metres (Nulsen, 1997) (Table 2-2).

Table 2-2. Ionic profile (mg L^{-1}) of ISW in WA

Parameter	30 ppt (*)		32 ppt (**)		35 ppt (*)	
	ISW	OW	ISW	OW	ISW	OW
Osmolality[1]	805.67	941.00	890.00	890.00	927.67	1140.33
Na^+	7720.00	8803.00	8856.00	9306.00	9385.00	10190.00
K^+	77.26	313.00	72.00	390.00	92.32	356.10
Ca^{2+}	530.80	320.90	469.00	355.00	640.60	363.90
Mg^{2+}	1375.00	1015.00	1650.00	1126.00	1674.00	1172.00
S^{2+}	560.00	706.30			647.40	819.30
Na^+: K^+ ratio	99.92:1	28.12:1	122.42:1	23.86:1	101.66:1	28.62:1
Mg^{2+}: Ca^{2+} ratio	2.59:1	3.16:1	3.18:1	3.52:1	2.61:1	3.22:1

Source: (*) Dinh, (2016) – original ISW salinity 45 ppt; (**) Prangnell & Fotedar, (2006b); ([1]) - mOsm kg^{-1}

The concentration of inorganic carbon (C) in groundwater is 100 times higher than organic carbon (Robards *et al*., 1994). The alkalinity of water is presented by the concentration of calcium carbonate ($CaCO_3$), which is much higher than in OW at the same salinity level (Bottomley *et al.*, 1994; Roy *et al*., 2010). The bicarbonate (HCO_3) concentration of ISW varies due to the location, and is almost equal to or lower than its concentration in normal OW (Boyd & Thunjai, 2003).

At the same salinity, the core trace-metal compositions of zinc (Zn), arsenic (As), copper (Cu), nickel (Ni) and lead (Pb) in ISW are lower than in OW. However,

manganese (Mn), barium (Ba), selenium (Se) and cobalt (Co) concentrations are higher (Partridge, Lymbery, & Bourke, 2008; Roy *et al*., 2010; Saoud *et al*., 2003).

2.2 Causes and Impacts of Inland Salinity

Salt in Australian land is common at 100–15,000 tonnes ha^{-1}, due mainly to the winds from the ocean and accumulated over years (Pannel, 2001). Secondary salinity (or salinisation of land and water) occurs as a sequence of human activities, for instance, irrigation or tree clearance (Kolkovski, 2010; Lymbery *et al*., 2007; Podmore, 2009). Plant clearance and the shallow-root crops using less rainfall water cause an increase in the level of groundwater, and the water is then salinised due to salts leaching from surrounding areas (Kumar, 2008) (Figure 2-2). In addition, a reduction of rainfall in the near future and temperature prediction cause more stress on the inland water (Argent, 2017). The salinity exhibits remarkable variation (Timms, 2009), owing to changes in rainfall and solar radiation (Prangnell, 2007), calcium concentrations fluctuation, and potassium ions (K^+) deficiency relatively to OW (Nulsen, 1997; Prangnell & Fotedar, 2006a). Rainfall reduction, irrigation water demand increase bring the higher salinity for inland water (Hillel *et al.*, 2008).

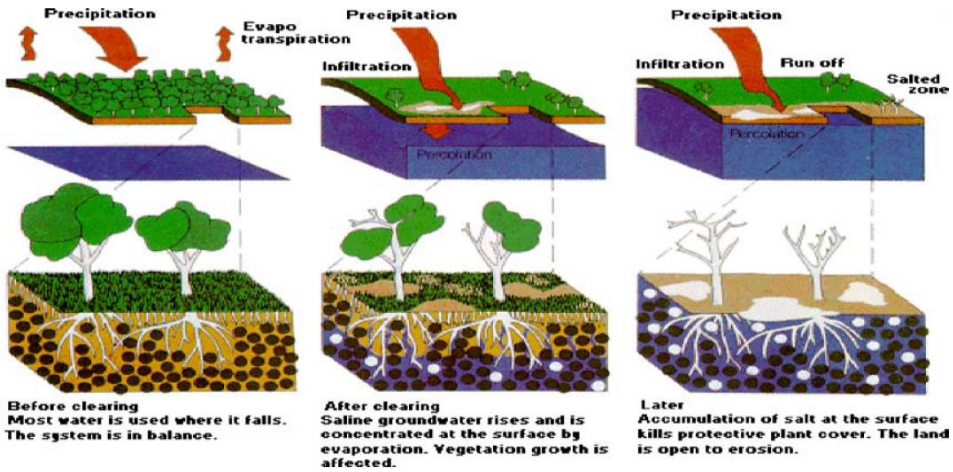


Figure 2-2. Cause of dryland salinity

Source: Primary Industries and Resources South Australia (1999)

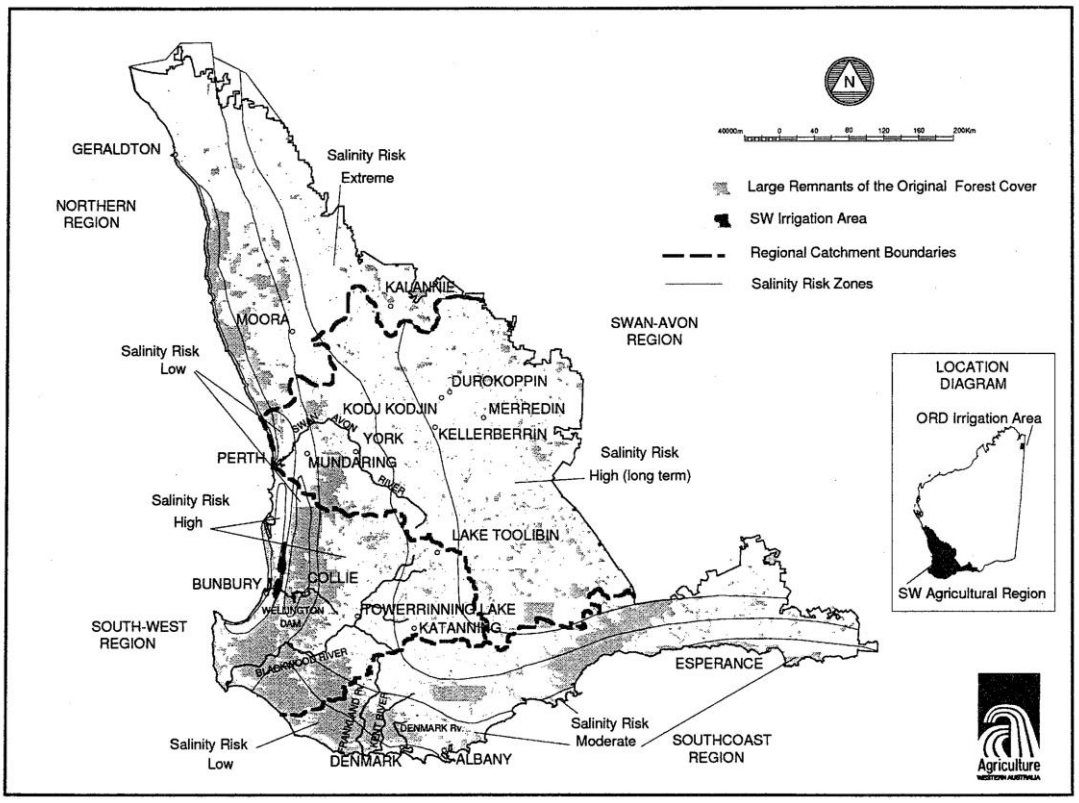


Figure 2-3. Salinity risk zone of south-west irrigation areas in southern part of WA

Source: George *et al.* (1997)

It is agreed that salinisation is one of the greatest environmental problems in Australia (Allan *et al*., 2008). Salinisation has spread from North Queensland to Tasmania, along the great Murray Darling River Basin and south-west Western Australia (Watts *et al.*, 2001) (for example see Figure 2-3). In Australia, land and water salinisation have severely negative influenced on wetlands, agriculture, water resources, infrastructure, biodiversity and communities (Allan *et al*., 2001; Kolkovski, 2010; NLWRA, 2001) (Table 2-3), with an estimated loss of around AUD12 billion year^{-1} (Ghassemi *et al*., 1995), and hundreds of million dollars for the Murray Darling Basin (Allan *et al*., 2008). The salinised area biological richness is threatened with extinction (Halse *et al.*, 2003), including aquatic invertebrates (Timms, 2009), aquatic insects (Carver *et al.*, 2009), waterbirds and plants (Halse *et al*., 2003). 20,000 farms in Australia show signs of salinity, and account for 14% of total national farms (Trewin, 2002). The Government had launched a National Action Plan for Salinity and Water Quality as a first and important step to tackle the salinisation (Watts *et al*., 2001). In Queensland,

the modelling and broadscale of natural resources have been created based upon the soil mapping, soils and landscape attribute (Brough *et al.*, 2006). The catchment management plan in WA is developed based upon the collected hydrological and ecological data in the period 1996–2006, target to maintaining the existing biodiversity richness of the areas affected by salinisation, reducing the biodiversity decline rate, and recovering the existing species richness (Wallace *et al.*, 2011).

There are 81 aquatic invertebrate families in these salinised ISWs (Kay *et al.*, 2001), such as 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 *et al.*, 2000; Lange-Bertalot *et al.*, 2003; Taukulis & John, 2009). Those species has been significantly affected by the rainfall and salinity (Kay *et al*., 2001).

Table 2-3. Aspects influenced by salinisation

Aspects	2000	2020	2050
Agricultural land (*1000 ha)	4650	6371	13660
Remnant and planted perennial vegetation (*1000 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

Source: NLWRA (2001), Watts *et al*. (2001).

The upper south-east part of the South Australia is a main salinised area which is forecasted to rise by 60% if the groundwater continues to expand at the pace in late 20 century (NLWRA, 2001). The salt interception schemes (SIS) have been developed in the state to reduce the negative impact of salinisation (Hutchinson, 1997). Wetlands are becoming saline wastelands, as it is predicted that in South Australia half the native vegetation of Chowilla wetlands will disappear (Watts *et al*., 2001).

Victoria's affected areas are mainly in the western, south-western, north-central and north-western parts (Gooley *et al.*, 1997). The water tables has increased which caused

a serious problem for irrigated farms and has attributed more risk of salinisation in these areas. The state government has studied and built pumping stations to alleviate the negative effect of water tables increasing and uses ISW for aquacultural activity (Gooley *et al*., 1997).

Queensland is still unaffected, with the exception of small salinised areas in the eastern region, but it is predicted to be at high risk of salinised areas expansion in the near future (NLWRA, 2001; Watts *et al*., 2001).

The northern and southern regions of New South Wales's have two huge underground basins, Great Artesian and Murray, respectively (Allan & Fielder, 1997), whereas the salinisation has also affected Sydney's western suburbs (Watts *et al*., 2001).

Adelaide's water supplies have been affected by salinisation in the past 20 years, despite huge efforts and resources being spent to reduce the negative effects of salinisation (Watts *et al*., 2001).

In Australia, the WA occupies the largest salinised areas (Figure 2-1). About 30% (equivalent to 5.7 million hectares) (NLWRA, 2001) of the land and lakes in south-west WA is affected by salinisation (Timms, 2005), and increase 14,000 ha annually (Furby *et al*., 2010). It was estimated that $1.5 billion of agriculture productivity was lost due to salinity expansion (Kay *et al*., 2001). The largest and most severely salinity effect in Australia is the wheat-belt, which accounts for 70% of the Australian salinised area (Doupé, Lymbery, & Starcevich, 2003; Kay *et al*., 2001), and is expected to be 50% of the national salinisation area in 2050 (Pannel, 2001). The affected area comprises of 38 affected towns (George *et al*., 2005), 6,918 farms and 1.24 million hectares of agricultural land is salinized (Trewin, 2002), and has rainfall of 300–700 mm annually (Halse *et al*., 2003; Kay *et al*., 2001). The South West of WA has experienced a reduction of rainfall since 1970 which resulted in a decline of water volume in reservoirs (Bennett & Gardner, 2014). The Northern, Central and the east of the Agricultural Regions of the South West WA are facing the highest salinity risk (Raper *et al.*, 2014). The areas of 567,000 hectares (45.7% of agricultural land affected by salinilization) are unable to be used for agricultural production (Trewin, 2002). In WA, 98,000 km of levees, bank and drains have been built for to prevent salinisation, which will cause the disappearance of 450 plant species in WA in the next 30 years

(Trewin, 2002). Hundreds of other plant species are subject to genetic changes and at risk of extinction as a result of salinisation (Watts *et al*., 2001). Salinisation also affects the richness of animal species (reduced by a third) by damaging their habitats. It is predicted that 60 water-bird species are at risk of extinction (Watts *et al*., 2001). One-third of aquatic invertebrate species is forecasted to be extinct in WA (Halse *et al*., 2003). About 850 plant and animal species has been threated to be extinct in WA (Wallace *et al*., 2011).

2.3 Potassium in Inland Saline Water

In comparison to the OW at the same salinity level, the ionic ratio of chloride (Cl), calcium (Ca), sodium (Na^+), sulphur (S) and bromine (Br) in ISW in Australia is almost the same (Fielder *et al*., 2001; Nulsen, 1997; Prangnell & Fotedar, 2006b). However, potassium (K^+) deficiency in ISW is common (Fielder *et al*., 2001; Nulsen, 1997; Saoud *et al*., 2003) due to clay soils' uptake of Na^+ (Stumm & Morgan, 1995) (Table 2-4).

The $[K^+]$ in the raw ground water is much lower than that in OW at the same salinity (Boyd *et al*., 2007; Fielder *et al*., 2001; Ingram *et al.*, 2002), and varies as per the depth (Nulsen, 1997; Nurmi *et al*., 1988). The $[K^+]$ in inland saline groundwater is 9.2 mg L^{-1} in New South Wales (Fielder *et al*., 2001), 25 mg L^{-1} in Victoria (Ingram *et al*., 2002), and 26–331 mg L^{-1} in WA (Nulsen, 1997).

Potassium deficiency in ISW adversely influences the growth and causes mortality of aquatic animals (Doroudi *et al*., 2006; Fielder *et al*., 2001; Forsberg *et al*., 1996; Ingram *et al*., 2002; Mourad *et al.*, 2012; Partridge & Creeper, 2004; Roy *et al.*, 2007; Shakeeb Ur *et al*., 2005; Tantulo & Fotedar, 2007). ISW should be fortified by K^+ from 50% to similar K^+ concentration in OW to cultivate fish, shrimp, molluscs (Dinh, 2016; Fielder & Allan, 2003; Fielder *et al*., 2001; Shakeeb Ur *et al*., 2005; Tantulo & Fotedar, 2006). Shrimps cultured in ISW with K^+ fortification at the same $[K^+]$ in OW have similar survival and growth rates as those cultured in OW, and the shrimp's osmo-regulation capacity is increased (Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006).

Table 2-4. Potassium deficiency in ISW worldwide

Country	Location	Sources
Australia	New South Wales	Doroudi *et al*. (2006); Doroudi *et al.* (2007); Fielder *et al*. (2001)
	Victoria	Ingram *et al*. (2002)
	Western Australia	George (1992); Prangnell and Fotedar (2005); Tantulo and Fotedar (2006)
China		Boyd and Thunjai (2003)
Thailand		Boyd and Thunjai (2003)
Ecuador		Boyd and Thunjai (2003)
India		Jain *et al.* (2002)
USA	Alabama	Boyd and Thunjai (2003); Davis *et al.* (2005); McNevin *et al.* (2004); Saoud *et al*. (2003)
	Arizona	Boyd and Thunjai (2003)
	Florida	Boyd and Thunjai (2003)
	Mississippi	Saoud *et al*. (2003)
	Texas	Boyd and Thunjai (2003); Forsberg *et al*. (1996); Saoud *et al*. (2003)

Although K^+ is vital to survival and growth rates and the osmo-regulation capacity of shrimp and fish, K^+ does not solely affect these mechanisms but does so in conjunction with $[Na^+]$. When K^+ is deficient in ISW, $[Na^+]$ in the haemolymph of shrimp increases which results in shrimp death (Tantulo & Fotedar, 2007). K^+ addition to blue algae cultured media, in accordance with the reduction of Na^+:K^+ ratio, increases the algae biomass (Subhashini & Kaushik, 1986). The low $[K^+]$ breaks the Na^+:K^+ ratio that causes the death of fish (Mourad *et al*., 2012). In ISW, K^+ is one of the three most important cations for the survival rates of *Panaeus monodon* (Shakeeb Ur *et al*., 2005) (Table 2-5, Table 2-8).

Table 2-5. Potassium (mg L^{-1}) range for optimal growth rate of marine species

Species	Range	Ref.
Bostrychia radicans	400–500 at 25ppt	Mourad *et al.* (1980)
Caloglossa leprieurii	200–400 at 15ppt	Yarish *et al*. (1980)
Chrysopkvys major	Dispensable in diet	Sakamoto and Yone (1978)
Argyrosomus japonicus	>50% of [K^+] in OW	Doroudi *et al*. (2006)
Litopenaeus vannamei	Na:K = 40:1–80:1	Perez-Velazquez *et al.* (2012)
Penaeus japonicas	10 g kg^{-1} diet supplementation	Deshimaru and Yone (1978)
Panaeus monodon	200 (at 12.5 ppt)	Shakeeb Ur *et al*. (2005)

2.4 Local Seaweed Species

2.4.1 Taxonomy and Distribution of Six Local Seaweed Species in Western Australia

Seaweeds, including the macroscopic plants inhabiting the intertidal regions of seashore, are algae (Fuhrer, 1981), which are divided into three main groups based upon their colors: the red algae, green algae, and brown algae (Huisman, 2000). In Australia, the red algae (Division Rhodophyta) consists of 1,300 species (out of 5,000–5,500 species worldwide); the brown algae (Division Heterokontophyta, class Phaeophyceae) includes 350 species (out of 1,500–2,000 species worldwide). Approximately, a quarter of the 8,000 species of the world's green algae (Division Chlorophyta) are considered to live in Australia (Huisman, 2000). Of these, six local species, *Grateloupia subpectinata*, *Lomentaria catenata* (a basionym of *Fushisunagia catenata*), *Ulva lactuca*, *Cystophora subfacinata*, *Sargassum linearifolium* and *Sargassum podacanthum* which are native or naturally distributed in Australia in generally, and in WA in particular, were chosen for this study. They were collected from the beaches and rivers of WA (Table 2-11).

Lomentaria genus includes approximately 40 species, three of which are distributed in southern Australia (Womersley, 1996). The *L. australis* grows wildly in Elliston (South Australia), Port Philip Heads (Victoria), and around Tasmania. *L. pyramidalis* can be found naturally in Point Peron (WA) and Flinders (Victoria) (Womersley,

1996). *L. monochlamypdea* is mainly located in Port Stanvac (South Australia), Port Phillip Heads (Victoria), and Coffs Harbour (New South Wales) (Millar, 1990; Millar & Kraft, 1993). The species *L. catenata* was considered as *L. australis* and as *L. ramsayana* from Port Jackson (Millar & Kraft, 1993). In Australia, the *L. catenata* is distributed in New South Wales (Millar & Kraft, 1993) and was first recorded in WA by the author of this study. This species is now moved to a separate genus as *Fushisunagia catenata*, originated in Japan (Filloramo & Saunders, 2016), however, the name *L. catenata* is used throughout the thesis. *L. catenata* is distributed in Korea (Lee, 1978; Yoo *et al.*, 2006), Japan and the Gulf of California (Lee, 1978), China (Guiry & Guiry, 2018).

Grateloupia is the largest genus in Halymeniaceae (Wilkes *et al.*, 2005). Its two main types, *G. subpectinata* and *G. luxurians*, are synonymous based upon their morphology and rbcL sequences (Verlaque *et al.*, 2005). The *G. subpectinata* originated in China and Korea, introduced to Australia initially at the harbour areas (Nelson *et al.*, 2013). It can now be found also in Tauranga, New Zealand (Guiry & Guiry, 2016; Nelson *et al*., 2013). *G. subpectinata* is a native species in WA, mainly distributed in the South West of WA (https://florabase.dpaw.wa.gov.au/browse/profile/36701, accessed on 11 Nov 2016).

Ulva is the widespread genera of the order Ulvales (Sze, 1998). It is difficult to identify *Ulva* to species due to the similarity in blade morphology among the species (Heesch *et al.*, 2009; Kraft *et al.*, 2010; Malta *et a*l., 1999). 50 of the 140 recorded *Ulva* species have been recognized worldwide (Hayden *et al.*, 2003). 562 *Ulva* species are named in AlgaeBase, 98 species of which are taxonomically recorded (Guiry & Guiry, 2016). 6 species (*U. australis* Aresch., *U. compressa* Forssk., *U. faciata* Delile, *U. intestinalis* L., *U. laetevirens* Aresch., *U. tanneri* H. S. Hayden et J. R. Waaland) in the Southern Australia are presented in GenBank accession data (Kraft *et al*., 2010). *Ulva* is naturally distributed in various locations in Australia (Kraft *et al*., 2010) such as WA, New South Wales and Tasmania in the depth of up to five meters below water surface. Southern sea lettuce (*U. australis*) is common in low intertidal and shallow subtidal habitats such as rocky shores or sheltered bays with moderate to strong waves (Morrison & Storrie, 2010). *U. lactuca* is also naturally found all over Australia including WA (Guiry & Guiry, 2016).

According to Womersley (1987, p. 366), *Cystophora* belongs to the Cystoseiraceae Family, while Edgar (2000) places it under phylum Phaeophyta. According to Egda (2000, p. 67), *C. subfarcinata* is a "dominant plant in the shallow subtidal zone of many areas of the southern coast" and grows naturally in Nickol Bay (WA), Wilsons Promontory (Victoria), and around Tasmania. Among the *Cystophora* genus, *C. subfacinata* is considered the most common species in the coast of Southern Australia (Baker & Gurgel, 2010; Womersley, 1987).

Sargassum is distributed all over Australia (Womersley, 1987). Its most common sub-species, *S. linearifolium*, is a shallow low-intertidal to subtidal species (Martin-Smith, 1993) and is commonly found in "in rock pools or the uppermost sublittoral on coasts of strong to moderate water movement" (Womersley, 1987, p. 441). *S. linearifolium* can be found in Port Denison, Houtman Abrolhos (WA), around southern Australia to New South Wale, possibly in the North coast of Tasmania (Baker & Gurgel, 2010; Huisman, 2000; Womersley, 1987). The distribution of the other sub-species of Sargassum, *S. podacanthum*, is narrower, from Point Peron (WA) to Port Noarlunga (South Australia) (Womersley, 1987). The *Sargassum* genus taxonomy is uncertain between and within "species" (Kilar & Hanisak, 1988).

2.4.2 Morphology

The family Lomentarianceaen algae is identified "with hollow thalli divided by multirowed cellular septa. Spermatangial sori are borne on specialized fertile ramuli" (Filloramo & Saunders, 2016, p. 348). Genus *Lomentaria* is described in detail by Womersley (1996) as "erecting or forming entangled clumps, much branched, with or without percurrent axes, branches terete or compressed, hollow, basally constricted with solid septa; holdfast discoid or hapteroid" (p. 134). The Australian species is distinguished by "thallus structure, external cystocarps and depressed tetrasporangial sori" (p135). The *Lomentaria catenata* is now described as *Fushisunagia catenata* (Filloramo & Saunders, 2016). The genus *Fushisunagia*, which is accommodated *L. catenata*, is "uniques from other lomentariaceaen taxa" (Filloramo & Saunders, 2016, p. 351). *L. catenata* is "thallus intertangled, forming a tufted mass, consisting of erect and creeping parts, cylindrical, cartilaginous, branching three to five times, monopodial in growth"… "attaching to substratum be means of discoid holdfast" (Lee, 1978, p. 125).

Grateloupia has the "mature auxiliary cell […] oblong in shape and conspicuously larger (13–15 µm long × 7–8 µm wide) than the other cells in the ampulla" (Faye *et al*., 2004, p. 63). The axes are "flattened (15–40 cm high, 4.5–10 mm wide, up to 1300 µm thick) with the mucilaginous texture, up to 17 cm long, 1–3 mm wide (common on the surfaces, up to 4.5 cm long, 1–2 mm wide)" (Faye *et al*., 2004, p. 61). From a discoid holdfast, up to 28 axes can be arisen (Faye *et al*., 2004).

Order Ulvales are characterized by basal bodies in the counterclockwise direction and the arrangement of micro tubular roots (Sze, 1998). *Ulva* spp. blades are composed of two cell layers, and the length can be up to one meter (Graham *et al*., 2009; Loughnane *et al*., 2008). Only *U. curvata* is attached to the stable substrates by rhizoidal branches (holdfast) (Graham *et al*., 2009; Malta *et al*., 1999; Skinner & Entwisle, 2007). All the other *Ulva* spp. are floating or lying on the sediments (Malta *et al*., 1999). *Ulva* spp. are unstable seaweeds. Their seasonal morphologies change under the environmental conditions (Loughnane *et al*., 2008; Malta *et al*., 1999). Womersley (1984) provides detailed description of the morphology of *Ulva lactuca*.

Cystophora subfarcinata has a special zigzag structure with wide axis ranging from 2 to 7 mm and thin main axis of 1–2 mm and lateral branches (Edgar, 2000; Womersley, 1987). The discoid-conical holdfast is not divided or lacerate. The basic type of *Cystophora* is the arrangement of conceptacles in two rows.

Sargassum have many branches growing from a short stipe (Huisman, 2000, p. 224). For more details of the morphology of *Sargassum*, see Womersley (1987, p. 418–419). The length of *Sargassum*'s thallus is about "0.1–2.0 m, while its stipes are 1–20 cm long from a discoid-conical holdfast". The typical structure of the *S. linearifolium*'s primary branches is terete. "Branches of *S. podacanthum* are also typically terete, but more angular at the top, usually with short, scatted spines, which branch out radically" (Womersley, 1987, p. 418).

Table 2-6. Taxonomy of the studied local seaweed

Taxonomy	***F. catenata* (*L. catenata*)**	***G. subpectinata***	***U. lactuca***	***C. subfarcinata***	***Sargassum* spp.**
Empire	Eukaryota				
Kingdom	Plantae			Chromista	
Phylum	Rhodophyta		Chlorophyta	Ochrophyta	
Subphylum	Eurhodophytina	Eurhodophytina	Chlorophytina		
Class	Florideophyceae	Florideophyceae	Ulvophycae	Phaeophyceae	
Subclass	Rhodymeniophycidae	Rhodymeniophycidae		Fucophycidae	
Order	Rhodymeniales	Halymeniales	Ulvales	Fucales	
Family	Lomentariaceae	Halymeniaceae	Ulvaceae	Sargassaceae	
Genus	Fushitsunagia/Lomentaria	Grateloupia	Ulva	Cystophora	Sargassum
Subgenus					Sargassum

Source: Guiry and Guiry (2018), Womersley (1987)

2.4.3 Reproduction and Growth Rate

Reproduction of *Lomentaria* is carpogonial branches 3-celled, tetrahedrally divided, gametangial thalli dioecious. Its life cycle is triphasic with isomorphic gametophytes and tetrasporophytes (Womersley, 1996). *L. catenata* is tetrasporic. One important character of *L. catenata* is the fertile ramuli development in spermatangium formation (Lee, 1978). Biomass growth is in winter and spring (Yoo *et al*., 2006). In the north hemisphere, the non-growth season is from March to August, and the tetrasporangia or spermatangia appear from September to January, the young cystocarps appear since October to December. In January and February, the branches are regenerated and a quick reduction of the number of fonds is recorded, particularly in March (Lee, 1978).

The *G. subpectinata* grow from the whole thalli except the basal and distal ends (Adharini *et al.*, 2016; Faye *et al*., 2004). The life stages of *Grateloupia* are isomorphic (Kawaguchi *et al.*, 2001), with an alternation of gametophytes and sporophytes (Adharini *et al*., 2016). October and November are the best time for the tetrasporophytes growth, while September and March are the season for the dominance of carposporophytes (Adharini *et al*., 2016).

Ulva spp. reproduction is typically isomorphic alternation of generations (Graham *et al*., 2009; Sze, 1998) and includes the sporophyte and gametophyte phases. Spores are produced in the sporophyte phase by meiosis and anisogametes are produced by mitosis during the gametophyte phase. The zoospores and gametes are produced from the cells in the edge of thallus. Haploid, biflagellate swimming gametes are produced from the haploid *Ulva* spp. individuals (Druehl, 2000). Female gamete is dark green while male gamete is yellow green due to its prominent eyespot (Pettett, 2009). The haploid male and female gametes fuse to become a diploid zygote which then develops to the diploid sporophyte. Then the diploid sporophytes produce quadriflagellate swimming haploid zoospores by meiosis. The zoospores grow into gametophyte of different sexes and they produce male and female biflagellate gametes by mitosis. The haploid form and diploid sporophyte are similar in morphology (Druehl, 2000) (Figure 2-4 (A).

Ulva spore release is dependent on the environmental conditions, particularly light, temperature and salinity (Han *et al.*, 2008). The light requirements for sporulation and

growth are similar. The maximal spore release is found at the light level of >30 µmol photon m^{-2} s^{-1}, pH level of 7–9, salinity level of 25–35 ppt and temperature from 15–20°C (Han *et al*., 2008). *Ulva* spp. has high reproduction rate, and can produce swarmers all year round from their tissues (Ramus & Venable, 1987).

Reproduction of *Cystophora* is sexual, oogamous and diplontic (Hotchkiss, 1999). Its sub-species, *C. subfacinata*, reproduces all year round (Shepherd & Edgar, 2013). The conceptacles of *C. subfacinata* are biosexual or occasionally unisexual, "with the ostioles scattered or in two rows near the base" and "thalli monoecious", "receptacles simple or often branched" (Womersley, 1987, p. 400). The growth rate of *C. subfacinata* shows no seasonal changes. It has two stages of growth, the length growth is in summer-autumn and the tissues is reproductive after the autumn (Hotchkiss, 1999; Shepherd & Edgar, 2013) (Figure 2-4 (B).

The *Sargassum*'s growth and development population are seasonal (Vuki & Price, 1994) and varies according to the species (McCourt, 1984). The fertile receptacles of temperate *Sargassum* are shed in summer and the tropical *Sargassum* is abundant in winter (McCourt, 1984). The annual life cycle of *S. yezoense* peaks in length and density in May–June when the water is about 12–15°C and is at its lowest in September–December, when the temperature is about 16–20°C. The germination and the maturation of *S. yezoense* peak when the maximum water temperature is 22°C, in June to August, and then decline as the temperature drops. The germination picks up again in spring when the temperature is about 15°C (Agatsuma *et al.*, 2002). *S. fulvellum* releases the eggs in early summer (March–April) (Hwang *et al.*, 2007). For the *S. baccularia*, the annual growth phase is in spring (Schaffelke & Klumpp, 1998). The maximal biomass and reproduction of Australian *Sargassum, S. tenerrimum, S. fissifolium, S. olygocystum* attain in December–February and March–May. Differently, only *S. linearifolium* receives the peak biomass in June–September and reproduction from September to January (Martin-Smith, 1993).

The reproduction of *S. linearifolium* is described as

> "Thalli monoecious. Receptacles unisexual or bisexual, forming dense clusters 3–10 mm long. Much branched furcately or laterally, terete, 0.6–1.2 mm in diameter, drying slightly verrucose apices rounded, with scattered ostioles. Conceptacles unisexual; oogonia sessile, ovoid to subspherical, (100–) 160–240 µm long and

(60–) 90–220 µm in diameter, few per conceptacle; antheridia sessile or on short, branched paraphyses, avoid, (18–) 20–28 µm long and (8–) 10–18 µm in diameter" (Womersley, 1987, p. 441).

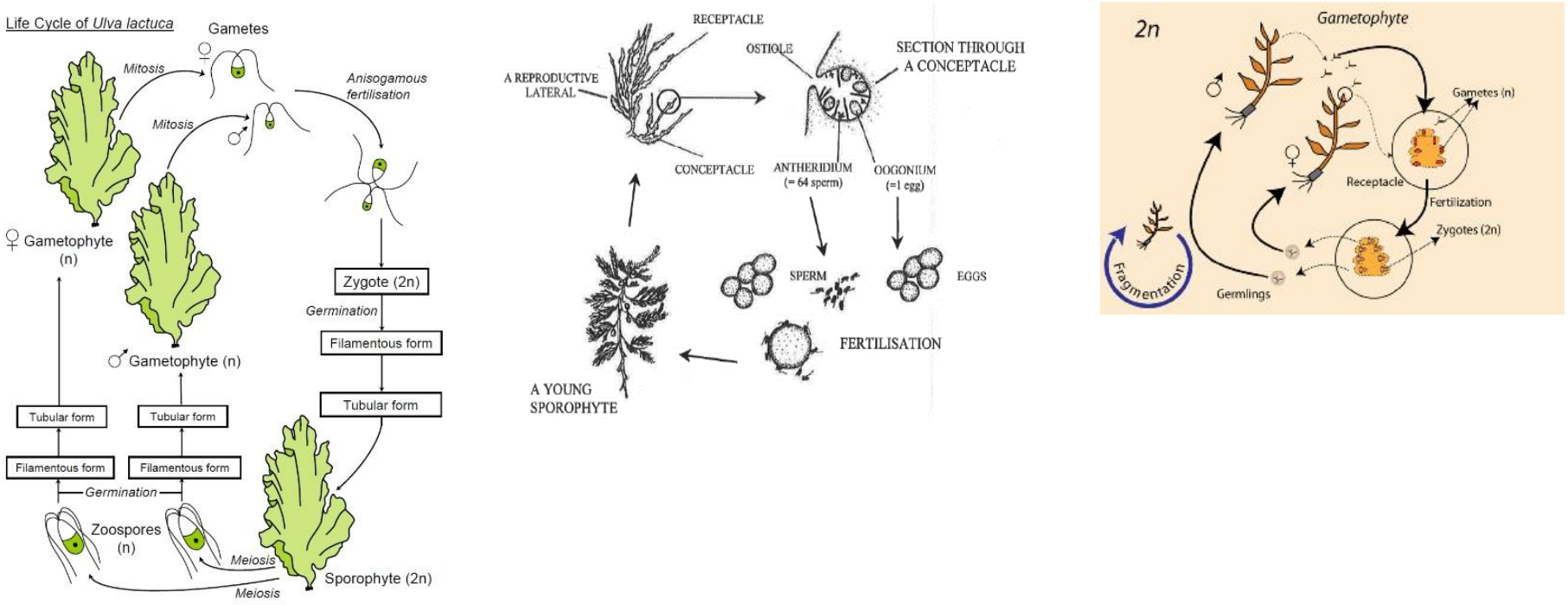
S. podacanthum is also thalli monoecious with biosexual receptacles, simple or branched. Its conceptacles are unisexual (p. 444), similar to those of *S. linearifoliu*m (Womersley, 1987) (Figure 2-4 (C).

The specific growth rate (SGR) of *Sargassum* is significantly affected by temperature and nutrients (Hwang *et al.*, 2004). The SGR of *S. baccularia* in continuous nutrient supply of 0–22 µM ammonium in 30 days is from 2.5% to 9% d^{-1}. At the field, the SGR of *S. baccularia* thalli are high from October to May, reaching their maximum of 3% d^{-1} from December to March, so the nutrient requirement is also higher than in winter (Schaffelke & Klumpp, 1998). The SGR of *G. subpectinata* reaches 2.4–2.8% d^{-1} at 20^{o}C (Adharini & Kim, 2016) (Table 2-12).

2.4.4 Seasonality of Local Seaweed Species

L. catenata growth season is from September to December in Japan, at the same time with the development of the new branches from tetraporangia (Lee, 1978). The *G. subpectinata* reaches the maximal growth in September in Korea and continues growing in autumn and winter (Adharini *et al.*, 2016). *Ulva*'s growing season is summer, when longer photoperiod and high temperature are expected (Ramus, 1978; Vermaat & Sand-Jensen, 1987). *Ulva* spp. die in winter under the icing surface, bury in the bottom of the water body and bloom again in the summer (Kamermans *et al.*, 1998). Sometimes, *Ulva* spp. can survive up to two months under the dark during winter in European coastline, and resume to grow when re-exposed to the light (Kamermans *et al.*, 1998). *U. lactuca* can grow and photosynthesize at the minimum range of 0.6–1.7 µE m^{-2} s^{-1} (Vermaat & Sand-Jensen, 1987).

In Australia, the *Cystophora*'s standing biomass reaches its peak in spring and declines in summer. Unlike *Cystophora, C. subfarcinata* achieves its maximal biomass in late winter and shrinks in spring (Hotchkiss, 1999). *S. linearifolium* also grows well in late winter, but reaches its maximal size and reproduction rate in spring (Martin-Smith, 1993). In contrast, other *Sargassum* spp. starts growing in summer, reaching its maximal growth rate in autumn. More specifically, its length peaks from January to March and bottoms out from July–September (Martin-Smith, 1993).



(A) **(B)** **(C)**

Figure 2-4. The life cycles of (A) *Ulva lactuca, (B) Cystophora, (C) Sargassum fusiforme*

(Source: (A) http://cronodon.com/BioTech/Algal_Bodies.html; downloaded 24/07/2012, 1:56PM), (B) Hotchkiss (1999), (C) Bast (2014)

Table 2-7. The growth rate of some seaweed species

Species	Place	Growth rate	Condition	References
G. subpectinata	Lab	2.38–2.83% d^{-1}	20°C, 40 µmol photon m^{-2} s^{-1} irradiance, 12:12 light:dark	Adharini and Kim (2016)
S. cymosum	Lab	0.0944±0.0105*	24ºC, 150–600 PDF µE m^{-2} s^{-1}	Hanisak and Samuel (1987)
S. filipendula	Lab	0.1071 ±0.0030*	30ºC	Hanisak and Samuel (1987)
S. fluitans	Lab	0.1089±0.0027*		Hanisak and Samuel (1987)
S. fluitans	Neritic	0.041–0.091*		Lapointe *et al*. (2014)
S. honeri	Ocean	4.6% d^{-1}		Gao and Hua (1997)
S. natans	Lab	0.0727 ± 0.0049*	24ºC, 36–42 ppt; 300–600 PDF µE m^{-2} s^{-1}	Hanisak and Samuel (1987)
S. natans	Neritic	0.031–0.093*		Lapointe *et al*. (2014)
S. natans	Ocean	0.005–0.020*		Lapointe *et al*. (2014)
S. polyceratium	Lab	0.0787 ±0.0153*		Hanisak and Samuel (1987)
S. pteropleuron	Lab	0.1117 ± 0.0080*	18ºC, 24–36ppt	Hanisak and Samuel (1987)
U. rigida	Field	0.75–2.91% d^{-1}	15–21ºC, 30–37ppt, 256–854 µE m^{-2} s^{-1}	de Casabianca *et al*. (2002)
U. lactuca	Lab	54.42±3.82 mg d^{-1}	22±2ºC, 14:10 light:dark	Kaladharan and Gireesh (2003)
U. lactuca	Lab	0.013–0.0014*	17 µE m^{-2} s^{-1}	Vermaat and Sand-Jensen (1987)
		0.042–0.043*	75 µE m^{-2} s^{-1}	
		0.094–0.121*	563 µE m^{-2} s^{-1}	
			Natural and inorganic N-enrich 0.56 mg L^{-1} water	
U. lactuca	Lab	16.4±0.18% d^{-1}	50 µM N by NH_4Cl	Ale *et al.* (2011)
		9.40±0.72% d^{-1}	50 µM N by $NaNO_3$	

(*) (doublings d^{-1})

Table 2-8. The optimal environmental factors for seaweed growth

Factors	Range	Result	References
Salinity			
U. lactuca	35–40	Optimal	Friedlander (1992)
U. lactuca	17–34	Cultured germlings and young blades grow well	Koeman and van den Hoek (1981)
S. thunbergia	30	Optimal growth	Cui *et al*. (2014)
S. ilicifolium	30	Oospore/receptacle/day: optimal	Ragaiah *et al.* (2012)
S. wightii	30–40 (25–35ºC)	Maximum shedding of oospores	Sukumaran and Kaliaperumal (2000)
S. muticum (Yendo)	34	Optimum growth	Hales and Fletcher (1989)
Temperature (ºC)			
G. subpectinata	16–22	Maximal growth	Adharini *et al*. (2016)
U. lactuca	10	Optimum growth	Malta *et al*. (1999)
S. thunbergii	22	germling growth 7–9% day^{-1}	Li *et al.* (2014)
S. sandei	25	Maximum growth	Hwang *et al*. (2004)
S. berberifolium	20–25		
S. polycystum	25		
S. siliqousum	30		
Light			
U. lactuca	0.6–1.7	Optimum growth	Vermaat and Sand-Jensen (1987)

Table 2-9. Water quality parameters for growth of seaweed

Parameter	Species	Range	Results	References
NH_4-N	*Ulva* sp.	Winter: max 3.6	Optimal growth	Campbell (2001)
NH_4-N	*Ulva* sp.	7.8 µM m^{-2} d^{-1}	Optimal growth	Bartoli *et al.* (2005)
NH_4-N	*U. curvata*		Significantly correlated	Ramus and Venable (1987)
NH_4-N	*S. enerve*	200 (N:P=16:1)	Increase 0.70g	Liu *et al.* (2004)
NH_4-N	*S. hemiphyllum*	3.97±0.81 µM	0.28% d^{-1} in length, 1.65% d^{-1} in weight	Yu *et al.* (2013)
NH_4-N	*S. hemiphyllum*	0.5±0.24 µM	0.92 % d^{-1} in length, 0.62% d^{-1} in weight	Yu *et al*. (2013)
NH_4Cl	*S. horneri*	50–100 mg L^{-1}	Inhibit germination	Ogawa (1984)
NH_4-N	*S . hemiphyllum*	25 mg L^{-1}	Inhibit germination	Ogawa (1984)
NH_4-N	*S. thunbergii*	25 mg L^{-1}	Inhibit germination	Ogawa (1984)
NO_3^--N	*S. enerve*	200 (N:P=16:1)	Increase 0.56 g	Liu *et al*. (2004)
NO_3^--N+NH_4-N	*S. sandei*	12 µM	Maximum growth	Hwang *et al*. (2004)
PO_4^{3-}-P		0.6 µM	Maximum growth	Hwang *et al.* (2004)
NO_3^--N+NH_4-N	*S. baccularia*	3–5 µM	Maximum growth	Schaffelke and Klumpp (1998)
PO_4^{3-}-P	*S. baccularia*	0.3–0.5 µM	Maximum growth	
K^+	*Ulva sp.*	400–500 mg L^{-1}	Maximum growth	Yamashita *et al.* (2009)
PO_4^{3-}-P	*U. reticulate*	10 µM PO_4^{3-}-P + 50 µM NO_3^--N		Ahmad *et al*. (2011)

Table 2-10. Proximate compositions of seaweed (% dry weight)

Species	Carbohydrate	Fibres	Protein	Sugar	Lipid	Ash	References
G. turuturu		60.4	22.9		2.6	18.5	Denis *et al.* (2010)
G. turuturu	1.61–4.16		16.2–21.8		2.81–5.44	14.4–15.9	Munier *et al.* (2013)
G. doryphore	41.82–54.72		22.9–30		0.81-1.3	6.98–11.85	Perfeto (1998)
S. echinocarpum	10.5±1.3		10.3±0.7		3.8±0.2		McDermid and Stuercke (2003)
S. hemiphyllum		56.8–62.9	9.76–10.1		3.04–4.42	19.6–21.5	Chan *et al.* (1997)
S. horneri			1.00		0.10	3.4	Murakami *et al.* (2011)
S. longifolium	16.8±0.7		18.65±1.21		8.2±1.57		Narasimman and Murugaiyan (2012)
S. naozhouense	47.73	4.83	11.20		1.06	35.18	Peng *et al.* (2013)
S. obtusifolium	12.3±1.1		13.0±1.1		2.6±0.2		McDermid and Stuercke (2003)
S. oligocystum		9.40±1.39	5.64±0.19			13.8±2.74	Muraguri *et al.* (2016)
S. polycystum		8.47±1.21	5.40 ± 0.07		0.29±0.01	42.40±0.41	Matanjun *et al.* (2009)
S. vulgare							
S. wightii	25.5±1.37		16.59±0.86				Murugaiyan *et al.* (2012)
Ulva spp. (hot water extraction)			4.30	41.70		23.70	Lahaye and Axelos (1993)
U. lactuca (insoluble fraction)			40.50	36.10		9.40	Lahaye *et al.* (1994)
U. lactuca (soluble fraction)		15.80	16.80			23.00	Lahaye and Jegou (1993)
U. lactuca			7.06		1.64	21.30	Wong and Cheung (2000)
U. lactuca		54.00	8.46		7.90	19.59	Yaich *et al.* (2011)
U. lactuca			10–21				Castro-González *et al.* (1996)

Species	Carbohydrate	Fibres	Protein	Sugar	Lipid	Ash	References
U. fasciata	17.1–20.6		8.8–12.3		3.6-5.1	25.4–32.2	McDermid and Stuercke (2003)
U. fasciata		7.10±0.32	10.06±0.90			19.92±3.42	Muraguri *et al*. (2016)
U. reticulate	15.37 ± 0.41		13.47±0.60				Manivannan *et al.* (2009)
U. fasciata	70.1		14.7		0.5		Rameshkumar *et al.* (2012)
Ulva sp.		6.9±4.1	18.6±7.3			23±7.4	Makkar *et al.* (2016)
U. compressa		26.62	41.16				Patarra *et al.* (2011)
U. stenophylla	66.9±1.66		20.43±4.85		1.24±0.59	22.1±0.88	Smith *et al.* (2010)

Table 2-11. Mineral composition (% dry weight) of seaweed species

Species	N	P	K	Mg	Na	Ca	S	References
G. doryphora		0.075–0.20	0.16–1.27					Perfeto (1998)
G. lithophila			3.82		6.56			Sivakumar and Arunkumar (2009)
S. echinocarpum	1.53	0.14	9.50	1.16		1.31	1.16	McDermid and Stuercke (2003)
S. hemiphyllum			0.06	0.01	0.01	0.02		Chan *et al*. (1997)
S. horneri				1.21–1.98		1.03–1.47		Murakami *et al*. (2011)
S. longifolium			6.38		6.10			Sivakumar and Arunkumar (2009)
S. myriocystum			12.14		5.60			Sivakumar and Arunkumar (2009)
S. muticum			7.46	1.94	1.39	1.68		Gorham and Lewey (1984)
S. obtusifolium	1.67	0.14	7.90	0.93		1.50	1.41	McDermid and Stuercke (2003)
S. polycytum			8.37	0.50	1.36	3.79		Matanjun *et al*. (2009)
S. vulgare	2.00±0.11							Lourenço *et al.* (2002)
S. wightii			3.59		4.63			Sivakumar and Arunkumar (2009)
Ulva sp.		2.7±2.1	22.1	16.7±3.2		29.2±28.9		Makkar *et al*. (2016)
U. fasciata	3.62–3.74	0.22	2.87–3.15	2.19–2.94		0.39–0.47	5.24–5.51	McDermid and Stuercke (2003)
U. lactuca			2.25		2.59			Sivakumar and Arunkumar (2009)
U. reticulate			5.04		8.85			Sivakumar and Arunkumar (2009)
U. stenophylla			7.9±3.9	192.3±142.1	1.9±1.8	12.9±3.6		Smith *et al*. (2010)

Table 2-12. Heavy metals composition (mg kg^{-1}) in seaweed

Species	Cu	Zn	Fe	Mn	References
G. doryphora	14.0±1.0	300±100	295±123		Caliceti *et al.* (2002)
Sargassum sp. (at Nha Trang Bay)	1.9–4.0	6.2–46.5	93–779	17.9–284.3	Chernova and Sergeeva (2008)
Sargassum sp. (at the Great Bay-Japan)	1.6–4.9	12.5–27.3	155–549	8.8–965.0	Chernova and Sergeeva (2008)
Sargassum sp.			813–2895		García-Casal *et al.* (2007)
S. binderi	10.2	2.8	31	3.6	Al-Shwafi and Rushdi (2008)
S. boveamum	17.2	7.4	60	5.4	Al-Shwafi and Rushdi (2008)
S. echinocarpum	11.0	7.0	92	6.0	McDermid and Stuercke (2003)
S. hemiphyllum	0.30	1.4–1.8	19.4–26.0	1.7–2.0	Chan *et al*. (1997)
S. horneri		3.49–5.52			Murakami *et al*. (2011)
S. longifolium	2.21	1.79	69.05	2.82	Murugaiyan and Narasimman (2012)
S. obtusifolium	9.0	16.0	129	15.0	McDermid and Stuercke (2003)
S. polycytum	0.30	21.5	682.1		Matanjun *et al*. (2009)
Enteromorpha compressa (at Romel)	65.7	58.5	3866		Khaled *et al.* (2014)
E. compressa (at El-Boussit)	4.0	5.0	1284		Khaled *et al*. (2014)
E. compressa	17.5	8.1	36	12.9	Al-Shwafi and Rushdi (2008)
E. compressa	13.8–20.1	37.8–47.2	449–1628	134.9–166.7	Abdallah and Abdallah (2008)
Enteromorpha sp. (southwest Sardinia)	1.9–41.3	28.2–722.0			Schintu *et al.* (2010)

Species	**Cu**	**Zn**	**Fe**	**Mn**	**References**
Ulva sp.	7–17	28–61	1052–1440	101.0	Makkar *et al*. (2016)
U. fasciata	1.0–5.0	6.0–9.0	86–141	12.0–17.0	McDermid and Stuercke (2003)
U. lactuca	7.2–14.5	27.4–63.1	515–709	33.2–74.0	Abdallah and Abdallah (2008)
U. rigida (southwest Sardinia)	1.9–4.2	28.2–50.7			Schintu *et al*. (2010)
U. rigida	3.1–3.2	5.6–6.1			Besada *et al.* (2009)
U. rigida C. Ag.	13.0±7.0	64.0±55.0	1033±564		Caliceti *et al*. (2002)
U. stenophylla	11.0±2.8	61.0±22.7	1227±522	192.3±142.1	Smith *et al*. (2010)

Table 2-13. Carbon, nitrogen and phosphorus concentration (% dry wt) and total C:N:P ratios in seaweed

Species	N	P	N:P	C:N:P	References
S. baccularia	0.65–1.05	0.05–0.12			Atkinson and Smith (1983)
S. berberifolium	1.67	0.12	14:1		Hwang *et al*. (2004)
S. echinocarpum	1.32 ±0.08	0.08±0.01	38:1		Larned (1998)
S. fluitans				271:10:1 (neritic water)	Lapointe *et al*. (2014)
S. fluitans				875:19:1 (oceanic water)	Lapointe *et al*. (2014)
S. natants				268:10:1 (neritic water)	Lapointe *et al*. (2014)
S. natants				719:17:1 (oceanic water)	Lapointe *et al*. (2014)
S. polycystum	1.99	0.15	13:1		Hwang *et al*. (2004)
S. sandei	1.38	0.16	9:1		Hwang *et al*. (2004)
S. siliqousum	2.36	0.19	12:1		Hwang *et al*. (2004)
S. siliquosum				1278:33:1	Martin-Smith (1993)
U. fasciata	2.69±0.29	0.12±0.01	48:1		Larned (1998)

2.4.5 Environmental Variables Affecting Seaweed Growth

2.4.5.1 Salinity

Salinity is the most important parameter that has a significant effect on seaweeds' growth, photosynthesis, chlorophyll-a content, spore biomass and their ability to absorb heavy metals (Kamer & Fong, 2001; Mamboya *et al*., 2009; Scherner *et al*., 2013; Sousa *et al*., 2007). Seaweeds' tolerance to salinity is dependent on the species. *Grateloupia* is able to resist the high variation of salinity in short-term, and grows well in 25–37ppt (Simon *et al.*, 2001; Simon *et al*., 1999). *Ulva* grows well in salted water of 5–40ppt (Choi *et al*., 2010; Kamer & Fong, 2001). Salinity toleration of *U. lactuca* and *U. ridiga* can be wide but these species prefer open-sea water (Friedlander, 1992; Zavodnik, 1975). On the other hand, *U. curvata*, *U. scandinavica* and *U. rigida* thrive in polyhaline water such as man-made lagoon and estuarine areas, but not in the open-sea water (Koeman & van den Hoek, 1981). Generally, salinity level of 30 ppt is supposed to be ideal for most of the *Ulva* species (Malta *et al*., 1999). The minimal salinity level that *U. scandinavica* can tolerate is 5 ppt, while at this level *U. curvata* would die (Malta *et al*., 1999). Like *Ulva*, *Sargassum* also grows well in a broad range of salinity levels, from 24 to 42 ppt (Hanisak & Samuel, 1987), but prefers salinity levels of 30–34 ppt for optimal growth (Cui *et al*., 2014; Hales & Fletcher, 1989) (Table 2-13).

The salinity of ISW in Australia widely varies with two-thirds of those areas has salinity 5–40 ppt (Mazor & George, 1992) are in the range of suitability for the above seaweed species to grow.

2.4.5.2 Temperature and pH

The effect of temperature on the growth of seaweeds varies depending on the species. The *G. subpectinata* prefers high temperature, growing best in hot seasons (summer and autumn), albeit with less density (Adharini *et al*., 2016). *Ulva*, on the other hand, grows better in the cooler weather, with temperature ranging from below zero up to 25^{o}C, depending on the species. *U. rigida*, for example, grows well in the temperature range of 7 to 25^{o}C and reaches the highest growth rate at 17^{o}C (de Casabianca *et al*., 2002), while the *U. curvata*'s growth peaks at 25^{o}C and decreases when the temperature is lower. The optimal temperature for the growth of *U. lactuca*, *U. rigida*

and U. scandinavica is 10ºC (Malta *et al*., 1999). *U. scandinavica* survival is recorded at -5ºC for 2 weeks in the darkness, anoxia and sulphide contact and can live under the freezing condition of winter (Kamermans *et al*., 1998; Vermaat & Sand-Jensen, 1987).

The effect of the temperature on *Sargassum* also varies significantly depending on the species. The ideal temperature for *S. thunbergii*'s germling growth is 22ºC (Li *et al*., 2014) with the germlings SGR in the range of 7–9% d^{-1}. At 15ºC, *S. horneri*'s optimal SGR is 5% d^{-1} (Choi *et al.*, 2009; Yamauchi, 1984), and at 20–25ºC, SGR is negative after 12 days of cultivation (Choi *et al*., 2009). The optimal growth of *S. muticum* is achieved at 25ºC (Hales & Fletcher, 1989), and *S. patens* grows best at 20–30ºC (Endo *et al.*, 2013) (Table 2-13).

The pH of water is another factor influencing the growth of seaweeds. Red seaweed *Gracilaria tikvahiae* prefers the pH level of 7.0–8.0 for high production (Lignell & Pedersén, 1989), and the *G. secundata* reaches its maximum growth rate at pH 8.0 (Skirrow, 1975). The maximal growth rate of *G. manilaensis* is recorded at pH 7.6–7.8 at 1.3% d^{-1} (Hidayat *et al*., 2015). *S. fulvellum* quantum yield is similar in the pH of 4–10 (Hwang *et al.*, 2006). A pH level of below 4 inhibits the zygote germination of *S. honeri*, which prefers the pH level of 5–10 (Ogawa, 1984). *Ulva* can grow in a high pH environment (Beer & Israel, 1990). However, when the pH is above 9, the growth of *Ulva* is negatively affected (Berndt, 1991; Maberly, 1992).

The pH also affects seaweeds' photosynthesis through the appearance of CO_2 or HCO_3^- (Aizawa & Miyachi, 1986; Drechsler & Beer, 1991), which in turn influences the growth of seaweed (Chen & Durbin, 1994). The concentration of CO_2 in air and water is 0.03–0.04% and 10–12 µmol at 25°C, respectively (Aizawa & Miyachi, 1986) and 14 µmol m^{-3} at 15°C (Axelsson *et al.*, 1995; Beer & Israel, 1990). These variations are caused by the changes in temperature and pH (Aizawa & Miyachi, 1986). Seaweeds have a CO_2-concentrating system, and CO_2 is the main source feeding into the cells through plasmalemma (Beer & Israel, 1990). However, HCO_3^- is the main source of carbon for photosynthesis of macroalgae in the high pH condition of OW (Björk *et al.*, 1992). Therefore, HCO_3^- concentration strongly affects seaweed photosynthesis (Maberly, 1992). The pH also affects the ion absorption of seaweed (Basha & Murthy, 2007). The metal absorption of *Sargassum* peaks at pH 4.5 (Davis *et al*., 2000; Figueira *et al*., 1997).

The temperature and pH are vital in the growth of seaweeds (Cui *et al*., 2014; Ding *et al*., 2013), and those factors vary widely in ISW in Australia (Nulsen, 1997; Nurmi *et al*., 1988; Taukulis & John, 2009). Currently, there is no information recorded about the effect of those factors to the seaweed growth in ISW yet. In addition to the species dependent effect, different species of seaweeds would need to be tested to finalize their growth ability in ISW, as well as the similarity and differences of temperature and pH effects in OW and ISW to seaweed growth.

2.4.5.3 Nutrients Uptake Capacity of Seaweeds

In the OW, the most common type of ammonia (NH_3-N) is ammonium (NH_4-N) (Burgess *et al*., 2003) which is the most preferable nitrogen source of *Ulva* (Ahmad *et al*., 2011; Liu *et al*., 2004) and *Sargassum* (Liu *et al*., 2004). *Ulva* requires high NH_4-N concentration for growing (Campbell, 2001), and the growth of *Ulva* is significantly correlated with NH_4-N uptake (Ramus & Venable, 1987). Nitrogen (N) required for maximal growth of *Ulva* in winter is 3.6 μM (Campbell, 2001). *Ulva* can consume up to 7.8 mmol m^{-2} of N per day^{-1} (Bartoli *et al*., 2005). The presence of 10 μM phosphate (PO_4^{3-}-P) in media of 50 μM NO_3^--N or 50 μM NH_4-N increases the *U. reticulata*'s capacity to absorb NO_3^--N/NH_4-N, better than that in the environment where NO_3^--N or NH_4-N is solely presented (Ahmad *et al*., 2011). *U. lactuca*'s ability to accumulate chlorophyll is positively linear with the nitrogen concentration in water (Ahmad *et al*., 2011). Nitrogen required for the maximal growth of *Ulva* in winter is 3.3 mg g^{-1} dry weight d^{-1} (Campbell, 2001). *U. lactuca* can reduce the NH_3-N concentration in water similar to zeolite (Burgess *et al*., 2003). The *Sargassum*'s growth rate drops when the continuously supplied NH_4-N is out of the range of 3–5 μM (Schaffelke & Klumpp, 1998) (Table 2-14).

As the importance of the NH_4-N and PO_4^{3-}-P in the growth of the seaweed, it is worth to value the effect of those parameters into the growth of seaweed in ISW to test the growth feasibility of seaweeds in ISW and their optimal growth condition for marine aquaculture application. In addition, there may be some differences of the effects of NH_4-N and PO_4^{3-}-P in OW and ISW on the seaweed growth that may take into account to determine.

2.4.5.4 The Role of Potassium in Seaweed Growth

K^+ cannot be replaced physiologically by other chemicals for algae growth (Yarish *et al*., 1980). The [K^+] makes up 1–2 % of dry biomass of plants (Evans & Sorger, 1966) and it plays a crucial role in the growth of the terrestrial plants (Blumwald *et al.*, 2000; Talling, 2010). It has been found to have an effect on the growth of *Platymonas subordiformis* and marine red alga *Porphyra leucosticta* (Escassi *et al.*, 2002; Kirst, 1977). It has also been recognised as an important internal cation in algae (Kirst, 1977), which accounts for 85 and 59% of the tropical seaweed *Eucheuma cottonii* and *Sargassum polycystum* major cations, respectively (Matanjun *et al*., 2009). In brown seaweeds, such as *Fucus vesiculosus, Laminaria digitata* and *Undaria pinnatifida,* K^+ accounts for 37, 67 and 49% of their internal total cations, respectively, whilst in red algae such as *Chondrus crispus* and *Porphyra tenera* the figures are 37 and 43%, respectively (Rupérez, 2002). The K^+ content can make up 3.2% of the dry weight of *Ulva*, and the tissue K^+ maybe 20 times higher than its concentration in OW (Yamashita *et al*., 2009). *U. ohnoi* grows best in OW, where the Na:K ratio is 47:1, and cannot grow where the Na:K ratio is 2:1 (Yamashita *et al*., 2009).

The physiological dependence on the K^+ to support seaweed growth is comparable to higher plants. In the higher plants, the role of K^+ is important in photosynthesis and respiration by activating several enzymes to synthesise protein and carbohydrates (Checchetto *et al.*, 2013). The intracellular [K^+] is regulated by K^+ exchange mechanism between internal and external [K^+] (Blumwald *et al*., 2000; Tromballa, 1978). During this regulatory process, the [K^+] in the outside medium determines a gradient between the internal and external environment of the cells, allowing the exchange between Na^+ and K^+. The different gradient is an important mean of nutrient transport within intracellular cells (Blumwald *et al*., 2000). The K^+ plays an important role in maintaining an osmotic gradient of aquatic plant cells (Malhotra & Glass, 1995) facilitated by keeping a normal ratio between Na^+ and K^+ internally, i.e. high K^+ (100–200 mM) and low Na^+ (1–10 mM) (Blumwald *et al*., 2000). The H^+-ATPase activities are also highly affected by [K^+] (Sekler & Pick, 1993), it allows active extrusion of H^+ out of cells which generate gradient to induce the Na^+/H^+ antiporters that move the H^+ into the cells while at the same time extrudes Na^+ out of cells (Blumwald, 2000; Blumwald *et al*., 2000).

A reduction in [K^+] of the external culture medium of estuarine red algae *Bostrychia radicans* Montagne and *Caloglossa leprieurii* (Montagne) J. Agardh leads to a reduction in their intracellular [K^+] (Yarish *et al*., 1980). A lower cells [K^+] (66.2–90.5 mmol) is exhibited when the external [K^+] ranges from 0.008 to 0.390 mmol (Allison & Walsby, 1981). Conversely, rising external [K^+] increases the [K^+] accumulation rate in *Anabaena flosaquae* cells up to 20 folds (107.6 mmol) compared with the initial external [K^+] (3–6 mmol) (Allison & Walsby, 1981).The [K^+] required for the protein synthesis is in the range of 100–150 mmol (Blumwald *et al*., 2000). In unicellular marine algae, [K^+] is accumulated internally and can be up to around 14 folds (110–120 mmol) of its external concentration (8.2 mmol) in lower salinity containing NaCl 0.1–0.3 mol. The [K^+] in cells rises up 25 folds (210 mmol) when external NaCl increases to 0.4–0.6 mol (Kirst, 1977).

Although the importance of the K^+ is recorded in the growth of seaweeds in OW, the efficient K^+ level in ISW to seaweed growth has not been studied yet, whereas the K^+ deficiency is common in ISW in Australia (Boyd *et al*., 2007; Fotedar *et al*., 2011; Ingram *et al*., 2002; Partridge & Lymbery, 2008; Saoud *et al*., 2003). In the purpose of growing seaweed in ISW, it is vital to determine the effect of K^+ levels in ISW to the growth of seaweeds, which is now still lacking in literature. Those information gaps will be filled by this study up to six species of seaweeds.

2.4.6 Proximate and Ionic Compositions of Seaweeds

The proximate and chemical compositions of seaweed varies with seasons and species (Perfeto, 1998). The nitrogen of *L. catenata* tissue is varied from 2.7–3.8% depending on the dissolved inorganic nitrogen concentration of water (Kim *et al.*, 2014). In autumn and winter, higher concentrations of protein, ashes, and phosphorus are recorded in *Grateloupia doryphore* whereas the carbohydrate reaches its maximal value in summer (Perfeto, 1998).

Four main components are fibre, ash, protein, and lipid, which account for 54, 20, 8.5, and 8%, respectively, in *U. lactuca* (Yaich *et al*., 2011). The total fibre content (soluble and insoluble extraction) is about 40% of the dry weight of *U. lactuca* (Lahaye & Axelos, 1993). Of the aqueous extraction of *U. lactuca*, sugars accounts for 36–42%, including gluco, sylose, rhamnose, and anduronic acid at 57, 11, 9, and 7 mol%, respectively (Lahaye & Axelos, 1993).

Sulphate share is 41.5% of the ash and 16% of extraction in the dry weight of the *Ulva* (Lahaye & Axelos, 1993). The dried *Ulva* also contains 12.2% polysaccharides (Lahaye & Axelos, 1993; Lahaye & Jegou, 1993), which is also known as Ulvan (Lahaye & Axelos, 1993; Ray & Lahaye, 1995). *Ulva* also contains vitamins, trace elements, enzymatic resistant proteins in their fibres (Lahaye & Jegou, 1993; Ray & Lahaye, 1995) and all the essential amino acids (except tryptophan) (Wong & Cheung, 2000; Yaich *et al*., 2011).

The total lipid and fatty acid of *Ulva* is 1.85–1.95 and 0.54–0.71 percent of dry weight, respectively (Fleurence *et al.*, 1994). Palmitic acid and oleic acid share in *Ulva* fatty acids is 60 and 16% of, respectively (Yaich *et al*., 2011). In *U. rotundata* neutral lipids, glycol lipid, and phosphor lipid account for 46, 38 and 16%, respectively of the total lipid (Fleurence *et al*., 1994).

The tissue-N of *Ulva* in Port Phillip Bay (WA) is 43.78 and 28.27 mg g^{-1} dry weight in the winter and summer, respectively (Campbell, 2001). Potassium and sodium contents in *Ulva* are about 0.21 and 0.089 M, respectively, depending on fresh weight specimen. In addition, the contents of potassium and sodium in *Ulva* are stable, and they are not affected by salinity and Na/K ratio in water (Yamashita *et al*., 2009).

The proximate compositions of *Sargassum*, likewise, vary widely among species, with protein ranging 5.4 to 14% of protein and lipid from 0.29 to 3% (Matanjun *et al*., 2009; McDermid & Stuercke, 2003). The $[K^+]$ in *Sargassum* ranges from 7.9–9.5% of dry weight (McDermid & Stuercke, 2003), much higher than in *Ulva* and *Grateloupia*, however the tissue-N and tissue-P are much lower (McDermid & Stuercke, 2003; Perfeto, 1998). These parameters will be measured in seaweeds culturing in ISW to see the effect of ISW to the $[K^+]$, tissue-N and tissue-P of the seaweeds, under the different condition of temperature, pH and K^+ levels (Table 2-15, Table 2-16, Table 2-17, Table 2-18).

2.4.7 Uses and Benefits of seaweeds

Seaweeds provide organic carbon for aquatic food chain (Duarte, 1995; Graham *et al*., 2009). They can be used as food, produce phycocolloid, for agriculture (Lindsey Zemke-White & Ohno, 1999), and can also be used as a biofilter for aquacultured species (Neori *et al*., 2004).

2.4.7.1 As a Source of Food and Medicine

Seaweeds have been used as a healthy source of protein for Asian people for centuries, gradually introduced into the western markets as nori rolls, salads, and agar gels (Lee, 2008).

The red seaweed *Grateloupia*, including *G. subpectinata*, is used as a high price source of food in Korea (Adharini *et al*., 2016). So is *G. filicina*, which has a potential antioxidant activity (Athukorala *et al.*, 2003).

Ulva is more widely used for human food and medicine in Vietnam, Italy, Portugal, Argentina, Canada, Chile, Hawaii, Japan, Malaysia, Indonesia and Philippines (Lahaye *et al*., 1994; Lindsey Zemke-White & Ohno, 1999; Sze, 1998). *U. lactuca*, which contains similar protein and energy to Lucerne hay, can be used as cattle-feed (Ventura & Castañón, 1998). As the consequence of the capability of holding K^+ in their thalli, *Ulva* presents itself a potential candidate for space agriculture (Yamashita *et al*., 2009).

The brown seaweeds, include *Cystophora* and *Sargassum*, have also been used commonly in Asia as a source of medicine for human and to produce alginate (Wiltshire *et al*., 2015; Yende *et al*., 2014). *Sargassum* has been cultivated in many countries, such as Korea, Japan, and India for human consumption (Bast, 2014). For instance, the *S. naozhouense* and *S. fusiforme* have been used widely as a source of food in Korea, Japan, China (Bast, 2014; B. Wang *et al*., 2010). The *S. naozhouense* has been used as a source of food and drugs for traditional orientation treatments (Hur *et al*., 2008; B. Wang *et al*., 2010). *Sargassum* also provides a source of sargaquinoic acid, sargachromenol for neurite growth and survival (Hur *et al*., 2008).

2.4.7.2 Extracts of Seaweeds

The extracts of seaweeds are useful for various purposes. For example, the extract of *G. lithophila* has effects against the mosquito larva (Poonguzhali & Nisha, 2012). The *U. fenestrate* extract has been found to possess allelopathic properties that inhibit growth and/or development of other macroalgae and animal larvae (Nelson *et al.*, 2003). Water-soluble polysaccharides with good gelling properties extracted from cell-wall of *Ulva* spp. (Lahaye & Axelos, 1993) can be used to produce compost, paper and gel (Ray & Lahaye, 1995) as well as enzyme for human digestion (Lahaye *et al*., 1994). Ulvan, which is the main polysaccharide, is simple and low cost to extract by using hot water (Paradossi *et al.*, 1999). *Ulva* by-products can reduce the oxidative stress and atherosclerosis development in hamsters (Godard *et al.*, 2009). The lipid in *Ulva* can be used as biodiesel (Petrus & Noordermeer, 2006). *Ulva* biomass can also be converted to fuels for transport and chemistry (Chung *et al.*, 2011).

The methanolic and aqueous extracts of *L. catenata* obtained at 20^{o}C contains angiotensin-converting enzyme, which is important in blood pressure control (Cha *et al.*, 2006). This species can also be used to produce DL-galactan hybrids (Cosenza *et al.*, 2017), polysaccharide PS1 and PS2 (Takano *et al.*, 1994), anticoagulant polysaccharide, which is sulfated proteoglycan containing high sulfate and protein content, that plays a crucial role in blood coagulation (Pushpamali *et al.*, 2008). The extract of *L. catenata* also contains the antiviral against the pathogenic infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) of fish (Kang *et al.*, 2008) and antioxidant source (Kim *et al.*, 2008). *L catenata* biomass can also be used to produce bioethanol (Sunwoo *et al.*, 2017).

The extract from *Sargassum* can be used as in the treatment of neurological disorders (Natarajan *et al.*, 2009), dementia (Pangestuti & Kim, 2010), and HIV (Thuy *et al*., 2015). It also provides important biochemical compounds for agriculture (Ara *et al.*, 1997). The fact that *Sargassum* is active in antioxidant activity, cholinesterase inhibition activity, neuroprotective activity, anti-cancer and cytotoxic activity has made it a popular ingredient in health enhancement products (Yende *et al*., 2014).

2.4.7.3 As a Source for Fertilizer

Brown seaweeds, typically *Sargassum*, are commonly used as a source of fertilizers and soil conditioners (Huisman, 2000). *Ulva* has also been widely used for agriculture as soil conditioners, fertilizers, and water cleansers (Lindsey Zemke-White & Ohno, 1999; Sze, 1998).

2.4.7.4 As a Source of Bio-filter

Ulva is a "good colonizer and tolerates pollution better than most macroalgae, often thriving where competition with other species is reduced" (Sze, 1998, p. 78). The bloom of *Ulva* can serve as an indication of water pollution (Ho, 1990; Villares *et al.*, 2001; Wan *et al.*, 2017). In addition, the high density of *Ulva*'s population may reduce the growth of eelgrass and other unbeneficial marine vegetables (Sugimoto *et al.*, 2007). Dried green algae and active carbon of *U. lactuca* can be used to remove Cr^{6+} in wastewater and other water solution (El-Sikaily *et al.*, 2007).

Seaweeds, including *Ulva* and *Sargassum*, can clean the water discharged from aquaculture (Neori *et al*., 2004; Partridge, 2008; Troell *et al*., 1999; Van Khoi & Fotedar, 2011). *Ulva* can also absorb trace metals in polluted water, such as sewage runoffs and marshes (Ho, 1984). Therefore, they can be cultured together with shrimp, seabream, or seabream and abalone; or seabream and sea urchin; which would result in higher aquaculture yields and income (Cruz-Suárez *et al*., 2010; Jin & Dong, 2003; Kitadai & Kadowaki, 2007; Mai *et al*., 2010; Neori *et al*., 2004; Neori *et al*., 1996).

Like *Ulva*, *Sargassum* also has the ability to absorb heavy metals (Davis *et al*., 2000) such as lead (Pb^{2+}) (Martins *et al.*, 2006), Cadmium (Cd) (Lodeiro *et al.*, 2006), copper (Cu^{2+}) (Karthikeyan *et al.*, 2007; Padilha *et al.*, 2005; Vijayaraghavan & Prabu, 2006), and zinc (Zn) (Valdman *et al.*, 2001).

2.4.8 Seaweed Aquaculture

Aquaculture remains the fastest food producing industry. In 2013, aquaculture produced 97.92 million tonnes of food, not including aquatic plants, accounting for 43% of fisheries food supply (FAO, 2015). While the marine capture production reduced gradually over the last decade, the marine aquaculture production steadily

increased (FAO, 2014). The marine aquaculture accounted for nearly 37% of total aquaculture production in 2012 (FAO, 2014). In 2012-2013, the aquaculture production of Australia was 80,066 tonnes, valued at $1 billion, accounting for 35 and 43% of the fisheries production and values (Stephan & Hobsbawn, 2014). The Oceania produced more mariculture production than the inland aquaculture, sharing 97.67% of total live weight aquaculture production in 2013 (FAO, 2015). However, the total aquatic plants produced by Oceania share only 0.07% of global aquatic plants production and 0.02% of its value (FAO, 2017). Aquatic plants, 95.5% of which were seaweed, contributed 27 million tonnes live weight to the total aquaculture production in 2013, an increase of 13.4% from 2012, valued at approximately USD 6.655 billion (FAO, 2015). This exceeded the 2013's estimated production (26.1 million tonnes) by almost a million tonnes (FAO, 2014). Mariculture of aquatic plants share 95.73% of total aquatic plants production and 97.19% of its values (FAO, 2017).

The Asian-Pacific dominated the world's aquaculture, contributing 89% to the world's total production (FAO, 2015) and nearly 80% to the total seaweed production (Chung *et al*., 2011). China is the world's largest seaweed producer, accounting for more than a half of the global seaweed production, followed by Indonesia, Philippines, Korea, Japan (FAO, 2015). In 2015, China still was the lead in aquatic plants producer, accounted for 47% of the global production and 50% of values (FAO, 2017). Japan is the second-most important seaweed producer in terms of value due to the Nori production (FAO, 2010). Seaweeds produced in Asia have been exported to Western markets, which have gradually accepted seaweeds as a food product, opening a potential growth for seaweed industry (Lee, 2010).

In 2008/09, the import of seaweed to Australia reached over 5,000 tonnes, valued at AU$17 million, mainly the fresh, dried or chilled seaweeds, from the major providers in China, Japan, Korea and Ireland (Lee, 2010). As the main source of seaweed consumption in Australia comes from import, the development of an alternative land-based source of seaweed culture in Australia is necessary for both domestic consumption and for export (Lee, 2008). Although it has been a potential food industry (Lee & Momdjian, 1997), seaweed culture in Australia has not been commercially developed. Currently, seaweed culture is limited to four major industries. These include (1) alginates by Australian kelps such as *Durvillea* sp. in Tasmania, (2)

fertilisers and feeds for agriculture by the bull kelp *Durvillea* sp. as liquid fertilizer and animal feed in Australia, (3) fucoidanc bioactive compounds extraction by *Undaria pinnatifida* in Tasmania, and (4) beta-caroten by micro-algae *Dunaliella salina* in WA (Lee, 2010). The seaweed culture in Australia is facing various market factor constraints, such as being the minor industry, the habit of using seaweed as food, application of seaweed food, and impacts of seaweed culture ecologically and sustainably, as well as the economic assessment of seaweed culture (Lee & Momdjian, 1997). Today, seaweed is becoming one of the hottest new trend in culinary landscape (Belton, 2013). However, in Western Australia, collecting the wild live seaweeds needs licence from both Department of Parks and Wildlife and the Department of Fisheries as well as the permission from relevant land managers. Therefore, growing seaweeds in ISW can contribute to environmental protection of not only the ISW expansion prevention but also reduce natural collection of seaweeds.

Ulva grows freely in the coastal pools and need substrates or rocks to live on (Sze, 1998). Their growth rate could reach 30% d^{-1} in northern region (Bruhn *et al.*, 2011) or 9.63 g m^{-2} d^{-1} in Japan (Yamashita *et al*., 2009). Suitable stocking densities for *U. lactuca* Linnaeus growth are from 0.13 to 4.5 kg wet weight m^{-2} (Lapointe & Tenore, 1981). At the stocking density of 0.8 kg wet weight m^{-2}, *U. lactuca* provides maximal yield of 4.6 g C m^{-2} day^{-1}. At at higher stocking density its yield starts to decline (Lapointe & Tenore, 1981). The global *Ulva* culture production is not documented, but Japan has been reported to solely produce 1,500 tonnes of dry weight of *Ulva* spp. annually (Lindsey Zemke-White & Ohno, 1999).

L. catenata have been widely studied on seasonal growth (Chang Geun *et al.*, 2010), the extracts (Cha *et al*., 2006; Kang *et al*., 2008; Kim *et al*., 2008; Khan *et al.*, 2008; Pushpamali *et al*., 2008), phylogenetics (Filloramo & Saunders, 2016; Gall *et al.*, 2008; Kanagasabhapathy *et al.*, 2008). However, the cultivation of this species has not been practiced yet opening a potential study subject in the future.

Seaweed can be grown as monoculture, such as *Laminaria*, *Porphyra*, *Gracilaria*, *Eucheuma* in China (Xiu-geng *et al.*, 1999), *Chondrus crispus* in land-based tanks and ponds in Canada (Chopin *et al.*, 1999), *Gracilaria* in tanks in USA (Capo *et al.*, 1999). China is the largest producer in the world of *Laminaria japonica* (Tseng, 2001). Seaweed can also be used as a biofilter for aquaculture species (Neori *et al*., 2004),

and can be integrated with shrimp (Cruz-Suárez *et al*., 2010; Mai *et al*., 2010; Van Khoi & Fotedar, 2011), seabream and shellfish, salmon (Neori *et al*., 2004), marine fish (Al-Hafedh *et al*., 2012). *Sargassum* have been cultivated, such as *S. fulvellum* in Korea (Hwang, Baek, *et al*., 2007; Hwang, Park, *et al*., 2007) and *S. fusiforme* (Pang *et al*., 2006), *S. horneri* (Pang *et al*., 2009), *S. naozhouense* (B. Wang *et al*., 2010) and *S. thunbergii* (Li *et al*., 2010) in China.

Farming of *C. subfacinata* and *S. linearifolium* has not been historically recorded, except for the growing of these seaweeds as transplanted material to be threaded onto rope in South Australia and the field growth rate is low (Wiltshire *et al*., 2015). The *S. linearifolium* grows poorly in summer and is only suitable for short-term culture (Wiltshire *et al*., 2015). Growing these seaweed species in ISW is not only contribute to the mariculture production, but also contribute to literature of recording the first attempt to grow those species in artificial conditions.

2.5 Inland Saline Water Aquaculture

Aquaculture remains the fastest growing food-production industry. Total production in 2009 was 55.1 million tonnes, excluding aquatic plants, and accounted for 46% of fisheries food supply (FAO, 2010), which was increased to 76.6 million tonnes in 2015, valued at US$163 billion (FAO, 2017). Although marine capture production has reduced stably over last decade, aquaculture production has steadily increased at the pace of 5.9% annually (FAO, 2010, 2017). Marine aquaculture accounted for nearly 40% of total aquaculture production in 2009, which was 8.1 kg per capita supply. Out of the above total recorded aquaculture production, aquatic plant production in 2008 was 15.8 million tonnes live weight, valued at approximately US$7.4 billion, and consisting of 99.6% total production of seaweed (FAO, 2010). In 2015, the aquatic plants production (mainly seaweeds) was double of that in 2008. 96% of the aquatic plants production was from aquaculture, which accounted for 27.7% of total aquaculture volume (FAO, 2017). Inland marine aquaculture using the ISW can contribute to salinisation management by helping to reduce agricultural land degradation and opening up a new farming practice for farmers (Doupé, Lymbery, & Starcevich, 2003).

Table 2-14. Species cultured in ISW worldwide

Common name	Scientific name	States	Reference
Brine shrimp	*Artemia salina*	USA	Brune *et al.* (1981)
Barramundi	*Lates calcarifer*	India	Jain *et al.* (2006)
Chinese shrimp	*Fenneropenaeus chinensis*	China	Liu (2001)
European carp	*Cyprinus carpio*	India	Swivedi and Lingaraju (1986)
Fathead minnow	*Pimephales promela*	USA	Burnham and Peterka (1975)
Giant freshwater prawn	*Macrobrachium rosenbergii*	India	Jain *et al.* (2007)
Grey mullet	*Mugil cephalus*	India	Barman *et al.* (2005); Swivedi and Lingaraju (1986)
Manila clam	*Venerupis* (Ruditapes) *philippinarum*	Netherlands	Van der Hiele *et al.* (2014)
Marine diatom	*Phaeodacrylum tricomutum*	USA	Brune *et al*. (1981)
Pearl spot	*Etroplus suratensis*	India	Swivedi and Lingaraju (1986)
Red drum	*Sciaenops ocellatus*	USA	Forsberg *et al*. (1996)
Tiger prawn	*P. monodon*	India	Dwivedi and Trombetta (2006); Shakeeb Ur *et al*. (2005)
White shrimp	*Litopenaeus vannamei*	USA	Davis *et al*. (2005); Gong *et al.* (2004); Roy *et al.* (2009)
		China	Liu *et al.* (2014)

Source: Dinh (2016)

Table 2-15 Cultured species in ISW in Australia

Common name	Scientific name	State	Reference
Agar	*Gracilaria cliftonii*	WA	Kumar *et al*. (2010)
Atlantic salmon	*Salmo salar*	VIC	Ingram *et al*. (2002)
Australian bass	*Macquaria novemaculeata*	VIC	Ingram *et al*. (2002)
Australian snapper	*Pagrus auratus*	NSW	Fielder *et al*. (2001)
		SA	Hutchinson and Flowers (2008)
		VIC	Ingram *et al*. (2002)
		WA	Partridge *et al*. (2006)
Barramundi	*Lates calcarifer*	WA	Partridge and Lymbery (2008); Partridge *et al*. (2006)
		SA	Hutchinson (1997)
Black bream	*Acanthopagrus butcher*	WA	Doupé *et al*. (2005)
Blue mussel	*Mytilus edulis*	WA	Dinh and Fotedar (2016)
Brine shrimp	*Artemia salina*	VIC	Gooley *et al*. (1997)
Banana prawn	*Penaeus merguiensis*	Qld	Collins *et al.* (2005)
European carp	*Cyprinus carpio*	VIC	McKinnon *et al.* (1998)
Greenlip abalone	*Haliotis laevigata*	WA	Fotedar *et al.* (2008)
Greenback flounder	*Rhombosolea tapirina*	SA	Hutchinson (1997)
		VIC	Ingram *et al*. (2002)
Kuruma prawn	*P. japonicus*	VIC	Ingram *et al*. (2002)
Marron	*Cherax tenuimanus*	WA	Paust (1997)
Microalgae	*Dunaliella salina*	SA	Kolkovski (2010)
		WA	Paust (1997)
Mulloway	*Argyrosomus japonicas*	NSW	Doroudi *et al*. (2006)
		SA	Hutchinson and Flowers (2008)
		WA	Partridge *et al*. (2006)
Pacific oyster	*Crassostrea gigas*	VIC	Ingram *et al*. (2002)
Rainbow trout	*Oncorhynchus mykiss*	VIC	Ingram *et al*. (2002); Lymbery
		WA	*et al*. (2007); Partridge *et al*. (2006)
Sand whiting	*Sillago ciliata*	VIC	Ingram *et al*. (2002)

Common name	Scientific name	State	Reference
Silver perch	*Bidyanus bidyanus*	VIC	Ingram *et al*. (2002), Doroudi *et al*. (2007)
		SA	Hutchinson (1997)
Sydney rock oyster	*Saccostrea glomerata*	VIC	Ingram *et al*. (2002)
Tommy rough/Australian herring	*Arripis georgiana*	SA	Hutchinson (1997)
Tiger prawn	*Penaeus monodon*	WA	Tantulo and Fotedar (2006)
Trochus	*Trochus niloticus*	NT	Lee (1996)
Western rock lobster	*Panulirus cygnus*	WA	Tantulo and Fotedar (2007)
Western king prawn	*Penaeus latisulcatus*	WA	Prangnell and Fotedar (2006b); Prangnell and Fotedar (2005)
Yabby	*Cherax albidus*	WA	Paust (1997)
Yellow-fin whiting	*Sillago schombergkii*	SA	Hutchinson (1997)
Yellowtail kingfish	*Seriola lalandi*		Hutchinson (1997)

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

(Modified from Dinh (2016)

Inland saline aquaculture has been practiced worldwide for many years. In the USA, experiments have been conducted on culturing marine prawn in low salinity water, such as on the Pacific white shrimp (*Litopenaeus vannamei* Boone) with potassium and magnesium fertiliser supplementation (Roy *et al*., 2010). Red drum aquaculture development is vitally based upon salinity and has expanded successfully using groundwater (Forsberg *et al*., 1996; Forsberg & Neill, 1997). Bivalve (*Ruditapes philippinarum*) (Baud & Bacher, 1990) and marbled spinefoot rabbitfish (Mourad *et al*., 2012) have also been trialed. In Australia, ISW aquaculture has gradually developed all over the country (Allan *et al*., 2001). Rainbow trout (*Oncorhynchus mykiss*) have been grown in WA (Allan *et al*., 2001), in addition to barramundi, mulloway, snapper and algae (Borowitzka, 1997; Partridge, Lymbery, & George, 2008; Partridge *et al*., 2006), Atlantic salmon (*Salmo salar*), Australian bass

(Macquaria novemaculeata), black bream (*Acanthopagrus butcheri)* (Ingram *et al*., 2002), and king prawn (*Penaeus latisulcatus* Kishinouye) (Prangnell & Fotedar, 2006b). Oysters have also been trialed in ISW but do not have good growth and survival rates (Ingram *et al*., 2002). Blue mussels *Mytilus edulis* (Linnaeus, 1758) can also survive and grow in ISW fortified with K^+ at similar concentrations as OW (Dinh, 2016) (Table 2-14).

In Australia, fish, crustaceans, and seaweed have been cultured in ISW (Table 2-15). However, their economic contribution to Australian national fisheries is still minor (Doupé, Lymbery, & Starcevich, 2003). Of these, seaweed is one of the good options because of its high nutrients and easy cultivation (Cordover, 2007), particularly in WA, which has abundant ISW and sunlight (Table 2-17, Table 2-18). Algae can also be a biofilter that cleans water for other aquaculture species development (Neori *et al*., 2004). Matting Rush *(Juncus kraussii)* has shown good results for removing nutrients and salinity from aquaculture effluent in WA wetlands. However, Matting Rush only grows well in salinity lower than 20 ppt (Lymbery *et al.*, 2006), whereas in WA, the ISW salinity level may be higher during summer (Mazor & George, 1992). Forsberg *et al.* (1996) and Mourad *et al.* (2012) recommend that salinity and specific ion concentrations should be measured for potential aquaculture in ISW.

Table 2-16. Effect of K^+ in aquaculture

Criteria	Species	Method	Result	Ref.
Osmoregulation, ionregulation	*Penaeus latisulcatus* Kishinouye	K^+-fortified ISW at 80 and 100% of $[K^+]$ in OW at 20, 25, 27ppt and raw ISW	dependent on K^+ in cultured medium	Prangnell and Fotedar (2006a)
Growth and survival	*Penaeus latisulcatus* Kishinouye	K^+-fortified ISW at 80 and 100% of $[K^+]$ in OW	is significantly dependent on the medium $[K^+]$	Prangnell and Fotedar (2006b)
Haemolymph K^+	*Penaeus latisulcatus* Kishinouye	Sudden change in K^+ in K^+-fortified ISW at 80 and 100% of $[K^+]$ in OW at 32 ppt	is crucial for osmolality maintenance Significantly increase the ingestion rate of prawns Effect the osmoregulatory capacity of prawns	Prangnell & Fotedar (2009)
Growth and survival	*Penaeus monodon* Fabricius	Increasing K^+	Improve growth and survival	Shakeeb Ur *et al*. (2005)
Growth and survival	*Litopenaeus vannamei* Boone	Increasing K^+	Improve growth and survival	Saoud *et al*. (2003)

Criteria	Species	Method	Result	Ref.
Mortality	*Americamysis bahia* Molenock	K^+ deficiency or excess	Significant mortality of mysid shrimp	Pillard *et al.* (2002)
Hemolymph K^+	(euryhaline green crab) *Carcinus maenas* Linnaeus	K^+ reduced in haemolymph	is not toxic (less than 24 h)	Lovett *et al.* (2006)

Table 2-17. Algal species that could be grown in saline groundwater sources

Species	Product or market	Salinity (g L^{-1} NaCl)	Status
Dunaliella salina	Beta-carotene	>200	Commercial
Aphanothece halophytica	Polysaccharides, phycobilin pigments	> 200	Research & Development
Isochrysis, Tetraselmis Chaetoceras, Pavlova	Feed used in the aquaculture of molluscs, crustaceans & fish	about 30	Presently produced in various hatcheries
Spirulina platensis	Health food	up to 30	Commercial (in USA, Thailand, China, India)
Porphyridium cruentum	Polysaccharides, pigments for cosmetics	up to 30	Research & Development
Gracilaria spp.	Feed used in aquaculture of abalone; source of agar	about 30	Mainly wild harvest, but also some abalone; source of agar culture overseas
Ulva spp.	Feed used in aquaculture of abalone	about 30	Some small-scale production of abalone overseas
Caulerpa spp.	Luxury food (sold mainly in Japan)	about 30	Presently farmed in the Philippines

Source: Borowitzka (1997, p. 36)

Table 2-18. 8 Potential seaweed species cultured in Integrated Multitrophic Aquaculture systems

Species	Family	Remarks
Ulva spp. (Green)	Ulvaceae	It contains 15% protein, 50% sugar and starch, <1% fat, high in some ion, vitamins, and trace elements
Gracilaria sp. (Red)	Gracilariaceae	About 70% of the world's agar is produced from this species
Porphyra sp. (Red)	Bangiaceae	Some other species: *Martensia* sp., Calosiphonaceae (*Schmitzia japonica*), *Caulerpa filiformis*, *Rhodoglossum* (Red tongue), *Placonium, Branchioglossum, Kallymenia rosea*
Asparogpsis armata (Red)	Bonnemaisoniaceae	Can be called as *Falkenbergia rufolanosa*
Grateloupia sp. (Red)	Halymeniaceae	
Geluduaceae	Red alga family	
Ecklonia radiate (Brown kelp)	Alariaceae	Abundant local species. Can be integrated cultured with abalone. It has large morphology so not convenient to culture in tanks
Sargassum sp. (Brown)	Sargassaceae	Provide a source of sea urchin food

Source: Winberg *et al.* (2009)

CHAPTER 3 METHODOLOGY

3.1 Preparation of Seaweed

Six local seaweed species were randomly collected along the Perth beaches and Swan river representative for brown, green and red seaweeds. These seaweeds were identified by Western Australia (WA) Herbarium as brown seaweed *Cystophora subfarcinata*, *Sargassum linearifolium*, *Sargassum podacanthum*, green seaweed *Ulva lactuca*, and red seaweed *Glateroupia suspectinata* and *Lomentaria catenata*.

Brown seaweed *C. subfarcinata*, *S. linearifolium*, *S. podacanthum* and red seaweed *G. suspectinata* whole thalli were collected from Point Peron, WA (latitude 32°16.3S, longitude 115°41.2E). The red seaweed *L. catenata* and green seaweed *U. lactuca* were collected from Matilda Bay, Swan River, WA (latitude 31°97.9S, longitude 115°82.2E). Some *U. lactuca* was also collected at Cottesloe beach (latitude 31°99.4S, longitude 115°75.1E) depended on the seasons. The seaweeds were transported in tanks holding ambient local salty water to Curtin Aquatic Research Laboratory (CARL) within two hours of collection. Upon arrival at the laboratory, the seaweeds were thoroughly cleaned in ocean water (OW) and all epibiotic were removed.

The seaweeds were then acclimated for one to three days in aerated OW at 22°C in white plastic 114 L aquaria, under a downwelling photo-lux of 120 µmol photon m^{-2} s^{-1} and a 14:10 h light:dark cycle, before use in experiments (Hanisak & Samuel, 1987).

After acclimation, the seaweed was removed and cut into pieces to achieve a pre-determined weight approximately 3,500 mg L^{-1} for cultivating in beakers or the whole thalli would be used for cultivating in the tanks, and was then immediately transferred into the trial beakers/tanks depend on the experiment.

3.2 Preparation of Culture Media

Inland saline water (ISW) at 45 ppt was procured from a lake in Wannamal, WA (31°15.8S, 116°05.0E). OW at 35 ppt was procured from Hillary Habour (31°83S, 115°74E). Both waters were brought to CARL, where the trials were carried out. The waters were stored and aged in separate 10,000 L reservoirs. OW, ISW and fresh water

were filtered through a 0.5 µm glass fibre membrane. ISW and OW were then diluted with filtered fresh water to achieve the required salinity of cultured media for each experiment.

3.2.1 Potassium-fortified Inland Saline Water

Potassium chloride (KCl) or potash of sulphate (K_2SO_4) were used to fortify ISW, the required K^+ were calculated from the difference between K^+ concentrations of OW and ISW and converted to equivalent quantities of KCl or K_2SO_4.

Potassium chloride (KCl) (Sigma-aldrich Pty. Ltd., Australia, holding 52.45% K^+) was used to fortify ISW (contain K^+ 84.4 mg L^{-1}) to approximately 100%, 66%, 33% (ISW100, ISW66, ISW33, respectively) of the [K^+] in ocean water (OW) at 351 mg L^{-1}. 508.5, 280.90, 59.99 mg L^{-1} of KCl were respectively added to ISW 35 ppt to achieve ISW100, ISW66, ISW33. This water preparation was applied for experiments in Chapter 4 to 6.

Cultured media at 30 ppt were prepared for red seaweed *L. catenata* and green seaweed *U. lactuca*. [K^+] at 30 ppt in OW and ISW was 313 and 77 mg L^{-1}, respectively. Therefore, 451, 248 and 50 mg L^{-1} of KCl were used to fortify ISW 30 ppt to achieve ISW100, ISW66, ISW33, respectively, for the waters using in Chapter 8, 9 and 10.

K_2SO_4 (Richgro soluble powder sulphate of potash 0-0-41-17, holding 41.5% K^+) 642 mg L^{-1} was used to fortify ISW 35 ppt to achieve ISW100 to use for water preparation in Chapter 4 and Chapter 7.

3.2.2 Nutrient Enrichment for Potassium-fortified Inland Saline Water

After finding the required [K^+] for each seaweed species growth in ISW, the nutrient enrichment experiments were set up.

Ammonium chloride (NH_4Cl) and sodium dihydrogen phosphate (NaH_2PO_4) were the sources of nitrogen and phosphorus provided in optimum K^+-fortified ISW (K^+ISW) for each studied species, to test the effect of nutrient enrichments on seaweeds. Each species required different concentrations of ammonium and phosphate, therefore the detail of experiment set up would be placed at related sections.

3.3 Experimental Setup

The experiments were conducted at CARL, for the time depend on each species and the tested criteria. The ambient OW and ambient ISW were used as control. The experiment set up was depended, and could be triplicates for the tanks or four replication in beakers.

The beakers/tanks were put under down-welling photo-lux density 90 µmol photon m^{-2} s^{-1} in a 14:10 h light:dark cycle (Hanisak & Samuel, 1987). During the experiment, salinity was maintained at 34–35 ppt or 30–31 ppt, dependent on cultured species, by adding fresh water to compensate for evaporation.

3.4 Data Collection

3.4.1 Water Quality

Nitrogen included ammonium (NH_4-N), nitrate (NO_3^--N), nitrite (NO_2^--N), and phosphorus (PO_4^{3-}-P) were measured every 14 days, using a Hach DR890 hand-held meter (Hach, Loveland, Colorado, USA). The Cadmium Reduction Method (Method 8171 and Method 8039) was used to measure NO_3^--N at low (0–5 mg L^{-1}) and higher concentrations, respectively. The Diazotization Method (Method 8507) was used to measure NO_2^--N at a lower range (0–0.350 mg L^{-1}), and the Ferrous Sulfate Method (method 8153) was used to measure NO_2^--N at a higher range (0–150 mg L^{-1}). The Salicylate Method (Method 8155; Method 10023) was used for NH_4-N at 0–0.05 mg L^{-1} and higher concentrations, and PO_4^{3-}-P was measured by the Amino Acid Method (Method 8178). Total Kjeldahl Nitrogen (TKN) in water was also determined every 14 days according to the Official Method of the AOAC (Helrich, 1990) (method 937.48) by analyzing N using a Kjeltec Auto 1030 analyzer (Foss Tecator, Höganäs, Sweden).

The pH and salinity were recorded daily at 9–11AM using a pH meter (CyberScan pH 300, Eutech Instrument, Singapore), and a portable refractometer (RHS-10ATC, Xiamen Ming Xin Instrument, Xiamen, Fujian, China), respectively.

The temperature was recorded hourly by data loggers (HOBO Pendant temperature/light Data Logger UA-002-08, UA-002-64).

The ionic profile of cultured medium was analysed using inductively coupled plasma (ICP) spectroscopy at CSBP Soil and Plant Laboratory, Bibra Lake, WA.

In addition, the total solids (TS), total volatile solids (TVS), total suspended solids (TSS) and volatile suspended solids (VSS) were also determined according to standard methods (American Public Health Association [APHA], 1998).

3.4.2 Seaweed Growth

The weight of seaweed was determined fortnightly, and at the termination of the experiment. All thalli were removed from the culture beakers/tanks by a small net and then dried using soft hand towels (Ahmad *et al*., 2011). All seaweed thalli were instantly weighed in a weighing scale (AW220, d=0.1 mg, Shimazu, Japan).

The specific growth rates (SGR) were calculated as: $\mu_a=(\ln A_t-\ln A_o)\times 100/t$.
Where: μ_a is the SGR of seaweed (% d^{-1}),

A_t: the weight of seaweed (mg) at the current time (t, day),

A_o: the weight of seaweed (mg) at the beginning of the calculated period (0, day),

t: the time of the calculated period (days).

3.4.3 Nutrient Analysis of Seaweed

The seaweeds were dried at 60^{o}C for 72 h to obtain a stable dried weight. All samples were weighed, ground with a pestle and mortar to a fine powder, and then stored in a freezer at -18^{o}C until analysis of the proximate and chemical compositions.

The standard methods described in Official Method of the AOAC (Helrich, 1990) were applied to measure the dried content and ash of the seaweeds. The dried content was calculated relative to the fresh biomass. The ash content was determined by burning dried seaweed at 550^{o}C for 30 min relative to the dried content.

A bomb calorimeter (C2000, IKA, Staufen, Germany) was used to determine the gross energy of the seaweeds.

Tissue N was determined according to the AOAC (Method 937.48) (Helrich, 1990) using a Kjeltec Auto 1030 analyser (Foss Tecator, Höganäs, Sweden). Crude protein was calculated by total N multiplied by 6.25.

The ionic compositions of the seaweed were analysed using the prepared freeze fine powder by ICP spectroscopy at the CSBP Soil and Plant Laboratory, Bibra Lake, WA. The total nitrogen and carbon contents of seaweed were also analysed at the CSBP Soil and Plant Laboratory, Bibra Lake, WA.

3.5 Data Analysis

The SPSS for Windows version 24.0 was used to analyse data. The significant differences among the means of variables at level of $P<0.05$ were tested by Analysis of variance (ANOVA, MANOVA), paired sample *t*-tests and least significant difference (LSD) post hoc tests. The normality and homogeneity of the data were tested by Shapiro-Wilk Test of Normality and the Lenene's test. When the data was not normally distributed, the overall difference was tested by Kruskal-Wallis test. When the homogeneity was violated, the Robust Test of Equality of Means was applied for means comparison instead of ANOVA. The relationships of the variables over time were determined by regression analysis.

CHAPTER 4 SELECTION OF SEAWEED SPECIES AND POTASSIUM SOURCE FOR CULTIVATION OF SEAWEED IN INLAND SALINE WATER

4.1 Introduction

Inland saline water (ISW) is considered as a potential source for aquaculture development in Australia, particularly for the agricultural farms where water is salty-affected (Allan *et al*., 2001; Partridge, Lymbery, & George, 2008). However, the deficiency of potassium (K^+) in ISW is the major constraint in culturing marine aquatic species (Davis *et al*., 2005; Partridge & Lymbery, 2008; Prangnell & Fotedar, 2006b). In order to remove the constraint, the K^+ concentration in ISW has to be increased to a level that similar to at least 50% of K^+ concentration in ocean water (OW) to culture marine species (Dinh, 2016; Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006). Dinh (2016) has summarised 29 marine species that have been cultivated in ISW with an exception of only one seaweed species *Gracilaria cliftonii* (Kumar *et al*., 2010), opening a chance for seaweed inland aquaculture development. It is important to find suitable seaweed species to culture in ISW, take into account their feasibility of cultivation in ISW as well as their contributions to aquaculture.

For the marine species culture using ISW, three sources of K^+ can be used to increase the $[K^+]$ in water. These sources are muriate of potash KCl (holding 49.8% of K^+ and 45.2% Cl^-), potassium sulfate K_2SO_4 (holding 42% K^+ and 51% SO_4^{2-}), and sulfate of potash magnesia $K_2SO_4.2MgSO_4$ (holding 17.8% K^+, 10% Mg^{2+}, 63.6%SO_4^{2-}) (Boyd & Thunjai, 2003). Grade KCl (containing 52.5% K^+) (Sigma-aldrich Pty. Ltd. (Aus) is common used salt to fortify ISW to culture marine species in laboratory (Dinh, 2016; Doroudi *et al*., 2006; Fielder *et al*., 2001; Mourad *et al*., 2012; Prangnell & Fotedar, 2005, 2006b; Shakeeb Ur *et al*., 2005; Tantulo & Fotedar, 2006), whereas K_2SO_4 in the form of Kmag® (containing 18.3% of K^+) has only been used to fertilize shrimp pond with muriate of potash (KCl) (Boyd *et al*., 2007; McNevin *et al*., 2004), although the K_2SO_4 is commercially cheaper than KCl. In Australia, the sulphate of potash K_2SO_4 (Richgro soluble powder sulphate of potash 0-0-41-17) contains 96% of K_2SO_4 and less than 4% of KCl, sharing 41.5% of K^+ and 17% of S (in the form of SO_4^{2-}), and is used to fertilise the soil. Since sulphate of potash K_2SO_4 has not been commonly

solely used in K^+ fortification for ISW, there has been no comparison of KCl and K_2SO_4 for ISW K^+ fortification yet.

This study attempts to examine the feasibility of cultivating the local seaweed species collected from beaches and rivers in Perth region in ISW, and testing the effects of two different sources of K^+ on the growth of seaweeds.

4.2 Materials and Methods

Two independent trials were set up to achieve two targets of this Chapter.

4.2.1 Seaweed Selection to Cultivate in Inland Saline Water

The trial was conducted in 42 days in winter in order to find out the candidate seaweed species who were able to cultivate in ISW and potassium-fortified inland saline water (K^+ISW) for further study. Six local seaweed species (as mentioned in Chapter 3), were used. Seaweed collection, culture media preparation of K^+ISW at 100%, 66%, 33% (termed as ISW100, ISW66, ISW33, respectively) of the [K^+] in ocean water (OW) at 35 ppt fortified by KCl, experimental setup, data collection and data analysis were described in Chapter 3. Five treatments were set up in four replicates, including four K^+ levels of ISW100, ISW66, ISW33 and ambient ISW (ISW0), and control treatment of ambient OW (OW).

After acclimation, *Sargassum linearifolium*, *S. podacanthum*, and *Cystophora subfarcinata* were cut in pieces, whereas the whole thalli of *Ulva lactuca*, *Lomentaria catenata*, and *Grateloupia suspectinata* were selected, to achieve the expected weight at a relative stocking density of 3,000 mg L^{-1} (Table 4-1). The seaweeds were transferred into 1.5 L beakers holding 1 L of culture media, which were randomly placed under the ambient room temperature was 20–22ºC.

4.2.2 Sources of Potassium Fortification for Inland Saline Water

4.2.2.1 Experiment Setup

The outcome of the previous trial shown that two *Sargassum* spp. presented the highest specific growth rate (SGR) in K^+ISW. Therefore, this experiment was conducted using *Sargassum* spp. only. *S. linearifolium* and *S. podacanthum* were collected from Point

Peron and were identified, treated as described in Chapter 3. The whole *Sargassum* spp. thallus, including holdfast, were chosen at the pre-selected weight of approximate 200 g a fond. *Sargassum* spp. thalli with a similar height and weight were used, and their holdfasts were attached to gravel to keep them submerged in water.

The ISW 35 ppt was also prepared follow the protocols in Chapter 3 using 642.65 mg L^{-1} K_2SO_4 or 508.5 mg L^{-1} KCl, termed as ISW_K_2SO_4, ISW_KCl, respectively,.

4.2.2.2 Experiment Design

The trial was conducted for 70 days in early summer under outdoor conditions. Six treatments, including two *Sargassum* species (*S. linearifolium* and *S. podacanthum*) and three water types (ISW_K_2SO_4, ISW_KCl and a controlled OW), were set up in four replications. A total of 24 white round plastic tanks of 114 L, holding 100 L of K^+ISW were randomly placed.

Data collection, included seaweed biomass, SGR, the water quality including the concentrations of ammonium (NH_4-N), nitrate (NO_3^--N), nitrite (NO_2^--N), phosphate (PO_4^{3-}-P), total Kjeldahl nitrogen (TKN) in water, the pH, salinity, temperature and light intensity of culture media were determined as methods described in Chapter 3. Data analysis was followed as in Chapter 3, section 3.5.

4.3 Results and Discussion

4.3.1 Seaweed Selection

Among the five treatments, each species of seaweed had a similar fresh biomass at the beginning of the trials. *L. catenata* and *U. lactuca* presented no significant difference in SGR as the trial progressed. *G. subpectinata* grew better in OW than in ISW in the first 14 days; however, total mortality occurred at this point. In the first 14 days, *C. subfarinata* grew significantly ($P<0.05$) higher in OW and ISW100 than ISW0, and the SGR of *Sargassum* in OW and ISW100 were significantly ($P<0.05$) higher than in ISW0 and ISW33. *S. linearifolium* cultured in OW resulted in higher SGR than in ISW100 in the first 14 days, but these became similar after this point (Table 4-1).

Of the six seaweed species, brown seaweed *S. linearifolium*, *S. podacanthum* and *C. subfarinata* survived after 42 days of growing in K^+ISW, and presented no significant

(P>0.05) difference from SGR in OW. However, all seaweeds died in ISW0. A positive SGR was only present in OW and ISW100 for *Sargassum*. Although *C. subfarinata* survived until day 42, the biomass decreased from day 28 onward. Total mortality of *L. catenata* and *U. lactuca* was recorded at day 42 in all treatments, and all *G. subpectinata* died in the second fortnight.

A repetition of the trial, culturing approximately 3.5 g L^{-1} *G. subpectinata* in OW, IWS0 and ISW100 at 35 ppt, was conducted in 14 days, and all *G. subpectinata* died in ISW0 in the second week. The SGR of *G. subpectinata* in ISW100 was 1.14% d^{-1}, which was similar to its SGR in OW but higher than the ISW100 from the initial experiment (data was not presented). Under similar conditions of temperature and light, the length SGR of *G. subpectinata* gametophyte culturing in OW is 2.38% d^{-1} (Adharini & Kim, 2016), a value higher than the weight SGR of mature thalli *G. subpectinata* from this study. *G. subpectinata* proved not to be able to successfully culture in ISW, since total mortality happened during the first month of the cultured period.

The *L. catenata* had a similar SGR as *G. subpectinata* in the first fortnight, although SGRs were all negative in all waters. However, *L. catenata* showed higher ISW tolerance than *G. subpectinata*, since the tissues lived longer.

Table 4-1. Initial biomass (g) and the SGR (% d^{-1}) of seaweeds

Treatments	*G. suspectinata*	*L. catenata*	*U. lactuca*	*C. subfarinata*	*S. linearifolium*	*S. podacanthum*
Initial biomass						
OW	3.53±0.76	3.03±0.01	3.05±0.01	3.45±0.10	3.17±0.13	2.88±0.13
ISW0	2.85±0.22	3.05±0.01	3.04±0.01	3.33±0.12	3.23±0.04	2.81±0.23
ISW33	2.70±0.26	3.03±0.01	3.05±0.01	3.40±0.07	3.01±0.20	3.06±0.04
ISW66	2.62±0.54	3.04±0.02	3.03±0.02	3.40±0.12	3.18±0.07	2.76±0.15
ISW100	3.42±0.70	3.02±0.01	3.05±0.01	3.30±0.17	2.92±0.13	2.83±0.12
SGR day 1−14						
OW	$_{1}$0.65±0.29^{a}	-0.87±0.23^{a}	-0.12±0.01^{a}	$_{1}$1.23±0.16^{ab}	$_{1}$2.29±0.08^{b}	0.32±0.50^{ab}
ISW0	$_{12}$-0.95±0.40^{ab}	-1.19±0.86^{a}	-0.32±0.17^{abc}	$_{2}$0.16±0.25^{bc}	$_{12}$1.69±0.19^{d}	0.12±0.72^{c}
ISW33	$_{2}$-1.70±0.67^{a}	-1.48±0.23^{a}	0.00±0.38^{b}	$_{23}$0.49±0.17^{b}	$_{12}$1.41±0.57^{c}	0.40±0.12^{b}
ISW66	$_{12}$0.55±0.94^{bc}	-1.28±0.30^{a}	-0.25±0.03^{ab}	$_{12}$0.75±0.17^{bc}	$_{12}$1.59±0.57^{c}	-0.13±0.12^{ab}
ISW100	$_{2}$-1.50±0.83^{a}	-1.83±0.69^{a}	-0.48±0.05^{ab}	$_{13}$1.04±0.22^{bc}	$_{2}$1.05±0.28^{c}	0.43±0.32^{bc}
SGR day 1−28						
OW		-0.87±0.23^{a}	-0.66±0.07^{a}	-0.20±0.15^{a}	$_{1}$0.04±0.09^{b}	$_{1}$0.52±0.32^{b}
ISW0		-1.00±0.34^{a}	-0.59±0.15^{a}	-0.59±0.14^{a}	$_{1}$0.27±0.15^{b}	$_{2}$-0.37±0.33^{b}
ISW33		-1.53±0.23^{a}	-0.25±0.25^{b}	-0.52±0.11^{b}	$_{2}$-0.65±0.36^{b}	$_{12}$-0.16±0.12^{b}
ISW66		-0.97±0.14^{a}	-0.38±0.19^{b}	-0.41±0.15^{b}	$_{1}$0.17±0.08^{b}	$_{12}$0.20±0.31^{b}

Treatments	***G. suspectinata***	***L. catenata***	***U. lactuca***	***C. subfarinata***	***S. linearifolium***	***S. podacanthum***
ISW100		-1.43±0.49[a]	-0.52±0.11[b]	-0.43±0.21[bc]	$_{1}$0.06±0.13[bc]	$_{12}$0.26±0.20[c]
SGR day 1−42						
OW				-0.30±0.30	0.12±0.14	0.28±0.20
ISW33				-0.44±0.44	-0.49±0.49	-0.08±0.07
ISW66				-0.09±0.09	-0.04±0.13	0.07±0.19
ISW100				-0.54±0.32[a]	0.13±0.06[ab]	0.13±0.17[b]

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; P>0.05; n=4)

The only representative of green seaweeds, *U. lactuca*, showed a decline in biomass from the first fortnight, and total mortality was encountered in the third fortnight. In the first two fortnights, when the survival of *U. lactuca* was recorded, its SGR was significantly ($P<0.05$) higher than the SGR of red seaweed cultured in K^+ISW, and similar to the brown seaweeds. This SGR was lower than previously found for *U. lactuca* growing in nitrogen enriched OW medium (Ale *et al*., 2011; Nielsen *et al.*, 2011). The higher SGR of *U. lactuca* is recorded at lower temperature which is constant at 15°C, light ranging 120–150 μmol photons m^{-2} s^{-1}, and salinity of 20 ppt (Nielsen *et al*., 2011), these environmental factors are different from the conditions of this study, opening a potential direction for further study.

Of the six studied species, brown seaweeds presented higher tolerance in captivity, and more successful culturing in ISW than the red and green seaweeds. In the first fortnight, only brown seaweeds had a positive SGR in either OW or K^+ISW, and *Sargrassum* spp. had a significantly ($P<0.05$) higher SGR than red seaweeds in all waters. In the second fortnight, the brown seaweeds resulted in a significantly ($P<0.05$) higher SGR than the remaining red seaweed, *L. catenata*. Of the brown seaweeds, *Sargassum* presented a significantly ($P<0.05$) higher SGR than *C. subfarinata* in OW and ISW0.

4.3.2 Sources of Potassium Fortification

4.3.2.1 The Growth of Two *Sargassum* spp. and Potassium Fortification

This study is the first attempt to compare the growth of *S. linearifolium* and *S. podacanthum* culturing in ISW fortified by either KCl or K_2SO_4 that resulted the same growth between these two species of *Sargassum*.

Previous research has shown the importance of K^+ on the growth, photosynthesis, respiration, chlorophyll content, etc., of plants (Gierth & Mäser, 2007; Jin *et al.*, 2011; Onanuga *et al.*, 2012; Sideris & Young, 1945; Terry & Ulrich, 1973; Zhao *et al.*, 2001; Zhao *et al.*, 2016), including seaweeds (Yarish *et al*., 1980; Zimmermann & Steudle, 1971). However, KCl has been commonly used for fortifying ISW than other K^+ sources (Table 4-2), and sulphate of potash K_2SO_4 has not been used in ISW yet. Although the standing biomass of *S. linearifolium* and *S. podacanthom* cultured in the three water types (OW, ISW_KCl, and ISW_K_2SO_2) varied during the experiment, no

significant (t-test, P>0.05) differences in the biomass of the two species were found in any of the types of water, and no significant (LSD test, P>0.05) differences of biomass of one *Sargassum* species were observed among three water types.

The two *Sargassum* spp. biomass reached maximum at days 28–42, and then declined (P<0.05) towards the end of the experiment. The standing biomass of *S. linearifolium* cultured in K^+ISW significantly (P<0.05) increased as time progressed, reaching the maximum at day 42. *S. podacanthum* showed a significant (P<0.05) increase at days 1–28. The biomass of *Sargassum* correlated (R^2>0.70) with time; however, no significant (P>0.05) difference of the correlations was observed among different water types (Table 4-3, Figure 4-1).

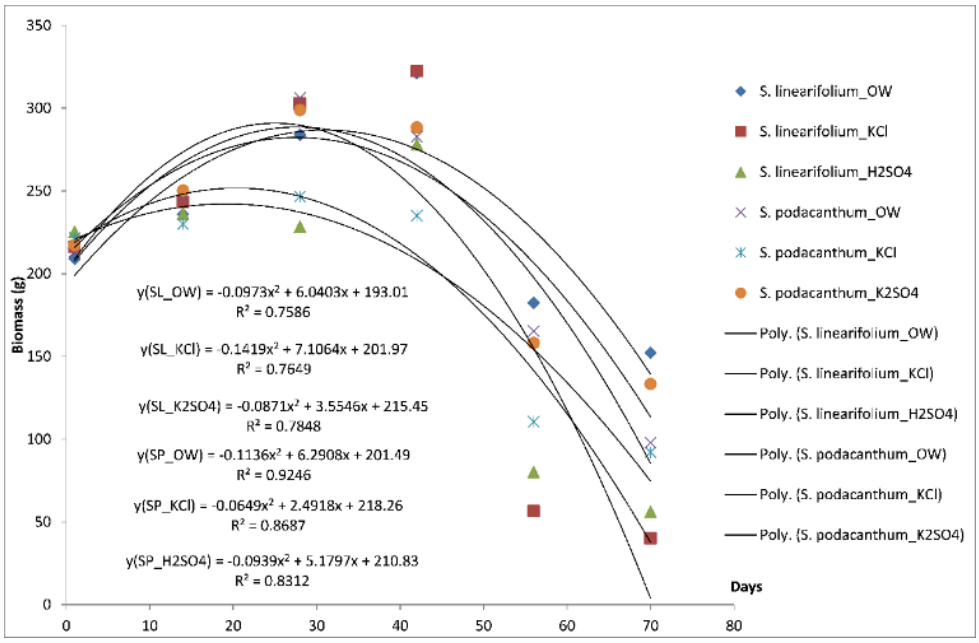


Figure 4-1. Correlation of the biomass (y) (g) of *Sargassum* spp. cultured in OW and two sources of K^+ISW over time (x) (days) (SL stands for *S. linearifolium*, SP stands for *S. podacanthum*)

Only during the first 14 days, there was a significant (P<0.05) effect of water on the SGR of both *Sargassum* spp. *S. linearifolium* in OW produced higher SGR and biomass increase than in ISW_K_2SO_4, whereas the SGR and biomass increase of *S. padocanthum* in OW was higher (P<0.05) than in ISW_KCl (Table 4-4). There were no significant differences in SGR or biomass between the two species in any of the three water types, except at between days 28–42, ISW_K_2SO_4 resulted in higher SGR

of *S. linearifolium* than *S. podacanthum*. In all time periods of the culture, *Sargassum* spp. SGRs were not significantly ($P>0.05$) influenced by either water types or species (Table 4-4, Table 4-8).

The needed quantity of K_2SO_4 (642 mg L^{-1}) was 1.26 times more than KCl (508.5 mg L^{-1}) to fortify the same volume of ISW to OW-equivalent [K^+]. However, the price of KCl is AU$115 kg^{-1}, which is 10.53 times expensive than K_2SO_4 (AU$10.92 kg^{-1}). Therefore, using the K_2SO_4 would be 8.33 times cheaper than KCl in order to receive the same [K^+] in ISW. As both sources of K^+ from KCl and K_2SO_4 resulted in a similar effect on the growth of both *Sargassum* spp., K_2SO_4 can also be used to fortify ISW. However, finance was not an issue for this research as all experiments had been conducted under laboratory conditions where KCl is available, therefore, KCl had been used throughout the thesis experiments more than K_2SO_4.

The other possible way to increase the [K^+] in ISW is mixing the OW with ISW to achieve the required K^+ concentration. However, this solution is only suitable when the required [K^+] is less than the [K^+] in OW, for instance for culturing snapper (*Pagrus auratus*) in ISW (Fielder *et al*., 2001).

4.3.2.2 Water Quality

The average temperature of cultured media collected at 9.30 AM was 24^{o}C and there was no significant difference in the temperature among the 6 treatments (Table 4-5). The average daily temperature over the experimental period recorded was 22.5^{o}C. However, the variation of temperature was extremely high. The largest range of temperature variation in a day was 20^{o}C. The maximum and minimum temperatures were 38.71 and 13.65^{o}C, respectively. The highest and lowest daily temperatures were recorded at approximately 2–4PM and 4–6AM, respectively.

Table 4-2. Sources of K^+ to fortify ISW to grow marine species

Species	Source of K^+	Results	Reference
Blue mussels (*Mytilus edulis* (Linnaeus, 1758)	KCl	K^+-ISW increases the survival and size of settling of blue mussel larvae	Dinh and Fotedar (2016)
Black tiger prawn (*P. monodon* (Fabricius, 1798) juveniles	KCl	100% K^+-ISW results in similarity of survival, SGR and osmoregulatory capacity of juvenile tiger shrimp as in OW at similar salinity except osmoregulatory capacity at 25 ppt	Tantulo and Fotedar (2006)
Juvenile mulloway (*Argyrosomus japonicas)*	KCl	$[K^+]$ in ISW > 14 ppt greater than 38% of $[K^+]$ in OW resulted in the survival rate 96% of juvenile mulloway after 8 months culture	Doroudi *et al*. (2006)
Snapper (*Pagrus auratus)*	KCl	40–100% K^+-ISW at 20 ppt results in similar survival rate of snapper as culturing in OW	Fielder *et al*. (2001)
Pacifc white shrimp (*Litopenaeus vannamei*)	KCl	The K^+ fortification significant inceases the postlarvae and juvenile white shrimp survival and growth culturing in low salinity waters	Roy *et al*. (2007)
Post larvae (PL) Black tiger prawn (*Penaeus monodon*)	KCl	At 12.5 ppt, the survival rate and growth of length and weight of PL shrimp in 116.3% K^+-ISW is similar to that in OW, higher than 87.3 and 58.2% K^+-ISW	Shakeeb Ur *et al*. (2005),
Rabbitfish (*Siganus rivulatus*)	KCl	Survival and length of rabbitfish in ISW_40 and ISW_100 are similar to that in OW at 15ppt after 10 weeks of culture period, whereas rabbitfish total mortality in ISW_0 occurs after 8 weeks.	Mourad *et al*. (2012)

Species	Source of K^+	Results	Reference
Western king prawn (*Penaeus latisulcatus*)	KCl	The survival rate of prawn in ISW_100 is significantly higer than in ISW60 and ISW40. K^+-ISW greater than 76% of $[K^+]$ in OW is necessary for prawn survival rate as similar as in OW.	Prangnell and Fotedar (2005)
Western king prawn	KCl	Similarity of prawn survival rate is recorded at 80 and 100% K^+-ISW and OW at 32 ppt.	Prangnell and Fotedar (2006b)
Western king prawn	KCl	Sudden increase of $[K^+]$ from ambient ISW to 80 and 100% of $[K^+]$ in OW does not affect the prawn survival rate osmoregulatory capacity, and ISW_100 results in higher prawn survival rate and osmoregulatory capacity than lower K^+-ISW levels	Prangnell and Fotedar (2009)
Shrimp	Muriate of potash, Kmag®	K^+ addition (muriate of potash holds 49.8 %K and potassium-magienium sulfate which holds 17.8%K) is necessary for shrimp pond using inland saline well water	Boyd *et al*. (2007)
Shrimp	Muriate of potash and K-Mag	K^+ addition (from initial of 6.25 to 40 mg L^{-1}) using muriate of potash (372–560 kg ha^{-1}) and K-Mag (0–1650–1868 kg ha^{-1}) significantly increases the survival and production of shrimp in pond using saline well water 2–8 ppt in Alabama	McNevin *et al*. (2004)

Table 4-3. Standing biomass (g) and biomass gain (g d^{-1}) of *Sargassum* spp. cultured in OW and K^+ISW fortified by two sources of K^+

Time	*S. linearifolium*			*S. padocanthum*		
	OW	**ISW_KCl**	**ISW_K_2SO_4**	**OW**	**ISW_KCl**	**ISW_K_2SO_4**
Standing biomass						
Day 1	$_{12}$207.18±1.78	$_{12}$213.48±4.98	$_{1}$220.90±15.30	$_{13}$211.15±13.40	$_{1}$222.42±9.37	$_{1}$212.97±12.48
Day 14	$_{1}$247.00±11.54	$_{23}$237.68±7.78	$_{12}$230.30±17.88	$_{12}$251.48±11.58	$_{1}$235.14±14.72	$_{12}$241.61±11.32
Day 28	$_{1}$251.94±32.22	$_{34}$289.75±16.46	$_{1}$225.60±25.18	$_{2}$316.24±15.06	$_{1}$260.16±24.66	$_{2}$287.38±30.86
Day 42	$_{1}$277.47±49.39	$_{4}$330.38±40.33	$_{2}$355.93±94.47	$_{2}$271.80±20.24	$_{1}$256.31±32.27	$_{12}$276.87±17.32
Day 56	$_{2}$144.71±43.22	$_{5}$69.62±13.84	$_{3}$85.83±20.51	$_{34}$136.67±55.29	$_{2}$112.96±8.23	$_{3}$161.60±26.79
Day 70	$_{2}$118.65±36.39	$_{5}$52.82±14.33	$_{3}$63.02±18.10	$_{4}$84.88±49.16	$_{2}$96.37±13.23	$_{3}$127.58±25.72
R^2	0.77±0.05	0.72±0.05	0.71±0.14	0.82±0.03	0.84±0.04	0.84±0.04
Biomass gain						
Day 1-14	$_{1}$2.84±0.94^{a}	$_{1}$1.73±0.23^{ab}	$_{12}$0.67±0.28^{b}	$_{12}$2.88±0.54^{a}	$_{1}$0.91±0.47^{b}	$_{1}$2.05±0.45^{ab}
Day 1-28	$_{12}$1.60±1.09	$_{1}$2.72±0.42	$_{12}$0.17±1.02	$_{1}$3.75±0.63	$_{1}$1.35±0.62	$_{1}$2.66±1.45
Day 1-42	$_{1}$1.67±1.14	$_{1}$2.78±0.94	$_{1}$3.21±2.31	$_{2}$1.44±0.40	$_{1}$0.81±0.61	$_{12}$1.52±0.61
Day 1-56	$_{23}$-1.12±0.74	$_{2}$-2.57±0.28	$_{2}$-2.41±0.58	$_{3}$-1.33±0.84	$_{2}$-1.95±0.05	$_{23}$-0.92±0.69
Day 1-70	$_{3}$-1.26±0.50	$_{2}$-2.30±0.23	$_{2}$-2.26±0.43	$_{3}$-1.80±0.51	$_{2}$-1.80±0.07	$_{3}$-1.22±0.52

R^2 is the significant value of the regression between the *Sargassum* biomass y (g) over time x (days). Values (mean±SE) within a row at one *Sargassum* sp. sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4).

Table 4-4. SGR (% d^{-1}) of *Sargassum* spp. cultured in OW and ISW fortified by two sources of K^+

Time	*S. linearifolium*			*S. podacanthum*		
	OW	**ISW_KCl**	**ISW_K_2SO_4**	**OW**	**ISW_KCl**	**ISW_K_2SO_4**
Day 1-14	$_{1}$1.23±0.38^{a}	$_{1}$0.76±0.09^{ab}	$_{1}$0.28±0.12^{b}	$_{1}$1.27±0.25^{a}	$_{1}$0.37±0.19^{b}	$_{1}$0.91±0.12^{ab}
Day 15-28	$_{2}$-0.05±1.39	$_{2}$1.39±0.19	$_{2}$-0.20±0.79	$_{1}$1.63±0.25	$_{1}$0.67±0.27	$_{1}$1.13±0.93
Day 29-42	$_{1}$0.50±0.53	$_{1}$0.82±0.94	$_{3}$2.58±1.65	$_{12}$-1.12±0.81	$_{2}$-0.18±0.24	$_{2}$-0.18±0.41
Day 43-56	$_{3}$-5.86±1.89	$_{3}$-11.35±1.26	$_{4}$-10.13±3.33	$_{3}$-7.31±3.13	$_{3}$-5.74±0.48	$_{3}$-4.13±1.13
Day 57-70	$_{2}$-1.92±0.74	$_{4}$-2.44±0.86	$_{2}$-2.82±0.88	$_{2}$-4.00±2.74	$_{4}$-1.29±0.52	$_{4}$-1.81±0.50
Day 1-28	0.59±0.51	1.08±0.13	0.04±0.42	1.45±0.24	0.52±0.23	1.02±0.55
Day 1-42	0.56±0.47	0.99±0.28	0.89±0.67	0.59±0.15	0.29±0.23	0.62±0.24
Day 1-56	-1.05±0.78	-2.10±0.34	-1.87±0.59	-1.38±0.84	-1.22±0.06	-0.57±0.42
Day 1-70	-1.22±0.75	-2.16±0.41	-2.06±0.63	-1.90±0.63	-1.23±0.15	-0.81±0.36

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4).

The light:dark was approximately 14:10 h per day, photoperiod was from 5AM to 7PM. The light intensity was highest at 9AM–3PM, at approximately 15,000 lux, and some days it was up to 20,000 lux (Table 4-5).

Table 4-5. Environmental factors measured daily at 9:30AM

	S. linearifolium			***S. padocanthum***		
Time	**OW**	**ISW_KCl**	**ISW_K_2SO_4**	**OW**	**ISW_KCl**	**ISW_K_2SO_4**
Light (lux)						
Surface	3453±302	3420±222	3516±172	4024±444	3931±431	4218±383
Bottom	1255±137	941±99	2783±1933	1359±283	736±153	1063±149
pH	8.23±0.02	8.25±0.01	8.21±0.02	8.23±0.03	8.25±0.03	8.26±0.01
^oC	23.71±0.59	23.94±0.61	24.53±0.63	23.47±0.55	23.69±0.59	23.39±0.60

Throughout the experiment, the light, pH and temperature of the waters were similar in all treatments.

The two K^+ISWs ionic profile were identical (Table 4-6). Of the main ions of saline waters, higher Na^+ and S were recorded in OW than in ISW, whereas the K^+ deficiency was clear in ISW than OW, and Ca^{2+} and Mg^{2+} in ISW were higher than in OW. The [Cl^-] in ISW was lower than in OW, and it was added to ISW_KCl to a similar concentration as in OW. [S] in ISW was also lower than in OW, and the K_2SO_4 addition elevated [S] in ISW_ K_2SO_4 was closed to that in OW (Table 4-6).

Study about main ions in OW related to seaweed limited on K^+ (Chen & Yeh, 2005; Ma & Liu, 2002; Scott & Hayward, 1953; Yamashita *et al*., 2009; Yarish *et al*., 1980), Ca^{2+} (Yarish *et al*., 1980), Ca^{2+} affects Zn^{2+} biosorption of *Sargassum* sp. (da Costa *et al.*, 2001; Pessôa de França *et al.*, 2002), Na^+ (Scott & Hayward, 1953, 1954; Yamashita *et al*., 2009), and some other studies also concern on transport of Na^+, K^+ and Cl^- ions in seaweed (Barr & Broyer, 1964; Gutknecht, 1966; MacRobbie & Dainty, 1958). Although the K^+ and Na^+ concentrations in OW and K^+ISW were different, their effects were not discussed in this current Chapter but the following ones. The only difference of the two K^+ISWs was [Cl^-] in ISW_KCl higher was than in ISW_K_2SO_4 and [SO_4^{2-}] in ISW_K_2SO_4 was higher than in ISW_KCl. As these anion concentrations in ISW were lower than in OW at the same salinity, fortifying the ISW by either KCl or K_2SO_4 elevated respective [Cl^-] or [SO_4^{2-}] in ISW closed to its

concentration in OW. There is no published information about the effect of anion, like Cl^- and SO_4^{2-}, on the growth of seaweed yet.

Table 4-6. Ionic profile (mg L^{-1}) in waters before culturing *Sargassum* spp.

Parameters	OW	ISW	ISW_KCl	ISW_K_2SO_4
Bo	3.95	0.66	0.68	0.66
Ca	371.6	583.00	587.60	583.00
Cl	19679.5	18297.0	19077.6	17907.8
Cu	<0.05	<0.05	<0.05	<0.05
Fe	<0.05	<0.05	<0.05	<0.05
Mg	1168	1525	1579	1565
Mn	<0.05	<0.05	<0.05	<0.05
P	<0.05	0.07	0.07	0.07
K	351.1	84.4	359.8	353.3
Na	10010	8719	8610	8574
S	805.4	602.4	642.6	769.2
Zn	<0.05	<0.05	<0.05	<0.05

Source: Modified from Dinh (2016)

Cl^- is the most abundant ion in the OW, with the average concentration of 19,000 mg L^{-1} (Feth, 1981) (Table 4-7), sharing 55.04% of total ions in OW (Brown *et al.*, 1989). Study of the Cl^- in ISW is limited. The role of Cl^- in the survival of red drum (*Sciaenops ocellatus*) in saline ground water is not clear. Increasing the Cl^- in saline ground water from 639 to 1,296 mg L^{-1} in conjunction with Ca^{2+} elevation from 36 to 336 mg L^{-1} significantly enhanced the survival of red drum, whereas the combination of Cl^- and Na^+ just slightly increase red drum survival (Forsberg *et al.*, 1996). Although KCl is more commonly used salt to fortify the ISW to culture marine species (Table 4-2), the role of K^+ is clearer to the fish growth and performance than the Cl^-. Cl^- and K^+ in ISW affect the growth of Australian snapper (*Pagrus auratus*) and the suitable K^+/Cl^- ratio is 0.007–0.018 (Fielder *et al.*, 2001). At 45 ppt, $[Cl^-]$ in ISW is 25,000 mg L^{-1}, which causes more severe chloride cell hyperplasia in juvenile barramundi (*Lates calcarifer*) than OW (Partridge & Creeper, 2004). However, as the SGRs of the two *Sargassum* spp. were the same in ISW_KCl and OW, which showed that Cl^- did not play any significant role on the growth of the two *Sargassum* spp.

Table 4-7. Cl^- and SO_4^{2-} concentrations in OW and ISW

Water	Salinity (ppt)	Concentration (mg L^{-1})	Reference
Cl^-			
OW	35	19,370	Harvey (1957)
OW	35	18,980	Brown *et al*. (1989)
OW		19,000	Feth, 1981; Goldberg (1966)
OW	35.3	20,000	Fielder *et al*. (2001)
OW	19.6	11,105	Fielder *et al*. (2001)
ISW	19.6	11,000	Fielder *et al*. (2001)
SO_4^{2-}			
OW	35	2,710	Harvey (1957)
OW	35	2,649	Brown *et al*. (1989)
OW		2,655	Goldberg (1966)
OW	35.3	2,500	Fielder *et al*. (2001)
OW	19.6	1,388	Fielder *et al*. (2001)
ISW	19.6	1,100	Fielder *et al*. (2001)

The third most abundant ion in OW is SO_4^{2-} which is 2,560 mg L^{-1} (Feth, 1981; McGraw & Scarpa, 2003) (Table 4-7), sharing 7.68% of total ions in OW (Brown *et al*., 1989). SO_4^{2-} accounts for more than 80% of total sulfur (S) in domestic water, includes precipitation (97%) and snow (88%) (Edwards *et al.*, 1992). However, SO_4^{2-} plays no role in shrimp hemolymph at the salinity lower than 25 ppt (Dall & Smith, 1981). The SO_4^{2-} itself also shows no significant effect on the short-term survival of Pacific white shrimp *Litopenaeus vannamei* (Boone), although in addition to the K^+, SO_4^{2-} and K^+ together strongly enhance the survival of white shrimp in freshwater (McGraw & Scarpa, 2003). SO_4^{2-} concentration of 0.46 mg L^{-1} in natural water at 2.56 ppt is fortified by Kmag® 1,650 kg ha^{-1} levels up to 75 mg L^{-1} and then reduces to 50 mg L^{-1} stably, has no significant influence on shrimp production as K^+, which results in higher survival and growth of shrimp when higher K^+ is applied (McNevin *et al*., 2004).

The role of Cl^- and SO_4^{2-} on the performance of plants have been investigated, such as in tobacco (*Nicotiana tabacum)*, where the low [SO_4^{2-}] significantly affects the leaf length and weight of tobacco but it should not exceed 96 ppm, and 280 ppm Cl^- is good

for tobacco leaf length and weight increase (Wedin & Struckmeyer, 1958). Although the high Cl^- shows inhibitive effect on tobacco leaf burn but not SO_4^{2-} (Wedin & Struckmeyer, 1958), higher Cl^- in the ISW_KCl did not affect the two *Sargassum* spp. growth in K^+ISW. The SO_4^{2-} deficiency (9.6 mg L^{-1}) provided by watering four times a day significantly reduces the photosynthesis capacity, lower chlorophyll a and b of tomato plants (*Lycopersicon esculentum* Mill. Cv. Trust) (Xu *et al.*, 1996). However, the lower SO_4^{2-} in the ISW_KCl than in OW and ISW_K_2SO_4 showed no significant effect on growth of the two *Sargassum* spp. when the SGRs of these two *Sargassum* spp. were similar at all water types. Therefore, it can be concluded that Cl^- and SO_4^{2-} played no significant role in the growth of the two *Sargassum* spp. in K^+ISW as the same growth rates were observed by two species.

The nitrogen and phosphorus of waters measured fortnightly were presented in Table 4-8. The [NO_2^--N] significantly ($P<0.05$) increased toward the end of the experiment and it was correlated ($P<0.05$) with the *Sargassum* spp. biomass. There was only one significant ($P<0.05$) difference in [NO_2^--N] between OW and ISW_KCl culturing *S. linearifolium*. No significant difference of [NO_2^--N] in waters was found between three water types or two species. Similar to NO_2^--N, the NO_3^--N and PO_4^{3-}-P concentrations significantly ($P<0.05$) rose by the end of the experiment. However, there was no significant ($P>0.05$) effect between water types and species on NO_3^--N, and no significant ($P>0.05$) difference of NO_3^--N concentration in water among water types of one *Sargassum* species, or between two species in one type of water. The only significant ($P<0.05$) difference in PO_4^{3-}-P between two species at OW was recorded at the day 28. The concentrations of NH_4-N and TKN were similar in all treatments, and they did not change over the duration of the experiment. The t-test showed no significant ($P>0.05$) difference of TKN between two species in a water. Although the concentrations of nitrogen and phosphorus in culture media including NO_2^--N, NO_3^--N, NH_4-N, TKN and PO_4^{3-}-P were significantly ($P<0.05$) correlated to biomass and SGR of the two *Sargassum* spp. (Table 4-9), there will be more detail discussion in the following Chapters.

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Table 4-8. The quality parameters of OW and ISW fortified by two sources of K^+ cultured *Sargassum* spp.

Param-eters	Time	*S. linearifolium*			*S. padocanthum*		
		OW	**ISW_KCl**	**ISW_K_2SO_4**	**OW**	**ISW_KCl**	**ISW_K_2SO_4**
NO_2^--N	Day 1	$_{1}$0.003±0.001	$_{1}$0.006±0.002	$_{1}$0.007±0.002	$_{1}$0.003±0.001	$_{1}$0.006±0.002	$_{1}$0.006±0.002
	Day 14	$_{12}$0.007±0.001	$_{1}$0.007±0.001	$_{1}$0.007±0.002	$_{1}$0.006±0.001	$_{12}$0.009±0.002	$_{1}$0.006±0.001
	Day 28	$_{12}$0.007±0.000	$_{1}$0.006±0.001	$_{1}$0.007±0.001	$_{1}$0.006±0.001	$_{12}$0.008±0.001	$_{12}$0.007±0.001
	Day 42	$_{12}$0.008±0.002	$_{1}$0.010±0.002	$_{1}$0.011±0.002	$_{2}$0.012±0.001	$_{1}$0.006±0.001	$_{12}$0.010±0.002
	Day 56	$_{23}$0.012±0.002	$_{1}$0.020±0.007	$_{12}$0.028±0.014	$_{2}$0.015±0.002	$_{2}$0.052±0.036	$_{2}$0.017±0.003
	Day 70	$_{3}$0.018±0.004^{a}	$_{2}$0.046±0.014^{b}	$_{2}$0.037±0.011^{ab}	$_{2}$0.016±0.002	$_{12}$0.028±0.007	$_{3}$0.023±0.007
NO_3^--N	Day 1	$_{1}$2.33±0.18	$_{1}$1.85±0.22	$_{1}$1.95±0.25	$_{1}$2.38±0.10	$_{1}$1.98±0.25	$_{1}$2.38±0.17
	Day 14	$_{1}$1.60±0.31	$_{1}$2.25±0.41	$_{1}$2.25±0.35	$_{1}$2.08±0.17	$_{1}$1.65±0.24	$_{1}$2.25±0.27
	Day 28	$_{1}$2.08±0.17	$_{1}$2.38±0.31	$_{1}$2.15±0.22	$_{1}$2.04±0.32	$_{1}$2.37±0.10	$_{1}$2.32±0.18
	Day 42	$_{1}$2.03±0.24	$_{1}$2.21±0.29	$_{1}$2.27±0.17	$_{1}$2.15±0.32	$_{1}$2.00±0.12	$_{1}$2.15±0.37
	Day 56	$_{1}$1.68±0.36	$_{1}$5.63±2.67	$_{1}$3.53±1.12	$_{12}$2.46±0.37	$_{1}$2.91±0.38	$_{1}$2.59±0.75
	Day 70	$_{2}$3.95±1.15	$_{2}$11.63±4.09	$_{2}$8.80±3.17	$_{3}$3.40±0.53	$_{2}$6.31±1.94	$_{2}$4.95±1.68
NH_4-N	Day 1	$_{1}$0.01±0.01	$_{1}$0.01±0.01	$_{1}$0.00±0.00	$_{1}$0.01±0.01	$_{1}$0.01±0.01	0.01±0.01
	Day 14	$_{1}$0.00±0.00	$_{1}$0.00±0.00	$_{1}$0.00±0.00	$_{1}$0.00±0.00	$_{1}$0.01±0.01	0.01±0.01
	Day 28	$_{2}$0.05±0.02	$_{12}$0.12±0.01	$_{2}$0.08±0.02	$_{2}$0.09±0.00	$_{1}$0.05±0.03	0.09±0.04
	Day 42	$_{3}$0.09±0.01	$_{3}$0.17±0.03	$_{3}$0.18±0.03	$_{2}$0.10±0.02	$_{2}$0.21±0.07	0.09±0.03
	Day 56	$_{12}$0.03±0.02	$_{12}$0.06±0.04	$_{2}$0.07±0.03	$_{1}$0.00±0.00	$_{1}$0.04±0.02	0.09±0.06
	Day 70	$_{1}$0.01±0.01	$_{1}$0.02±0.01	$_{12}$0.02±0.02	$_{1}$0.00±0.00	$_{1}$0.00±0.00	0.00±0.00

Parameters	Time	*S. linearifolium*			*S. padocanthum*		
		OW	**ISW_KCl**	**ISW_K_2SO_4**	**OW**	**ISW_KCl**	**ISW_K_2SO_4**
TKN	Day 1	0.81±0.21	$_{1}$1.09±0.18	$_{12}$1.12±0.09	0.81±0.23	1.00±0.18	$_{12}$1.12±0.04
	Day 14	0.95±0.24	$_{1}$1.17±0.27	$_{12}$1.13±0.25	0.85±0.27	0.93±0.24	$_{12}$1.27±0.06
	Day 28	1.18±0.57	$_{1}$0.96±0.41	$_{1}$0.57±0.33	1.25±0.42	0.36±0.14	$_{1}$0.55±0.29
	Day 42	1.38±0.33	$_{12}$1.73±0.20	$_{2}$1.95±0.22	1.47±0.23	1.66±0.48	$_{2}$1.90±0.44
	Day 56	2.08±0.80	$_{2}$2.29±0.38	$_{3}$4.56±0.44	1.55±0.65	3.19±1.14	$_{3}$3.05±0.54
	Day 70	0.80±0.32	$_{12}$1.78±0.29	$_{2}$1.89±0.32	1.05±0.18	1.85±0.10	$_{12}$1.48±0.22
PO_4^{3-}-P	Day 1	$_{1}$0.45±0.12	$_{1}$0.75±0.17	$_{1}$0.60±0.18	$_{1}$0.58±0.03	$_{1}$0.73±0.16	$_{1}$0.58±0.03
	Day 14	$_{1}$0.68±0.09	$_{1}$0.85±0.22	$_{1}$0.75±0.16	$_{1}$0.58±0.17	$_{1}$0.83±0.12	$_{1}$0.85±0.05
	Day 28	$_{1}$0.36±0.05	$_{1}$0.55±0.06	$_{1}$0.42±0.07	$_{1}$0.72±0.16	$_{1}$0.51±0.04	$_{1}$0.48±0.07
	Day 42	$_{2}$1.47±0.43	$_{1}$1.11±0.26	$_{1}$1.28±0.32	$_{1}$0.93±0.31	$_{1}$1.26±0.19	$_{2}$1.58±0.10
	Day 56	$_{13}$0.77±0.06^{a}	$_{1}$1.69±0.50^{b}	$_{12}$1.38±0.14^{ab}	$_{1}$0.82±0.25	$_{1}$0.93±0.21	$_{1}$0.92±0.17
	Day 70	$_{23}$1.43±0.33^{a}	$_{2}$3.71±1.12^{b}	$_{2}$2.65±0.98^{ab}	$_{2}$1.47±0.18	$_{2}$2.18±0.52	$_{2}$1.71±0.43

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4).

Table 4-9. Pearson correlations of *Sargassum* spp. biomass and water quality parameters

Criteria	Dependent variable	NO_2^--N	NO_3^--N	PO_4^{3-}-P	NH_4-N	TKN
Biomass	All	-0.424**	-0.459**	-0.372**	0.358**	-0.275**
	SL_OW	-0.414*	-0.382	0.133	0.215	0.153
	SL_ISW_KCl	-0.641**	-0.616**	-0.626**	0.484*	-0.352
	SL_ISW_K_2SO_4	-0.486*	-0.420*	-0.292	0.467*	-0.347
	SP_OW	-0.492*	-0.474*	-0.396	0.545**	-0.164
	SP_ISW_KCl	-0.380	-0.430*	-0.351	0.249	-0.510*
	SP_ISW_K_2SO_4	-0.652**	-0.624**	-0.456*	0.276	-0.378
SGR	All	-0.420**	-0.417**	-0.368**	0.246**	-0.312**
	SL_OW	-0.474*	-0.367	0.040	-0.015	0.053
	SL_ISW_KCl	-0.622**	-0.587**	-0.610**	0.433	-0.413
	SL_ISW_K_2SO_4	-0.439	-0.357	-0.242	0.488*	-0.310
	SP_OW	-0.722**	-0.544*	-0.445*	0.445*	-0.148
	SP_ISW_KCl	-0.396	-0.419	-0.375	0.218	-0.508*
	SP_ISW_K_2SO_4	-0.622**	-0.517*	-0.521*	0.019	-0.525*

(*) – 2-tail significance (P<0.05) (**) – 2-tail significance (P<0.001) SL – *S. linearifolium* SP – *S. podacanthum*

4.4 Conclusions

Among the six studied species, *Sargassum* is the most suitable candidate to cultivate in ISW. In addition, *L. catenata* showed longer survival than *G. subpectinata*, thus *L. catenata* can be chosen as a representative of the red seaweeds. Same SGR of *S. linearifolium* and *S. podacanthum* was achieved when cultured in ISW fortified by either KCl or K_2SO_4 to OW-equivalent [K^+]. The potash of sulphate K_2SO_4 is recommended to fortify ISW as an alternate for KCl.

BROWN SEAWEED

CHAPTER 5 [*]PRODUCTIVITY OF *Sargassum linearifolium* AND *S. podacanthum* IN POTASSIUM-FORTIFIED INLAND SALINE WATER

5.1 Introduction

Inland saline water (ISW) in Australia is abundant in natural rivers, lakes and in shallow aquifers (Nulsen, 1997). Over 2.5 million hectares of land is salt-affected as a consequence of a reduction in perennial, deep-rooted natural vegetation (Nulsen, 1997). The increase in inland saline areas has negatively affected agricultural land, wildlife habitats and native vegetation (Allan *et al*., 2008).

One possible way to convert ISW-affected land into a valuable resource is to use the same land for aquaculture of marine species (Allan *et al*., 2008). ISW is available in the form of large reserves of underground water (Nulsen, 1997), which could provide a source of water for inland marine aquaculture (Partridge, 2008). At the same salinity, the ionic profile of ISW is similar to seawater, except for the lower [K^+] in ISW (Boyd *et al*., 2007; Fielder *et al*., 2001; Fotedar *et al*., 2011; Ingram *et al*., 2002; Partridge & Lymbery, 2008; Prangnell & Fotedar, 2006b), which is around 26–331 mg L^{-1} in Western Australia (WA) (Nulsen, 1997). The K^+ deficiency in ISW has been shown to negatively affect survival and growth of juvenile mulloway (*Argyrosomus hololeptidotus*) (Doroudi *et al*., 2006), juvenile snapper (*Pagrus auratus*) (Fielder *et al*., 2001), and red drum (*Sciaenopsocellatus*) (Forsberg *et al*., 1996). Similarly, mortalities of giant tiger prawn (*Panaeus monodon*) (Ingram *et al*., 2002; Shakeeb Ur *et al*., 2005; Tantulo & Fotedar, 2007), Japanese tiger prawn (*Marsupenaeus aponicus*), Pacific white shrimp (*Litopenaeus vannamei*) (Roy *et al*., 2007), barramundi (*Lates calcarifer*) (Partridge & Creeper, 2004; Partridge & Lymbery, 2008) and snapper (Fielder *et al*., 2001) in ISW have been recorded due to K^+ deficiency. It is necessary to add K^+ to ISW, equivalent to 50–100% that of ocean water (OW) for culturing snapper (Fielder & Allan, 2003; Fielder *et al*., 2001), shrimp

[*] A part of this Chapter entitled 'Productivity of *Sargassum linearifolium* in Potassium-Fortified Inland Saline Water under Laboratory Conditions', was previously published in the journal of *Aquaculture Research* as a research article, 2017, volume 48, issue 11, pages 5631–5639.

(Shakeeb Ur *et al*., 2005; Tantulo & Fotedar, 2006), mulloway (Doroudi *et al*., 2006), and rabbitfish (*Siganus rivulatus*) (Mourad *et al*., 2012). Prawn (*Penaeus latisulcatus*, *Penaeus monodon*) cultured in OW-equivalent K^+ISW yields a similar survival and growth rate as in OW due to increasing osmo-regulation capacities (Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006). K^+ can be supplemented by adding muriate of potash, a product that contains at least 95% KCl (49.8% K^+) (Boyd *et al*., 2007; McNevin *et al*., 2004; Partridge & Creeper, 2004), Kmag, a product that holds 18.3% of K^+ (Boyd *et al*., 2007; McNevin *et al*., 2004), or chemical grade potassium chloride (KCl) holding 52.3% K^+ (Prangnell & Fotedar, 2006a, 2006b; Tantulo & Fotedar, 2007).

The ambient temperature in most of the salinity-affected areas of Australia is 12.3–28.4^{o}C (Partridge *et al*., 2006), and may not be suitable for shrimp farming (Partridge, Lymbery, & George, 2008), but this temperature may be suitable for seaweed culture. ISW aquaculture has gradually developed in Australia, mainly targeting fish and shrimp (Allan *et al*., 2001; Allan *et al*., 2008). To date, 29 shrimp, fish, and mollusc species have been researched and some have been commercially cultured in ISW in Australia as summarized by Dinh (2016), yet the research on seaweed culture in ISW remains restricted to only *Gracilaria cliftonii* (Kumar *et al*., 2010).

Of the 350 species of brown seaweeds in Australia, the order Fucales (including genera *Sargassum* and *Cystophora*) are common in Southern Australia (Huisman, 2000). The growth, chemical and nutrient uptakes have been studied worldwide on seaweed (Ahmad *et al*., 2011; Bird *et al*., 1978; Campbell, 1999; Coffaro & Sfriso, 1997; Coutinho & Zingmark, 1993; Dailer *et al*., 2012; de Casabianca *et al.*, 2002; Flindt *et al*., 1997; Gordillo *et al*., 2001; Kitadai & Kadowaki, 2007; Larned, 1998; Pérez-Mayorga *et al*., 2011; Perini & Bracken, 2014), and *Sargassum* spp. in particular (Andrew & Viejo, 1998; Chen & Zou, 2014; Choi *et al*., 2009; Cui *et al*., 2014; Gao & Hua, 1997; Hanisak & Samuel, 1987; Keesing *et al.*, 2011; Lapointe, 1986; Rao & Rao, 2002; Reef *et al.*, 2012; Schaffelke & Klumpp, 1998; Yuan *et al*., 2014). However, there is no available information about growing *Sargassum* in ISW, whereas the K^+ deficiency in ISW is currently a concern in using ISW for aquaculture.

As in animals, the role of K^+ is important to terrestrial plants and seaweed (Blumwald *et al*., 2000; Talling, 2010). K^+ is physiologically important for algal growth and

cannot be totally substituted by any other chemical element as it is an "activator and cofactor of enzymes in respiration and carbohydrate metabolism" (Yarish *et al*., 1980, p. 236). The K^+ concentration in water is an important factor in maintaining turgor regulation and external osmotic pressure of *Chaetomorpha linum*, a littoral green alga (Zimmermann & Steudle, 1971). K^+ is also important in photosynthesis and respiration as it activates several enzymes to synthesise protein and carbohydrates (Checchetto *et al*., 2013). Similarly, K^+ is important in regulating the osmotic pressure of *Platymonas subordiformis* and *Chlamydomonas reinhardtii* (Kirst, 1977; Malhotra & Glass, 1995). Therefore, this laboratory-based study was conducted to investigate the relationship between the growth of *S. linearifolium* and *S. podacanthum* and the levels of K^+-fortification in ISW in order to provide a platform to cultivate *Sargassum* spp. in ISW. These results would be fundamental for further research on seaweed aquaculture.

5.2 Materials and Methods

Two separate trials were conducted in winter of two years for two *Sargassum* spp. Seaweed collection, water preparation at 35 ppt, data collection and data analysis were followed as described previously in Chapter 3.

5.2.1 Experiment Setup

The growth rate of *Sargassum* spp. was determined in three levels of K^+-fortified ISW (K^+ISW). Five treatments were OW, ambient ISW (ISW0), ISW fortified with K^+ as 100, 66, 33% of the [K^+] in OW at 35 ppt, by KCl 508.5, 280.90, 59.99 mg L^{-1} respectively, namely ISW100, ISW66, ISW33.

5.2.1.1 *S. linearifolium* Cultured in Potassium-fortified Inland Saline Water

After acclimation, *S. linearifolium* was removed and cut into pieces to achieve the pre-determined weight of approximate 3,500 mg L^{-1} and then transferred immediately into 1.5 L beakers. Similar parts of the thallus and from the same stage of development, hence the same physiological state, was used in all treatments. A total of 20 1.5 L glass beakers were used for the trial of 84 days.

5.2.1.2 *S. podacanthum* Cultured in Potassium-fortified Inland Saline Water

A similar trial lasting 56 days of the following year was repeated with *S. podacanthum* in 15 L tanks, using the whole thalli of *S. podacanthum* at stocking density of 3,500 mg L^{-1}, in order to have sufficient *S. podacanthum* tissue for analysing protein and chemical compositions during and at the end of the experiment.

5.2.2 Data Collection

The ammonium (NH_4-N), nitrate (NO_3^--N), nitrite (NO_2^--N) and phosphate (PO_4^{3-}-P), Total Kjeldahl Nitrogen (TKN) concentration in water, *Sargassum* spp. biomass, *Sargassum* spp. dried powder, *Sargassum* spp. specific growth rates (SGR), crude protein, gross energy, the pH, temperature and salinity of cultured media, the total solids (TS), total volatile solids (TVS), total suspended solids (TSS) and volatile suspended solids (VSS) were determined as presented in Chapter 3.

In the *S. podacanthum* experiment, the ionic profile of cultured media, ionic compositions of the *S. podacanthum*, total nitrogen and carbon contents of *S. podacanthum* were analysed as described in chapter 3.

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5.3 Results

5.3.1 *S. linearifolium* Cultured in Potassium-fortified Inland Saline Water

5.3.1.1 Biomass of *S. linearifolium*

Since the commencement of the trial, the biomass of the *S. linearifolium* increased significantly ($P<0.05$) in ISW100 and OW. Culturing *S. linearifolium* in ISW100 and ambient OW resulted in significantly ($P<0.05$) higher biomass than in the other water types (Table 5-1). The *S. linearifolium* biomass in ISW100 and OW shared similar growth pattern and reached a peak around the third fortnight and then remained static for the rest of the trial before declining. From the second fortnight, *S. linearifolium* cultured in ISW100 grew significantly ($P<0.05$) lower than in OW. In the last fortnight of the trial, the biomass of *S. linearifolium* cultured in ISW100 declined at a rate of 1.5% and became significantly ($P<0.05$) lower than in OW.

Table 5-1. Biomass (mg) of *S. linearifolium* cultured in OW and K^+ISW

Time	OW	ISW0	ISW33	ISW66	ISW100
Day 1	$_{1}3.38\pm0.10^{a}$	$_{1}3.38\pm0.12^{a}$	$_{1}3.16\pm0.10^{a}$	$_{1}3.39\pm0.06^{a}$	$_{1}3.28\pm0.09^{a}$
Day 14	$_{2}4.50\pm0.26^{a}$	$_{1}3.38\pm0.20^{b}$	$_{2}4.52\pm0.55^{a}$	$_{23}4.51\pm0.21^{a}$	$_{2,3}4.81\pm0.38^{a}$
Day 28	$_{3}6.46\pm0.51^{a}$	$_{2}2.46\pm0.32^{b}$	$_{1}3.34\pm0.09^{b}$	$_{23}4.56\pm0.15^{c}$	$_{2,3,4}5.39\pm0.20^{c}$
Day 42	$_{4}7.91\pm0.44^{a}$	$_{3}1.43\pm0.55^{b}$	$_{1}2.62\pm0.17^{c}$	$_{23}4.28\pm0.21^{d}$	$_{3,4}5.79\pm0.38^{e}$
Day 56	$_{4}7.84\pm0.30^{a}$	$_{3}1.23\pm0.47^{b}$	$_{3}1.44\pm0.60^{b}$	$_{13}3.93\pm0.36^{c}$	$_{4}5.90\pm0.51^{d}$
Day 70	$_{34}7.15\pm0.21^{a}$	$_{4}0^{b}$	$_{4}0^{b}$	$_{13}3.76\pm0.26^{c}$	$_{4}5.83\pm0.42^{d}$
Day 84	$_{34}6.97\pm0.50^{a}$	$_{4}0^{b}$	$_{4}0^{b}$	$_{1}3.34\pm0.57^{c}$	$_{2}4.74\pm0.28^{d}$
R^2	0.91 ± 0.01^{ac}	0.93 ± 0.01^{a}	0.83 ± 0.03^{bc}	0.70 ± 0.07^{b}	0.88 ± 0.03^{ac}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript are not significantly different (LSD test; P>0.05; n=4). R^2 is the power regression correlation coefficient between biomass (y) and time (fortnight) (the data were transformed to arcsin before conducting comparison)

As the time progressed, lower [K^+] in ISW resulted in lower *S. linearifolium* biomass. During the first three fortnights, *S. linearifolium* biomass cultured in ISW100 and ISW66 showed a hyperbolic trend as the time progressed, plateaued around three to five fortnights and then declined (Figure 5-1). There was no significant (P>0.05) difference in *S. linearifolium* biomass between the ambient ISW and ISW33 except at second and fourth fortnights, where *S. linearifolium* biomass was significantly (P<0.05) higher in ISW33 than in ISW. Complete mortality was observed in ISW and ISW33 at the day 56 of the trial (Table 5-3).

The relationship between biomass and time was correlated (R^2 >0.7) in all water types (Figure 5-1), however it was significantly (P<0.05) stronger when no K^+ was fortified to ISW (Table 5-1).

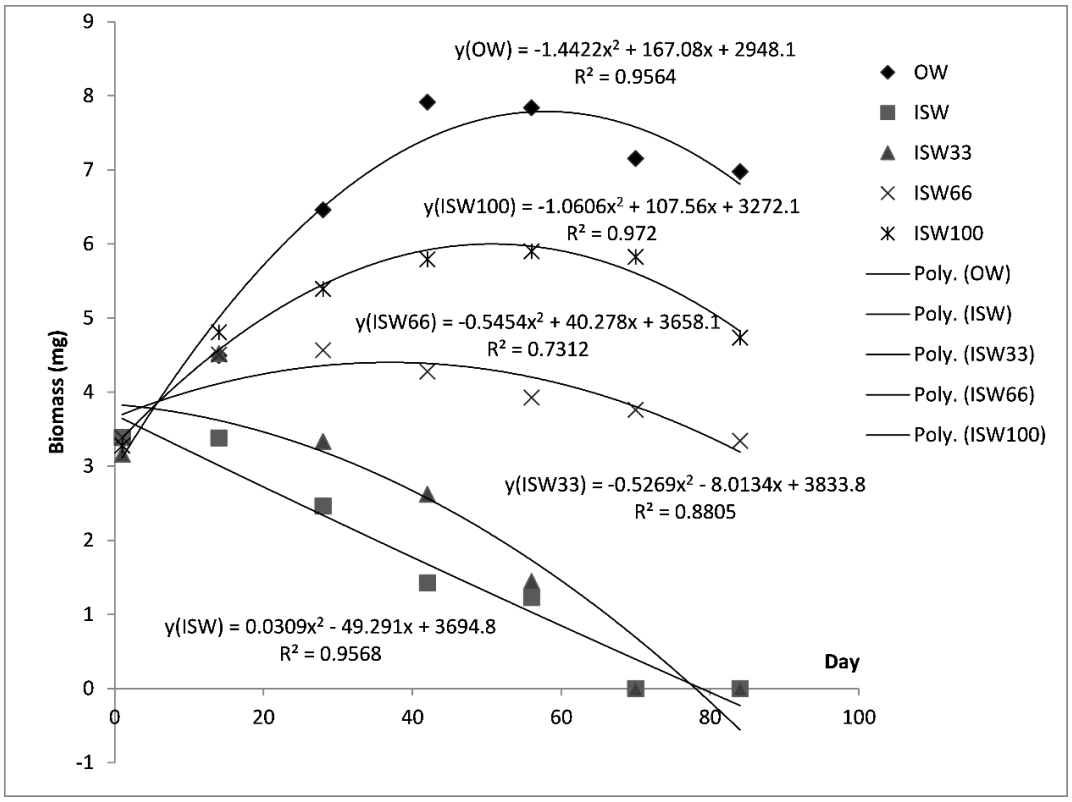

Figure 5-1. The correlation of *S. linearifolium* biomass (y) cultured in K^+ISW with time in days (x) (R^2 is the power regression correlation coefficient between biomass (y) and time (fortnight).

5.3.1.2 Specific Growth Rate of *S. linearifolium*

The SGR of *S. linearifolium* steadily declined in all water types, however, it was positive in OW and ISW100, and negative in the other types of ISW (Table 5-2). In the OW, the *S. linearifolium* grew at a constant rate of 2% d^{-1} from the commencement to the third fortnight and then fell to 1% d^{-1}, but was significantly ($P<0.05$) higher than in ISW and in ISW33. The SGR of *S. linearifolium* in the ISW100 and ISW66 was similar to OW in the first four fortnights, and significantly ($P<0.05$) lower in all types of ISW from the fifth fortnight onward.

The *S. linearifolium* biomass and SGR were significantly ($P<0.01$) correlated with pH and the concentrations of NO_3^--N and TKN in water whereas PO_4^{3-}-P showed significantly ($P<0.05$) less strong relationship (Table 5-3).

Table 5-2. Cumulative SGR (% d^{-1}) of *S. linearifolium* in K^+ISW

Time	OW	ISW0	ISW33	ISW66	ISW100
Day 1–14	1.92 ± 0.12^{a}	-0.01 ± 1.62^{b}	2.31 ± 0.71^{a}	1.91 ± 0.96^{a}	2.57 ± 0.66^{a}
Day 1–28	$_{1}2.29\pm0.18^{a}$	$_{1}-1.22\pm0.50^{b}$	$_{1}0.19\pm0.14^{c}$	$_{2}1.06\pm0.06^{d}$	$_{12}1.78\pm0.05^{ad}$
Day 1–42	$_{1}2.02\pm0.10^{a}$	$_{2}-3.78\pm2.33^{b}$	$_{12}-0.46\pm0.17^{a}$	$_{23}0.55\pm0.10^{a}$	$_{23}1.34\pm0.14^{a}$
Day 1–56	$_{12}1.50\pm0.03^{a}$	$_{2}-3.15\pm1.80^{b}$	$_{2}-2.95\pm1.99^{b}$	$_{34}0.24\pm0.19^{ab}$	$_{23}1.03\pm0.14^{a}$
Day 1–70	$_{23}1.07\pm0.03^{a}$			$_{34}0.14\pm0.12^{b}$	$_{23}0.81\pm0.09^{c}$
Day 1–84	$_{3}1.03\pm0.08^{a}$			$_{4}-0.08\pm0.27^{b}$	$_{3}0.52\pm0.09^{c}$

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript are not significantly different (LSD test; P>0.05; n=4)

Table 5-3. Pearson correlations of *S. linearifolium* biomass and water quality parameters of K^+ISW (N=140)

Dependent variable	pH	Tempe-rature	NO_2^--N	NO_3^--N	PO_4^{3-}-P	NH_4-N	TKN
Biomass	0.255^{**}	-0.140	-0.064	-0.321^{**}	-0.210^{*}	0.145	-0.394^{**}
SGR	0.319^{**}	-0.026	-0.111	-0.529^{**}	-0.356^{**}	0.249^{**}	-0.537^{**}

(*) – 2-tail significance (P<0.05) (**) – 2-tail significance (P<0.001)

5.3.1.3 Quality of Potassium-fortified Inland Saline Water

The pH, NO_3^--N, TKN and PO_4^{3-}-P were significantly (P<0.05) correlated to *S. linearifolium* biomass; whereas the NO_2^--N, NH_4-N were not (Table 5-3). The [K^+] showed no significant fluctuation over time in all treatments.

At the beginning and termination of the trial, pH of the water were similar among different water types. Except ISW100, pH of the water increased in all water types at the end of the trial (Table 5-4).

The NH_4-N concentration showed negligible value in all types of waters over the trial period. Except in ISW, the NO_2^--N level in all water types were low and similar to the initial value by the termination of the trial (Table 5-4).

The NO_3^--N increased significantly (P<0.05) as the trial progressed and remained higher than the initial values at all water types. In the ISW and ISW33, nitrate was

highest at the end of the second fortnight, when the *S. linearifolium* died and then remained constant over the rest of the trial (Table 5-4).

TKN significantly increased over the trial period and was significantly different among water types due to the presence of organic nitrogen. The TKN concentration was highest in ISW and ISW33, and rapidly increased right from the first fortnight and reached the peak when *S. linearifolium* biomass declined. TKN was significantly correlated with the growth of *S. linearifolium* and its concentration in OW and ISW66 were significantly (R^2>0.75) correlated with the progression of time (Table 5-4).

Table 5-4. Water quality parameters of K^+ISW cultured *S. linearifolium*

Par.	OW	ISW0	ISW33	ISW66	ISW100
pH					
Day 1	$_{1}$7.92±0.01	$_{1}$8.04±0.03	$_{1}$7.95±0.00	$_{1}$7.97±0.00	$_{1}$8.06±0.01
Day 14	$_{23}$8.84±0.04^{a}	$_{2}$8.48±0.02^{b}	$_{123}$8.36±0.02^{d}	$_{2}$8.56±0.04^{b}	$_{2}$8.73±0.03^{d}
Day 28	$_{2}$8.70±0.04^{a}	$_{2}$8.44±0.01^{b}	$_{2}$8.44±0.03^{b}	$_{3}$8.36±0.03^{c}	$_{23}$8.61±0.01^{d}
Day 42	$_{3}$8.85±0.05	$_{3}$8.63±0.05	$_{23}$8.37±0.20	$_{2}$8.53±0.06	$_{3}$8.52±0.11
Day 56	$_{23}$8.83±0.08	$_{4}$8.83±0.02	$_{4}$8.80±0.07	$_{4}$8.71±0.01	$_{2}$8.76±0.07
Day 70	$_{4}$8.24±0.03	$_{5}$8.27±0.03	$_{123}$8.23±0.02	$_{5}$8.24±0.01	$_{1}$8.21±0.02
Day 84	$_{2}$8.55±0.24	$_{5}$8.30±0.04	$_{123}$8.27±0.03	$_{35}$8.27±0.03	$_{1}$8.25±0.02
NO_2^--N (mg L^{-1})					
Day 1	$_{14}$0.021±0.002	$_{1}$0.044±0.018	$_{1}$0.022±0.002	$_{13}$0.021±0.001	$_{1}$0.028±0.006
Day 14	$_{1}$0.027±0.007^{a}	$_{2}$0.705±0.208^{b}	$_{2}$0.199±0.066^{a}	$_{1}$0.023±0.005^{a}	$_{2}$0.041±0.006^{a}
Day 28	$_{12}$0.028±0.005^{a}	$_{1}$0.135±0.021^{b}	$_{23}$0.112±0.005^{b}	$_{2}$0.035±0.004^{a}	$_{2}$0.036±0.003^{a}
Day 42	$_{3}$0.008±0.000^{a}	$_{1}$0.024±0.002^{a}	$_{13}$0.043±0.013^{b}	$_{3}$0.014±0.001^{a}	$_{3}$0.008±0.000^{a}
Day 56	$_{34}$0.011±0.001^{a}	$_{1}$0.014±0.002^{ab}	$_{1}$0.023±0.003^{c}	$_{13}$0.020±0.001^{bc}	$_{13}$0.013±0.002^{a}
Day 70	$_{34}$0.013±0.000^{a}	$_{1}$0.017±0.000^{ab}	$_{1}$0.017±0.001^{ab}	$_{13}$0.019±0.003^{b}	$_{13}$0.014±0.001^{ab}
Day 84	$_{124}$0.021±0.007	$_{2}$0.017±0.004	$_{1}$0.023±0.005	$_{1}$0.027±0.001	$_{13}$0.014±0.003
NO_3^--N (mg L^{-1})					
Day 1	$_{1}$1.20±0.10^{a}	$_{14}$2.10±0.22^{b}	$_{1}$2.05±0.22^{b}	$_{12}$2.13±0.10^{b}	$_{1}$1.47±0.13^{a}
Day 14	$_{2}$1.75±0.21	$_{14}$2.70±0.39	$_{13}$2.43±0.43	$_{2}$2.05±0.09	$_{12}$2.05±0.41
Day 28	$_{2}$1.33±.14^{a}	$_{2}$8.88±1.78^{b}	$_{2}$7.38±0.13^{b}	$_{12}$2.2±0.40^{a}	$_{13}$1.67±0.13^{a}
Day 42	$_{12}$1.53±0.62^{a}	$_{3}$5.80±1.25^{b}	$_{25}$6.03±0.77^{b}	$_{12}$2.80±0.49^{a}	$_{24}$2.93±0.53^{a}
Day 56	$_{3}$2.80±0.22^{ab}	$_{34}$3.77±0.33^{a}	$_{35}$3.83±0.53^{a}	$_{12}$2.90±0.37^{ab}	$_{235}$2.47±0.17^{b}
Day 70	$_{3}$3.18±0.13	$_{134}$3.98±0.20	$_{3}$3.43±0.56	$_{1}$3.200±0.55	$_{4}$3.63±0.33
Day 84	$_{4}$5.70±1.52	$_{134}$3.80±0.68	$_{5}$5.47±0.93	$_{4}$5.13±0.85	$_{45}$3.60±0.84

Par.	OW	ISW0	ISW33	ISW66	ISW100
NH_4-N (mg L^{-1})					
Day 1	$_{1}$0.825±0.175^{a}	Neg.^{b}	Neg.^{b}	Neg.^{b}	Neg.^{b}
Day 14	$_{2}$Neg.^{b}	0.250±0.250	Neg.^{b}	Neg.^{b}	0.250±0.250
Day 28	$_{3}$0.500±0.289	Neg.	Neg.	Neg.	0.500±0.289
Day 42	$_{2}$Neg.	Neg.	Neg.	Neg.	Neg.
Day 56	$_{2}$Neg.	Neg.	Neg.	Neg.	0.250±0.250
Day 70	$_{2}$Neg.	Neg.	Neg.	Neg.	Neg.
Day 84	0.005±0.005	Neg.	0.005±0.005	Neg.	Neg.
TKN (mg L^{-1})					
Day 1	$_{1}$0.21±0.05	$_{1}$0.21±0.06	$_{1}$0.14±0.07	$_{1}$0.21±0.04	$_{1}$0.14±0.09
Day 14	$_{1}$0.08±0.03^{a}	$_{24}$1.56±0.19^{b}	$_{2}$1.77±0.05^{b}	$_{23}$0.84±0.26^{c}	$_{2}$1.05±0.13^{c}
Day 28	$_{1}$0.26±0.06^{a}	$_{35}$2.90±0.07^{b}	$_{3}$2.69±0.07^{b}	$_{23}$0.86±0.15^{c}	$_{3}$1.84±0.23^{d}
Day 42	$_{2}$0.72±0.24^{a}	$_{25}$2.15±.027^{bc}	$_{3}$2.80±0.29^{b}	$_{2}$1.17±0.06^{a}	$_{35}$1.61±0.22^{ac}
Day 56	$_{1}$0.26±0.06^{a}	$_{4}$1.19±0.33^{bc}	$_{2}$1.31±0.44^{c}	$_{13}$0.58±0.11^{ac}	$_{12}$0.54±0.18^{ac}
Day 70	$_{3}$1.63±0.08^{a}	$_{3}$2.97±0.04^{b}	$_{3}$2.85±0.03^{b}	$_{4}$2.12±0.02^{c}	$_{4}$2.64±1.10^{b}
Day 84	$_{3}$1.59±0.58^{ab}	$_{5}$2.71±0.66^{ab}	$_{3}$2.92±0.72^{a}	$_{5}$3.25 ±0.42^{a}	$_{25}$1.10±0.25^{b}
PO_4^{3-}-P (mg L^{-1})					
Day 1	$_{12}$1.50±0.09^{a}	$_{1}$1.68±0.05^{ac}	$_{1}$2.00±0.15^{b}	$_{13}$1.78±0.08^{bc}	$_{1}$1.55±0.05^{ac}
Day 10	$_{12}$1.50±0.21^{ab}	$_{13}$2.08±0.15^{ac}	$_{1}$2.07±0.29^{ac}	$_{1}$1.45±0.13^{b}	$_{1}$2.23±0.18^{c}
Day 28	$_{123}$1.70±0.37^{a}	$_{2}$4.30±0.68^{b}	$_{12}$3.43±0.30^{b}	$_{13}$1.70±0.29^{a}	$_{1}$2.30±0.19^{a}
Day 42	$_{2}$1.30±0.08^{a}	$_{13}$2.10±0.29^{bc}	$_{1}$2.50±0.29^{b}	$_{1}$1.43±0.12^{ac}	$_{1}$2.10±0.35^{bc}
Day 56	$_{13}$1.97±0.08^{a}	$_{34}$2.97±0.19^{ab}	$_{2}$4.57±1.07^{b}	$_{2}$3.03±0.35^{ab}	$_{2}$4.33±1.40^{ab}
Day 70	$_{3}$2.10±0.15^{a}	$_{24}$3.32±0.41^{b}	$_{1}$2.50±0.16^{a}	$_{3}$2.18±0.10^{a}	$_{12}$2.53±0.29^{a}
Day 84	$_{3}$2.80±0.64	$_{13}$2.23±0.13	$_{1}$2.60±0.28	$_{3}$2.20±0.21	$_{1}$2.30±0.39

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4). Par. Means parameters

The PO_4^{3-}-P concentration in water varied significantly among water types and over the trial period, and was higher when the *S. linearifolium* biomass started to decline. It remained constantly in the first three fortnights and then increased significantly in OW and ISW66. The PO_4^{3-}-P fluctuated slightly during the middle of the trial in ISW, ISW33 and ISW100 but had the similar concentration at the termination as at the commencement of the trial. The PO_4^{3-}-P was significantly higher in ISW, ISW33 than in other water types (Table 5-4).

There was no significant (P>0.05) difference among treatments of some other water parameters measured at the end of the experiment, including TSS, VSS, TS, TVS, chlorophyll-a and primary productivity, except the respiration (Table 5-5).

Table 5-5. Other water parameters (mg L^{-1}) at the end of the experiment tested the growth of *S. linearifolium* in K^+ISW

Time	OW	ISW	ISW33	ISW66	ISW100
TSS	621±39	653±35	748±112	746±26	662±33
VSS	374±60	397±35	455±45	391±15	389±38
TS	68456±7990	41670±11563	76204±20522	83346±23113	52167±17787
TVS	38259±1820	20104±7975	27321±6848	40123±22238	46958±15555
Chlorophy-	0.0027±	0.0181±	0.0227±	0.0470±	0.0171±
ll-a	0.0027	0.0128	0.0227	0.0279	0.0155
Primary Productivity					
Gross	1.32±0.14	1.90±0.13	1.22±0.28	2.50±0.50	2.75±1.24
Net	1.00±0.10	1.10±0.10	0.79±0.19	1.54±0.34	1.62±0.77
Respiration	$0.32±0.13^{a}$	$0.80±0.07^{ab}$	$0.44±0.10^{ab}$	$0.96±0.22^{ab}$	$1.13±0.49^{b}$

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4).

5.3.2 *S. podacanthum* Cultured in Potassium-fortified Inland Saline Water

5.3.2.1 Biomass and Growth Rate of *S. podacanthum*

The fresh biomass of *S. podacanthum* did not change as the trial progressed in OW and ISW100. There was no significant (P>0.05) difference of the standing biomass and SGR of *S. podacanthum* between the OW and ISW100. Total mortality of *S. podacanthu*m cultured in ISW0 was recorded in the first 14 days of the trial, whereas in ISW33 and ISW66, it occurred in the following 14 days. In addition, a decline biomass was recorded from the day 14, along with a negative SGR, which was significantly (P<0.05) lower than the SGR in OW and ISW100 (Table 5-6, Table 5-7).

Table 5-6. Fresh biomass (g L^{-1}) of *S. podacanthum* cultured in K^+ISW

Time	OW	ISW0	ISW33	ISW66	ISW100
Day 1	3.38±0.13	$_1$3.38±0.04	$_1$3.344±0.11	$_1$3.40±0.10	3.38±0.08
Day 14	3.54±0.27^{ad}	$_2$0.00^{b}	$_2$1.55±0.54^{c}	$_2$2.28±0.14^{ac}	3.60±0.05^{d}
Day 28	2.91±0.64	$_2$0.00	$_3$0.00	$_3$0.00	2.86±0.39
Day 42	2.94±0.77	$_2$0.00	$_3$0.00	$_3$0.00	3.39±0.65
Day 56	2.92±0.78	$_2$0.00	$_3$0.00	$_3$0.00	2.96±0.48

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4).

Table 5-7. SGR (% d^{-1}) of *S. podacanthum* in K^+ISW

Time	OW	ISW0	ISW33	ISW66	ISW100
Day 1–14	-0.16±0.43^{a}		-5.68±3.93^{b}	-2.20±1.08^{ab}	0.97±0.41^{a}
Day 1–28	-0.47±0.71				-0.46±0.47
Day 1–42	-0.32±0.54				-0.17±0.33
Day 1–56	-0.26±0.41				-0.17±0.24
Day 14–28	-0.77±1.44				-1.90±1.13
Day 28–42	-0.03±0.38				0.42±0.97
Day 42–56	-0.08±0.11				-0.19±0.05

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4).

The dried content and protein of *S. podacanthum* remained unchanged over time in OW and ISW100, and no significant (P>0.05) difference of the dried content and protein between the two waters was recorded except the dried content at day 14 (Table 5-8). Also, the energy of dried *S. podacanthum* did not change as time progressed, and it remained as 10,500 J g^{-1}.

The detail of certain chemical compositions of dried *S. podacanthum* were presented in Table 5-9, of which C and N were slightly increased after 28 days of culturing, whereas P decreased. Therefore, the C:N:P ratio was higher at the day 28 than at the beginning of the trial. In addition, the [K] in *S. podacanthum* declined as time progressed (Table 5-9).

Table 5-8. Dried content (%) and protein (%) of *S. podacanthum* cultured in K^+ISW

Time	OW	ISW	ISW33	ISW66	ISW100
Dried content					
Day 1	13.32±0.28	13.32±0.28	13.32±0.28	13.32±0.28	13.32±0.28
Day 14	12.46±0.37[a]		14.35±0.19[b]	14.64±0.01[b]	14.32±0.50[b]
Day 56	14.28±0.13				14.19±0.19
Protein					
Day 1	9.41±1.10	9.41±1.10	9.41±1.10	9.41±1.10	9.41±1.10
Day 14	11.38±0.10		11.48±0.10	11.36±1.00	11.42±0.16
Day 56	8.44±0.06				10.33±0.14

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4).

Table 5-9. Chemical composition of *S. podacanthum* cultured in K^+ISW

Ion	Units	Day 1	Day 14				Day 28	
			OW	ISW33	ISW66	ISW100	OW	ISW100
Bo	mg kg^{-1}	115.29	153.22	244.00	189.24	131.85	213.89	106.42
Ca	%	1.62	1.34	2.84	1.56	1.57	1.55	1.62
C	%	26.60	28.00	30.90	26.80	29.90	29.10	29.30
Cu	mg kg^{-1}	50.55	11.71	19.33	12.57	12.74	12.18	10.28
Fe	mg kg^{-1}	80.31	42.87	144.28	55.32	57.49	49.33	52.15
Mg	%	0.68	0.68	1.25	1.20	0.86	0.89	0.80
Mn	mg kg^{-1}	7.95	6.52	36.86	30.44	18.05	14.94	23.48
P	%	0.14	0.12	0.12	0.15	0.13	0.11	0.11
K	%	12.17	11.36	3.40	7.02	8.14	8.35	7.92
Na	%	2.39	2.20	2.61	4.76	1.88	1.81	1.60
S	%	1.12	1.24	1.46	1.56	1.40	1.48	1.30
Total N	%	1.31	1.11	1.75	1.94	1.58	1.28	1.67
Zn	mg kg^{-1}	29.08	459.43	546.21	805.20	474.55	1087.17	584.56
C:N:P		190:9:1	233:9:1	258:15:1	179:13:1	230:12:1	265:12:1	266:15:1

5.3.2.2 Quality of Potassium-fortified Inland Saline Water

The environmental factors, temperature, light intensity, dissolved oxygen and pH of the water cultured *S. podacanthum* were presented in Table 5-10 and Figure 5-2, Figure 5-3, Figure 5-4. No significant difference of these factors were found among the treatments.

As *S. podacanthum* died in ISW0, ISW33 and ISW66 at day 28 therefore no water quality parameter from these treatments was collected. The ionic profile of waters was presented in Table 5-11, which showed a significant variation in terms of higher K^+ and lower Na^+ in ISW100 at day 28 than the day 1.

Table 5-10. Environmental factors in the trial tested *S. podacanthum* growth in K^+ISW

Factors	OW	ISW	ISW33	ISW66	ISW100
Temperature (^oC)	20.58±0.06	20.70±0.07	20.80±0.07	21.00±0.08	20.90±0.09
DO (mg L^{-1})	7.27±0.01	7.24±0.02	7.24±0.01	7.22±0.01	7.22±0.01
pH	7.98±0.04	7.94±0.03	7.94±0.03	7.91±0.04	7.89±0.04

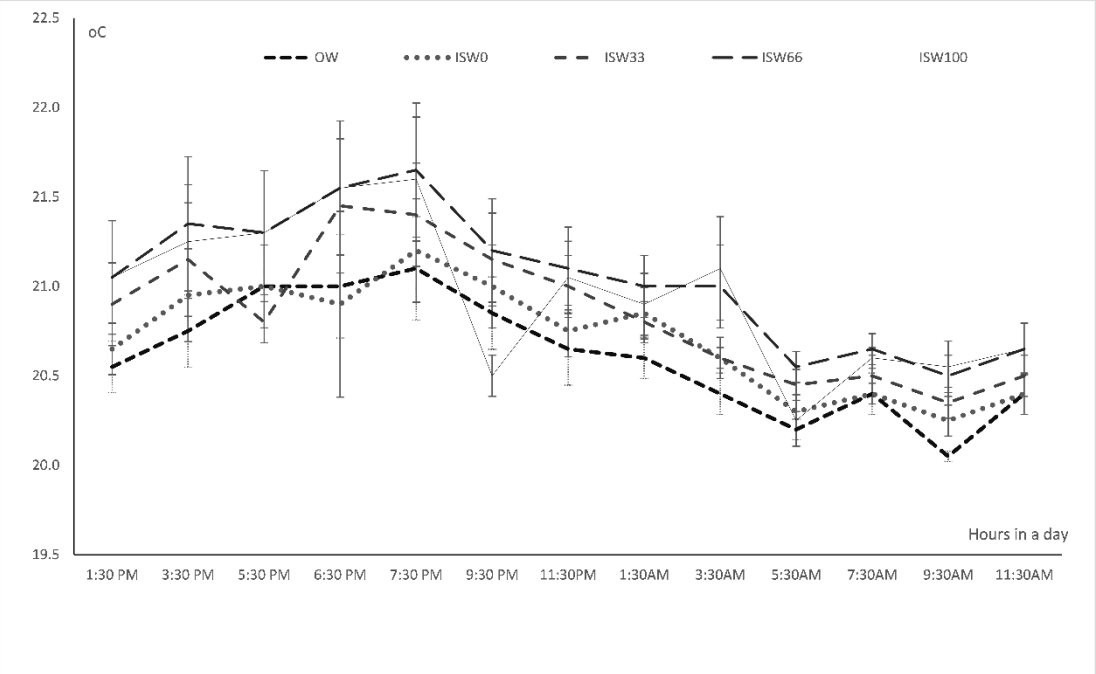

Figure 5-2. Hourly temperature variation of the K^+ISW cultivating *S. podacanthum*

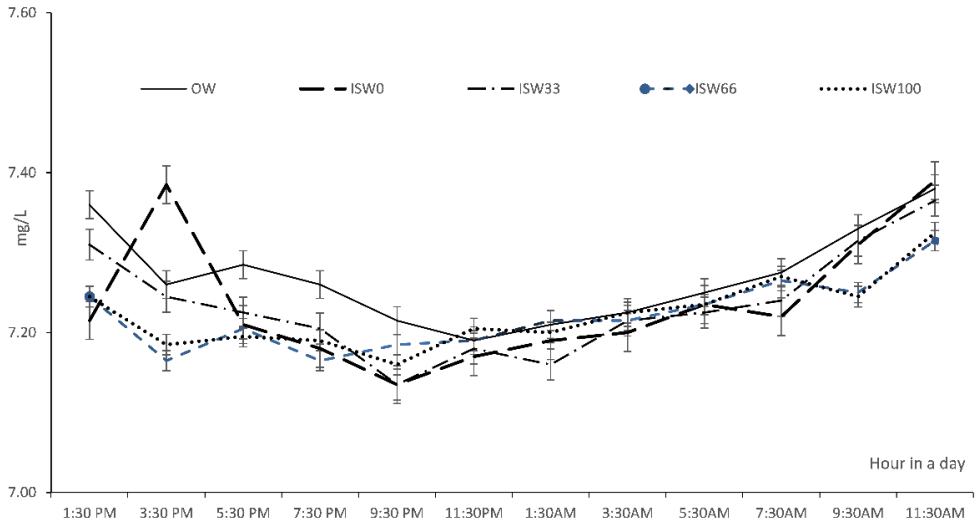

Figure 5-3. Hourly DO variation of the K^+ISW cultivating *S. podacanthum*

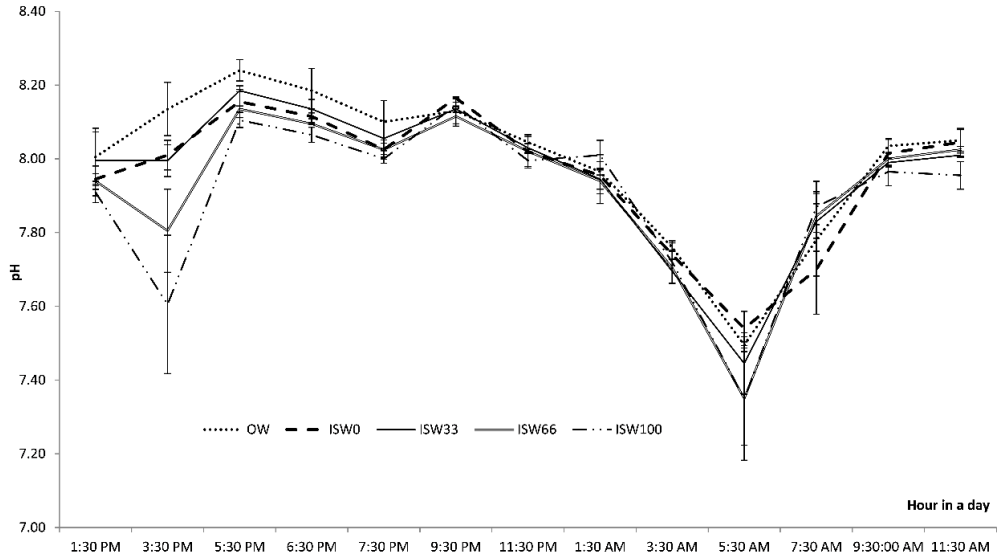

Figure 5-4. Hourly pH variation of the K^+ISW cultivating *S. podacanthum*

Since day 28 of the trial, [NO_2^--N] in OW and ISW100 significantly ($P<0.05$) declined, contrary to [NO_3^--N]. However, both of them remained unchanged from day 28 until the end of the trial. Meanwhile, [PO_4^{3-}-P] fluctuated during the trial period and, together with [NO_3^--N], they were significantly higher at the end of the trial than at the beginning. No significant differences in the water quality parameters between OW and ISW100 were recorded as the trial progressed (Table 5-12).

Table 5-11. Ionic profile (mg L^{-1}) in waters cultured *S. podacanthum*

Parameters	OW day 1	ISW100 day 1	OW day 28	ISW100 day 28
Bo	3.95	0.66	0.91	0.74
Ca	371.6	583.00	549.70	537.70
Cu	<0.05	<0.05	<0.05	<0.05
Fe	<0.05	<0.05	<0.05	<0.05
Mg	1168	1525	1671.00	1424.00
Mn	<0.05	<0.05	<0.05	<0.05
P	<0.05	0.07	<0.05	<0.05
K	351.1	351.5	369.50	410.80
Na	10010	8719	9506.00	8144.00
S	805.4	602.4	887.60	620.2
Zn	<0.05	<0.05	<0.05	<0.05

Table 5-12. Quality parameters (mg L^{-1}) of water cultured *S. podacanthum*

Par.	OW	ISW0	ISW33	ISW66	ISW100
NO_2^--N					
Day 1	$_{1}$0.022±0.002	$_{1}$0.041±0.024	0.023±0.002	0.022±0.003	$_{12}$0.026±0.010
Day 14	$_{1}$0.028±0.006^{a}	0.016±0.004^{a}	0.163±0.066^{b}	0.026±0.005^{a}	$_{1}$0.032±0.006^{a}
Day 28	$_{2}$0.001±0.001^{a}				$_{3}$0.007±0.002^{b}
Day 42	$_{2}$0.007±0.003^{a}				$_{23}$0.018±0.002^{b}
Day 56	$_{2}$0.011±0.001				$_{23}$0.016±0.005
NO_3^--N					
Day 1	$_{1}$1.10±0.00^{a}	2.17±0.30^{b}	2.13±0.28^{b}	2.13±0.15^{b}	$_{1}$1.40±0.16^{a}
Day 14	$_{1}$1.83±0.18^{a}	3.30±0.15^{c}	2.83±0.15^{bc}	2.17±0.15^{ab}	$_{1}$1.76±0.18^{a}
Day 28	$_{2}$3.60±0.29				$_{2}$4.30±0.46
Day 42	$_{2}$3.46±0.19				$_{3}$3.30±0.27
Day 56	$_{2}$3.17±0.41				$_{3}$3.13±0.15
PO_4^{3-}-P					
Day 1	$_{1}$1.47±0.12^{a}	1.70±0.06^{a}	2.10±0.26^{b}	1.80±0.10^{ab}	$_{1}$1.57±0.12^{a}
Day 14	$_{12}$1.60±0.21^{a}	2.50±0.23^{b}	2.50±0.23^{b}	1.80±0.15^{a}	$_{2}$2.23±0.20^{ab}
Day 28	$_{12}$0.97±0.12				$_{3}$0.97±0.07
Day 42	$_{12}$1.33±0.23				$_{1}$1.70±0.23
Day 56	$_{2}$1.80±0.31				$_{2}$2.27±0.18

Par. means parameters

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript (number) are not significantly different (LSD test; P>0.05; n=4).

Table 5-13. Comparison of the cumulative SGR (% d^{-1}) of the two *Sargassum* spp. cultured in K^+ISW

Time	OW		ISW		ISW33		ISW66		ISW100	
	S. L	S. P	S. L	S. P	S. L	S. P	S. L	S. P	S. L	S. P
Day 1–14	1.92±0.12^{a}	-0.16±0.43^{b}	-0.01±1.62		2.31±0.71^{a}	-5.68±3.93^{b}	1.91±0.96^{a}	-2.20±1.08^{ab}	2.57±0.66^{a}	0.97±0.41^{a}
Day 1–28	2.29±0.18^{a}	-0.47±0.71^{b}	-1.22±0.50		0.19±0.14		1.06±0.06		1.78±0.05^{a}	-1.90±1.13^{b}
Day 1–42	2.02±0.10^{a}	-0.32±0.54^{b}	-3.78±2.33		-0.46±0.17		0.55±0.10		1.34±0.14^{a}	0.42±0.97^{b}
Day 1–56	1.50±0.03^{a}	-0.26±0.41^{b}	-3.15±1.80		-2.95±1.99		0.24±0.19		1.03±0.14^{a}	-0.19±0.05^{b}

S. L – *S. linearifolium*; S. P – *S. podacanthum*
Values (mean±SE) within a row at one water type sharing a common superscript are not significantly different (t-test; P>0.05; n=4).

Table 5-14. Comparison of the discrete SGR (% d^{-1}) of the two *Sargassum* spp. cultured in K^+ISW

Time	OW		ISW0		ISW33		ISW66		ISW100	
	S. L	S. P	S. L	S. P	S. L	S. P	S. L	S. P	S. L	S. P
Day 1–14	$_{1}$1.92±0.12^{a}	-0.16±0.43^{b}	$_{1}$-0.01±1.62		$_{1}$2.31±0.71^{a}	-5.68±3.93^{b}	$_{1}$1.91±0.96^{a}	-2.20±1.08^{b}	$_{1}$2.57±0.66^{a}	0.97±0.41^{b}
Day 15–28	$_{1}$2.53±0.57^{a}	-0.77±1.44^{b}	$_{12}$-2.00±0.66		$_{12}$-1.18±0.61		$_{2}$0.51±0.22		$_{2}$1.26±0.34^{a}	-0.46±0.47^{b}
Day 29–42	$_{1}$1.48±0.38^{a}	-0.03±0.38^{b}	$_{2}$-8.90±6.51		$_{12}$-1.77±0.55		$_{23}$-0.48±0.24		$_{23}$0.48±0.43^{a}	-0.17±0.33^{b}
Day 43–56	$_{2}$-0.05±0.41	-0.08±0.11	$_{12}$-1.24±0.30		$_{2}$-10.41±7.61		$_{3}$-0.69±0.50		$_{234}$0.09±0.28	-0.17±0.24

S. L – *S. linearifolium*; S. P – *S. podacanthum*
Values (mean±SE) within a row at one water type sharing a common superscript are not significantly different (t-test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript are not significantly different (LSD test; P>0.05; n=4).

5.4 Discussion

Since K^+ is important in the growth of algae in general, this study demonstrated that the concentration of K^+ in water strongly affected *Sargassum* growth. K^+ release may damage the cell membrane (Peterson *et al.*, 1995), but K^+ in the form of potassium permanganate would help to aggregate the cells of algae (Chen & Yeh, 2005). The demand for K^+ depends on marine species for the optimal growth rates (Yarish *et al*., 1980). In this study, at 35 ppt *Sargassum* showed similar K^+ needs to the red seaweed *Caloglossa leprieurii* (Montagne) J. Agardh at [K^+] 230–350 mg L^{-1} (Yarish *et al*., 1980). However, the red seaweeds *Bostrychia radicans* Montagne grow better at 400–500 mg L^{-1} (Yarish *et al*., 1980), a higher [K^+] than in natural OW.

After reaching a peak, biomass of *Sargassum* spp. constantly declined over time in all water types, and this decline was associated with the [K^+] in the culture medium, as a result of K^+ being uptaken and accumulated internally by *Sargassum* spp. The decline in growth and the low survival rate of *Sargassum* spp. biomass cultured in K^+-deficient ISW may be due to [K^+] in ISW being too low to support growth and survival of the cultured *Sargassum* spp. The [K^+] of *Sargassum* tissues reduced over the culture period, due to the release of K^+ from internal cells to the environment, resulting in a reduction of the *Sargassum* spp. biomass and an increase in [K^+] in water as the trial progressed in the low [K^+] ISW. K^+ is actively and passively uptaken in both low and high [K^+] media, hence K^+ can be accumulated and maintained at high concentrations in cells, while Na^+ is extruded and kept at lower concentrations (Tromballa, 1978). As K^+ was accumulated intracellularly by *Sargassum* spp., the tissue K^+ became depleted below the threshold levels and was no longer able to support the growth of *Sargassum* spp. The active uptake of K^+ by *Sargassum* spp. in the lower [K^+] medium in K^+-deficient ISW needed proportionally higher energy compared to the passive K^+ uptake in the higher [K^+] medium in OW or ISW100. The demand of energy for this active K^+ uptake resulted in the poor survival and growth of *Sargassum* spp. in ISW, which only occurred for *S. linearifolium* until day 56 and *S. podacanthum* in the first 14 days. The low concentration of medium [K^+] caused intercellular K^+ to be released from higher concentrations, which was then replaced by Na^+ accumulation from the culture medium. The inhibition of nutrient transport due to the K^+ deficiency in ISW caused lower survival *Sargassum* spp. in ISW, ISW33 and ISW66. As the *S. podacanthum*

total mortality was observed after the first 14 days in ISW and ISW33, a higher [K^+] requirement was indicated for this species than for *S. linearifolium*. In addition, the survival of *S. linearifolium* for a longer duration in ISW100 with a positive cumulative SGR until day 84, without the nutrient supplement, showed its higher ability for growing in K^+ISW than *S. podacanthum*. The results of the positive growth of *S. linearifolium* until day 56 was applied for the *S. podacanthum* trial, which was run for 56 days to achieve sufficient biomass until the termination of the trial. The *S. linearifolium* presented higher SGR than *S. podacanthum* at all periods of cultured duration in OW and ISW100 (Table 5-13, Table 5-14), showing *S. linearifolium* has a higher potential to be cultured in ISW.

The [K^+] in ISW100 was similar to [K^+] in OW, but *Sargassum* spp. growth was significantly ($P<0.05$) lower, indicating other factors influence the growth of *Sargassum* spp. in ISW. The concentration of Na^+ in ISW (8,719 mg L^{-1}) is 13% lower than [Na^+] in OW (10,010 mg L^{-1}) at 35 ppt. In normal OW, the intercellular K^+ is maintained at a high concentration (Allison & Walsby, 1981). Under normal growth conditions, *Chlorella pyrenoidosa* maintains a high intracellular K^+ level, whilst Na^+ is maintained at a lower concentration by active and passive Na^+ influx and efflux (Barber & Shieh, 1973). The *Sargassum* spp. cultured in ISW100 spent more energy to accumulate Na^+ in cells through the Na^+ exchange mechanism, which in turn negatively affected the survival and growth of *Sargassum* spp. Moreover, the [Na^+] in ISW was lower than ambient [Na^+] in OW, and coupled with lower [K^+], a disruption in the exchange mechanism between Na^+ and K^+ in *Sargassum* spp. cells may occur. The concentration of Na^+ in *S. podacanthum* tissues increased by day 14 in ISW33, compared with the beginning of the experiment. As a result, cells may accumulate and exceed the [Na^+] that is required for proper protein synthesis (Blumwald *et al*., 2000), which could contribute to the mortality of *Sargassum* spp. cultured in ISW and ISW33.

Although the biomass and SGR of *Sargassum* spp. cultured in ISW100 was lower than in OW, both growth trends exhibited similar patterns over time, as they plateaued and started to decrease towards the end of the trial. This growth pattern follows the life cycle of sub-tropical and temperate *Sargassum*, where their maximum growth occurs during late winter and early spring, and starts to decline in early summer (Agatsuma *et al*., 2002; Martin-Smith, 1994). The life cycle of *Sargassum* and the different growth

period when cultured in indoor conditions were affected by daylight (Uchida, 1993). This experiment was conducted indoors where the daylight was constantly regulated using fluorescent lights of 90 µmol photon m^{-2} s^{-1} on a 14:10 h light:dark cycle, following the method proposed by Hanisak and Samuel (1987) under constant temperature, hence the light and temperature influences on *Sargassum* spp. were negated in these experiments. The water temperature was around 20–22ºC with no significant ($P>0.05$) difference among any water types, reflecting the same OW temperature during this season, similar to suitable temperature conditions for *Sargassum yezoense* maximum growth (Agatsuma *et al*., 2002).

During the trial, there was an evidence that other macronutrients, such as N and P, also had an important influence on the growth of *Sargassum* spp. As enrichment with N and P increases both nutrient concentrations in *Fucus vesiculosus* tissues (Perini & Bracken, 2014). N is a limiting factor in the *Sargassum* spp. growth in Taiwan (Hwang *et al*., 2004). NH_4-N is a more preferable source of N for seaweed than NO_3^--N (Liu *et al*., 2004); however, the [NH_4-N] in the experiment water was usually negligible. When NH_4-N is limited, the seaweed can use NO_3^--N instead (Jie *et al*., 2008). In natural water, NO_3^--N is more prevalent than NO_2^--N or NH_3-N (Robards *et al*., 1994). In these experiments, [NO_3^--N] increased significantly over culture periods, giving an indication of the negative correlation with the biomass of *Sargassum* spp. At the end of experiment, [NO_3^--N] in OW and ISW100 increased by 5.7- and 2.4-fold compared with their initial levels in *S. linearifolium* culture in the K^+ fortification experiment. These results are similar to the increase in *Gracilaria lemaneiformis* growth rate when [NO_3^--N] in OW is elevated from 300 to 600 µM (Zheng & Gao, 2009). The [NO_3^--N] in K^+-deficient ISW and ISW33 showed significant increases compared to NO_3^--N levels in OW, ISW66 and ISW100. The [NO_3^--N] increase in ISW and ISW33 only occurred when *Sargassum* spp. decomposed, releasing NO_3^--N back into the medium. In contrast, lower [NO_3^--N] in both OW and ISW100 during the trial showed that NO_3^--N was either uptaken by *Sargassum* spp. or converted to free nitrogen by nitrifying bacteria (Zhang *et al.*, 2014). This is similar to the increased biomass of *Ulva rigida* C. Agardh, which resulted in the simultaneous depletion of [NO_3^--N] from the water column (Naldi & Viaroli, 2002).

The growth of *Sargassum* spp. in these trials was lower than in natural OW (Gao & Hua, 1997), as the only source of nutrients in the trial were those leached from decomposing of *Sargassum* spp. The P limitation on macroalgae growth and productivity is more common than N limitation (Lapointe, 1986). In this trial, P was also produced by the decomposition of *Sargassum* spp. As a consequence, *Sargassum* spp. biomass decreasing over the trial time, the [P] significantly increased and was negatively correlated with the *Sargassum* spp. biomass. Also, the availability of P in the culture medium was positivelly correlated with [NO_3^--N]. The [P] increased when the [NO_3^--N] occurred above the detectable limit concentration in the water column, whilst P in *Fucus vesiculosus* tissues also increase when the ambient concentration of NO_3^--N increased (Perini & Bracken, 2014). A stable [P] in *S. podacanthum* tissues in the first 28 days of the culture period indicated a similar growth rate of *Sargassum* in OW and ISW100. However, the accumulation of N in *S. podacanthum* tissues in ISW and ISW33 was negatively correlated with the growth of *S. podacanthum*: the higher the N, the lower the SGR.

These trials have shown the negative effects of [K^+] deficiency in ISW on the survival and growth of *Sargassum* spp. cultured in ISW. This effect was species dependent, since there was a higher SGR in *S. linearifolium* than in *S. podacanthum* at all culture periods, as well as a longer survival time in both ISW and K^+ISW (Table 5-13, Table 5-14). The role of K^+ in the growth of *Sargassum* spp. was important for supporting the survival of *Sargassum* spp., and had a complexity in terms of its relation and compounded effect to other cations such as Na^+, and H^+. Therefore, a comprehensive understanding of interaction process among the cations in an intercelullar context, in relation to K^+ deficiency ISW in *Sargassum* culture, requires further investigation.

5.5 Conclusions

To sustain the growth of *S. linearifolium* and *S. podacanthum* in ISW, K^+ fortification to similar concentrations found in OW is necessary. *S. linearifolium* grows faster and survived longer than *S. podacanthum* in ISW100. However, neither of them could live in ISW without K^+ fortification. It is recommended that the *Sargassum* culture should be considered for two months only to obtain higher biomass and less investment, as the species shows seasonality of growth.

CHAPTER 6 [*] PRODUCTIVITY OF *Sargassum linearifolium* AND *S. podacanthum* IN NUTRIENT ENRICHED POTASSIUM-FORTIFIED INLAND SALINE WATER

6.1 Introduction

Mariculture, including seaweed culture, in inland saline water (ISW) is considered as a potential expansion and diversification of aquaculture industry in Australia (Allan *et al*., 2001). Seaweed culture can make use of salt-affected agricultural farms as it is less constrained by additional requirement for resources and changes in infrastructure than the culture of marine finfish and crustacean species. Therefore, growing *Sargassum* spp., in ISW can provide another source of commodity to the farmers with a lower capital investment than farming in the sea (Borowitzka, 1997) and can be an additional tool to protect the inland environment in Australia by combating the salinity problems (Ogburn, 1997).

At the same salinity, the level of potassium (K^+) concentration in ISW is lower than in ocean water (OW) in Australia (Allan & Fielder, 1997; Dinh, 2016), and USA (Boyd & Thunjai, 2003; Forsberg *et al*., 1996) although other ionic profiles can be similar (Fotedar *et al*., 2011; Prangnell & Fotedar, 2006a). K^+ is vital for aquaculture and K^+ deficiency in ISW can negatively affect the growth of the aquatic animals (Mourad *et al*., 2012). For example, the survival of juvenile mulloway (*Argyrosomus hololeptidotus*) (Doroudi *et al*., 2006), juvenile snapper (*Pagrus auratus*) (Fielder *et al*., 2001), and red drum (*Sciaenops ocellatus*) (Forsberg *et al*., 1996) are adversely affected when cultured in low K^+ environment. Therefore, fotifying ISW with K^+ to achieve similar concentration in OW is essential to sustain the growth of shrimp (Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006), fish (Fielder & Allan, 2003),

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red seaweed *Lomentaria catenata* (Bui, Luu, & Fotedar, 2017) and brown seaweed *Sargassum linearifolium* (Bui, Luu, Fotedar, *et al*., 2017).

Sargassum spp. commonly used as a source of fertilizers and soil conditioners (Huisman, 2000), are the dominant taxa in nearshore reef areas along Perth beaches (Womersley, 1996). *Sargassum* spp. are prevalent sources of compounds used in pharmaceutical (Hur *et al*., 2008), and agriculture industries (Ara *et al*., 1997). The extraction from *Sargassum* spp. can be used as in the treatment of neurological disorders (Natarajan *et al*., 2009), dementia (Pangestuti & Kim, 2010), and HIV (Thuy *et al*., 2015). The fact that *Sargassum* spp. are active in antioxidant activity, cholinesterase inhibition activity, neuroprotective activity, anti-cancer, and cytotoxic activity has made it a popular ingredient in health enhancement products (Yende *et al*., 2014). *S. fusiforme* has been cultivated in Korea and Japan as a food source (Bast, 2014).

Sargassum has many branches growing from a short stipe (Huisman, 2000). The length of *Sargassum*'s thallus is about 0.1–2 m, while its stipes are 1–20 cm long from a discoid-conical holdfast (Womersley, 1987). Of the *Sargassum* species, *S. podacanthum* is distributed from Point Peron (Western Australia (WA) to Port Noarlunga (South Australia) (Womersley, 1987), and thus can be an important candidate for growing in local ISW. The branches of *S. podacanthum* are typically terete, but more angular at the top, usually with short, scatter spines, which branch out radically (Womersley, 1987). *S. podacanthum*'s thallus is monoecious with bisexual receptacles, simple or branched, and its conceptacles are unisexual (Womersley, 1987). Although nutrient uptake and nutrient enrichment of various species of seaweeds, including some *Sargassum* spp. have been extensively studied (Coutinho & Zingmark, 1993; Pérez-Mayorga *et al*., 2011; Perini & Bracken, 2014; Reef *et al*., 2012; Schaffelke & Klumpp, 1998), there is no information available on the impacts of ammonium (NH_4-N) and phosphate (PO_4^{3-}-P) supplementation on *S. podacanthum* productivity, particularly when cultured in K^+-fortified ISW (K^+ISW).

Nitrogen (N) and phosphorus (P) in the water are not always present in appropriate quantities to meet algal demand (Robards *et al*., 1994) and are limiting factors in photosynthesis of seaweeds (Larned, 1998). N is a limiting nutrient in the growth of *Sargassum* spp. cultured in Hawaii (Larned, 1998) and Taiwan (Hwang *et al*., 2004),

P is also considered a limiting factor for *S. natans* and *S. fluitans* growth in the western North Atlantic (Lapointe, 1986). The range of atomic N:P ratio of *Sagassum* spp. varies from 20:1 to 38:1 and the average requirement of N:P for seaweed, in general, is from 10:1 to 30:1 (Atkinson & Smith, 1983). In OW, the most common type of ammonia is ammonium (NH_4-N), as a result of its relation to pH, and it is less toxic than unionized ammonia (Burgess *et al*., 2003), and is more preferably consumed by seaweed (Liu *et al*., 2004). The phosphate (PO_4^{3-}-P) concentration in media, alongside nitrate (NO_3^--N) or NH_4-N, increase the seaweed NO_3^--N/NH_4-N uptake capacity, respectively, better than in environments where the availability of only either NO_3^--N or NH_4-N is presented (Ahmad *et al*., 2011). As nutrient limitation on the growth of seaweed is species dependent (Larned, 1998), the majority of seaweed species grow faster in ammonium-enriched than in phosphate-enriched media (Larned, 1998). Supplying NH_4-N is more efficient than nitrate (NO_3^--N) for seaweed growth (Atkinson & Smith, 1983). Thus, the combined NH_4-N and PO_4^{3-}-P has a stronger effect on the growth of *S. baccularia* than a single nutrient (Schaffelke & Klumpp, 1998). However, the information on the impacts of nutrient supplementation during *Sargassum* spp. culture, in K^+-fortified ISW (K^+ISW), is lacking. The present study aims to examine the effects of different N and P concentrations, through the supplementation of NH_4-N and PO_4^{3-}-P, on the growth of *S. linearifolium* and *S. podacanthum* cultured in K^+ISW of Western Australia (WA) under the laboratory conditions.

6.2 Materials and Methods

S. linearifolium and *S. podacanthum* were collected and identified as described in Chapter 3. ISW at 355 ppt was also prepared followed the methods in Chapter 3. K^+ISW was prepared as fortifying ISW at 100% of [K^+] in OW by adding 508.5 mg L^{-1} of anhydrous potassium chloride as in Chapter 3.

A total of 96 1.5 L glass beakers with 1 L of water were used for the 84-day trial in late autumn and early summer. The growth of *Sargassum* spp. was determined at five levels of nutrients in OW and K^+ISW. The nutrients were provided as molar NH_4-N:PO_4^{3-}-P equal to 10:1 by the weekly addition of ammonium chloride (NH_4Cl) and sodium dihydrogen phosphate (NaH_2PO_4) mixtures (Campbell, 2001). Five different

concentrations of NH_4-N:PO_4^{3-}-P were 80:8, 120:12, 160:16, 200:20, and 240:24 µM (Liu *et al*., 2004). The required amounts of NH_4Cl and NaH_2PO_4 for NH_4-N:PO_4^{3-}-P 80:8, 120:12, 160:16, 200:20, and 240:24 µM were, respectively, 4.28 and 0.96, 6.42 and 1.44, 8.56 and 1.92, 10.70 and 2.40, 12.84 and 2.88 mg L^{-1}, weighed and stirred to dilute in a part of the cultured media which were taken out from the cultured beakers. The waters were then returned back to the beakers and diluted in the 1 L cultured medium by using a small glass stick to stir water. Twelve treatments were set up in four replicates (including five nutrient concentrations in OW (OW_80, OW_120, OW_160, OW_200, OW_240), five nutrient concentrations in K^+ISW (ISW_80, ISW_120, ISW_160, ISW_200, ISW_240), and the controls of ambient OW (OW_0), ambient K^+ISW (ISW_0), without any supplementation of nutrients.

Experimental setup, data collection and data analysis were followed as described in Chapter 3.

6.3 Results

6.3.1 Biomass and Growth Rate of *S. linearifolium*

Two water types (OW and K^+ISW) did not affect the fresh biomass of *S. linearifolium* in the first 28 days of culture period, but they strongly ($P<0.05$) affected the *S. linearifolium* biomass from the day 42. The concentrations of nutrient enrichment were highly ($P<0.05$) affected the *S. linearifolium* biomass. However, no interaction between water types and nutrient-enriched levels in water was found as the experiment progressed (Table 6-1). The biomass of *S. linearifolium* was correlated ($R^2>0.7$) with the time progress of the experiment expressed in fortnight (Table 6-2).

The only *S. linearifolium* fresh biomass increase was recorded in OW_0 in the first 28 days of the experiment, an average of 3 mg d^{-1}. Also, only in this water, total mortality did not occur in all replicates. In the nutrient enriched waters, the *S. linearifolium* biomass remained the same in the first 28 days in OW and in the first 14 days in K^+ISW, and then decreased significantly ($P<0.05$) as the experiment progressed (Table 6-3).

Table 6-1. P-values of two way ANOVA tests the effects of water types and nutrient enrich levels on biomass of *S. linearifolium* cultured in NH_4-N:PO_4^{3-}-N enrichment waters

Factor	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
Water	0.008	0.002	0.000	0.000	0.000	0.064
Nutrient	0.301	0.059	0.008	0.002	0.000	0.192
Water * nutrient	0.758	0.413	0.211	0.305	0.002	0.192

Table 6-2. The regression correlation of the *S. linearifolium* biomass in mg (y) with the time in fortnight (x) in OW and K^+ISW at controls and five additional nutrient levels of NH_4-N:PO_4^{3-}-P (µM)

Water	NH_4-N:PO_4-P	Regression	R^2
OW	Control	$y = -20.90x^2 + 88.18x + 483.84$	$R^2 = 0.96$
	80:8	$y = -26.10x^2 + 130.72x + 418.10$	$R^2 = 0.99$
	120:12	$y = -21.05x^2 + 59.30x + 537.02$	$R^2 = 0.92$
	160:16	$y = -14.90x^2 + 18.60x + 563.86$	$R^2 = 0.95$
	200:20	$y = -9.42x^2 + 18.60x + 575.35$	$R^2 = 0.94$
	240:24	$y = -14.28x^2 + 5.91x + 587.30$	$R^2 = 0.92$
K^+ ISW	Control	$y = -23.19x^2 + 96.08x + 436.81$	$R^2 = 0.97$
	80:8	$y = 3.70x^2 - 125.61x + 673.01$	$R^2 = 0.94$
	120:12	$y = -1.90x^2 - 93.89x + 683.43$	$R^2 = 0.91$
	160:16	$y = -4.20x^2 – 73.16x + 654.00$	$R^2 = 0.93$
	200:20	$y = 12.36x^2 – 202.394x + 774.08$	$R^2 = 0.91$
	240:24	$y = -0.11x^2 – 108.83x + 711.44$	$R^2 = 0.90$

When nutrient enrichment levels were higher, total mortality occurred sooner. From day 70, total mortality was recorded in the two highest nutrient enrichment levels in OW, and all nutrient enrichment levels in K^+ISW.

The SGR of *S. linearifolium* showed no significant difference among the nutrient enrichment levels in the same water types in the first 14 days. However, the nutrient enrichment levels significantly affected the SGR of *S. linearifolium* in OW since the day 56 onward, and in K^+ISW from the day 28. The higher nutrient enrichment levels resulted in a greater reduction rate of *S. linearifolium* biomass than the lower nutrient

levels. The SGR of *S. linearifolium* in OW was higher than in K^+ISW at the 80:8 level over the experimental period, and they were similar in other nutrient enrichment levels (Table 6-4). The biomass and SGR of the *S. linearifolium* were significantly ($P<0.05$) correlated with the pH, temperature of culture media, and all measured water quality parameter (Table 6-10).

6.3.2 The Quality of Water Culturing *S. linearifolium*

The pH and temperature varied during the culture period, but were similar among the different treatments in the *S. linearifolium* cultured medium, except a higher pH in ISW_240 than ISW_0 and ISW_80 from day 56 onward was recorded (Figure 6-1).

The water quality parameters varied widely during the culture period, and more so in K^+ISW. Nitrite (NO_2^--N) remained unchanged in the first 28 days in OW and 70 days in K^+ISW and then significantly rose by the end of the experiment, particularly in control of OW and K^+ISW (Table 6-5).

NO_3^--N was greater in higher nutrient enrichment levels, and was significantly higher at the end of the experiment than at the beginning in 200:20 and 240:24 levels. NO_3^--N in K^+ISW was higher than in OW at the same nutrient enriched levels (Table 6-6).

Only in the non-enriched level was TKN in K^+ISW similar to OW, and in all other levels, TKN in K^+ISW was higher than in OW at the same nutrient enriched levels. TKN rose significantly ($P<0.05$) toward the end of the experiment (Table 6-7).

At the beginning, the NH_4-N level was greater in the higher nutrient enrichment levels; however, its concentration became negligible over the first 28 days of the culture period, and then remained less than 0.3 mg L^{-1}. At the same nutrient enriched level, NH_4-N was similar in both OW and K^+ISW, except a higher concentration was recorded in ISW_160 than in OW_160 (Table 6-8).

(a)

(b)

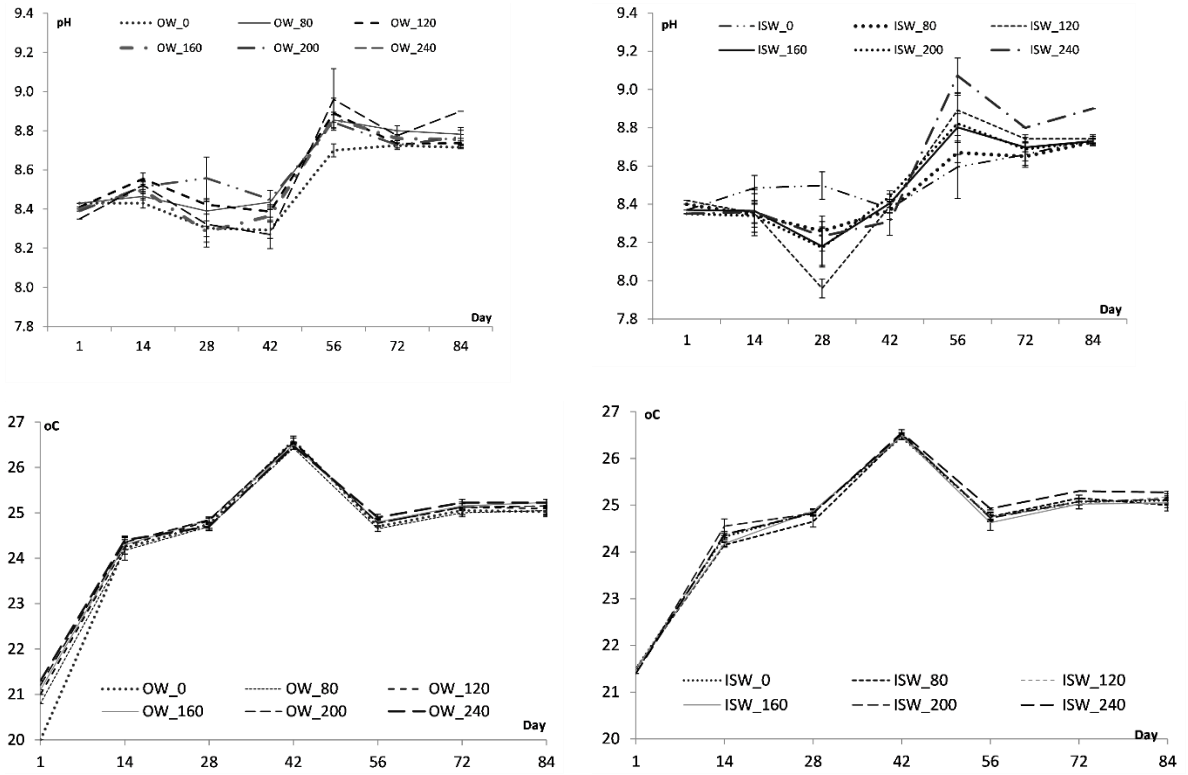

Figure 6-1. The pH (a) and temperature (b) of the nutrient-enriched waters culturing *S. linearifolium*

In contrast with the increasing trend of all sources of N in waters, the PO_4^{3-}-P remained the same in the non-enriched level, and was reduced by the end compared to the beginning in all other waters, except ISW_200. However, PO_4^{3-}-P concentrations in K^+ISW were significantly higher than in OW at the same nutrient levels, and higher than for the 120:12 level (Table 6-9).

Table 6-3 Fresh standing biomass (g) of *S. linearifolium* cultured in control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM) waters

Water	Time	Control	80:8	120:12	160:16	200:20	240:24
	Day 1	$_{13}3.59\pm0.01$	$_{1}3.60\pm0.05$	$_{12}3.62\pm0.03$	$_{1}3.62\pm0.07$	$_{1}3.61\pm0.03$	$_{1}3.66\pm0.07$
	Day 14	$_{2}4.42\pm0.26$	$_{1}4.20\pm0.23$	$_{1}4.35\pm0.13$	$_{1}4.31\pm0.30$	$_{1}3.76\pm0.13$	$_{23}4.22\pm0.29$
	Day 28	$_{23}4.22\pm0.20^{a}$	$_{1}4.04\pm0.32^{ab}$	$_{12}4.19\pm0.48^{a}$	$_{1}3.67\pm0.22^{ab}$	$_{2}2.95\pm0.25^{b}$	$_{13}3.81\pm0.31^{ab}$
OW	Day 42	$_{14}3.21\pm0.22$	$_{1}3.48\pm0.54$	$_{2}3.24\pm0.35$	$_{23}2.40\pm0.37$	$_{3}2.28\pm0.14$	$_{4}2.37\pm0.13$
	Day 56	$_{4}2.71\pm0.14^{ab}$	$_{1}3.14\pm0.63^{a}$	$_{3}1.65\pm0.68^{b}$	$_{3}2.10\pm0.30^{ab}$	$_{4}1.88\pm0.09^{ab}$	$_{4}1.93\pm0.15^{ab}$
	Day 70	$_{5}1.79\pm0.24^{a}$	$_{2}1.75\pm0.29^{a}$	$_{4}0.32\pm0.32^{b}$	$_{4}0.63\pm0.36^{b}$	$_{5}0^{b}$	$_{5}0^{b}$
	Day 84	$_{5}0.67\pm0.39^{a}$	$_{3}0.41\pm0.41^{ab}$	$_{4}0^{b}$	$_{4}0^{b}$	$_{5}0^{b}$	$_{5}0^{b}$
	Day 1	$_{1}3.59\pm0.05$	$_{1}3.58\pm0.09$	$_{1}3.63\pm0.11$	$_{1}3.61\pm0.03$	$_{1}3.57\pm0.18$	$_{1}3.64\pm0.05$
	Day 14	$_{1}3.71\pm0.36$	$_{1}3.65\pm0.27$	$_{1}3.88\pm0.12$	$_{1}3.85\pm0.21$	$_{1}3.57\pm0.18$	$_{1}4.20\pm0.30$
	Day 28	$_{1}3.65\pm0.63^{a}$	$_{2}2.13\pm0.73^{b}$	$_{1}3.28\pm0.09^{ab}$	$_{1}3.35\pm0.56^{a}$	$_{2}2.51\pm0.31^{ab}$	$_{1}3.27\pm0.23^{ab}$
K^+ISW	Day 42	$_{1}3.12\pm0.56^{a}$	$_{2}1.36\pm0.50^{ab}$	$_{2}2.00\pm0.12^{ab}$	$_{2}1.96\pm0.32^{abc}$	$_{3}0.76\pm0.45^{c}$	$_{2}1.36\pm0.79^{bc}$
	Day 56	$_{1}2.73\pm0.47^{a}$	$_{2}1.30\pm0.48^{b}$	$_{3}0.48\pm0.48^{bc}$	$_{3}0.88\pm0.57^{bc}$	$_{4}0^{c}$	$_{2}1.01\pm0.58^{bc}$
	Day 70	$_{2}0.77\pm0.46^{a}$	$_{3}0^{b}$	$_{4}0^{b}$	$_{3}0^{b}$	$_{4}0^{b}$	$_{3}0^{b}$
	Day 84	$_{2}0$	$_{3}0$	$_{4}0$	$_{3}0$	$_{4}0$	$_{3}0$

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column of one water-type sharing a common subscript are not significantly different (LSD-test; P>0.05; n=4)

Table 6-4. SGR (% d^{-1}) of *S. linearifolium* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM) waters

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1–14	OW	$_1$1.44±0.41^a	1.08±0.44^{ab}	1.32±0.20^{ab}	1.20±0.43^{ab}	0.28±0.26^b	0.98±0.38^{ab}
	K^+ISW	$_2$0.12±0.66	0.09±0.37	0.47±0.26	0.44±0.40	-0.01±0.38	0.97±0.48
Day 1–28	OW	0.57±0.16^a	$_1$0.38±0.28^{ab}	0.45±0.45^a	0.03±0.19^{ab}	-0.76±0.26^b	0.11±0.32^{ab}
	K^+ISW	-0.16±0.76^a	$_2$-2.69±1.60^b	-0.37±0.17^{ab}	-0.39±0.26^{ab}	-1.33±0.40^{ab}	-0.41±0.25^{ab}
Day 1–42	OW	-0.29±0.17	$_1$-0.16±0.35^a	$_1$-0.30±0.27	-1.09±0.42	$_1$-1.11±0.12	-1.04±0.17
	K^+ISW	-0.47±0.46^a	$_2$-1.68±0.45^b	$_2$-1.43±0.14^b	-1.54±0.35^b	$_2$-2.01±0.17^b	-0.72±0.24^{ab}
Day 1–56	OW	-0.51±0.10^{ab}	$_1$-0.34±0.33^a	-1.01±0.42^b	-1.04±0.28^b	-1.17±0.10^b	-1.16±0.17^b
	K^+ISW	-0.58±0.32^a	$_2$-1.35±0.36^b	-	-1.42±0.67^b	-	-1.07±0.10
Day 1–70	OW	-1.01±0.20a	-1.05±0.21^a	-	-	-1.47±0.08^b	-
	K^+ISW	-1.21±0.28	-	-	-	-	-
Day 1–84	OW	-1.18±0.10	-0.91±0.09	-	-	-	-
	K^+ISW	-	-	-	-	-	-

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column of one time period sharing a common subscript are not significantly different (t-test; P>0.05; n=4) (The SGR could not be calculated for some cases when mortality occurred in one or more replicates)

Table 6-5. The [NO_2^--N] (mg L^{-1}) in waters cultured *S. linearifolium* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	0.011 ± 0.001^{a}	$_1 0.007\pm0.000^{b}$	$_1 0.006\pm0.000^{b}$	$_1 0.007\pm0.000^{b}$	$_1 0.007\pm0.000^{b}$	0.006 ± 0.000^{b}
	K^+ISW	0.012 ± 0.000^{ab}	$_2 0.012\pm0.000^{ab}$	$_2 0.014\pm0.000^{c}$	$_2 0.011\pm0.002^{b}$	$_2 0.015\pm0.001^{c}$	0.007 ± 0.001^{d}
Day 14	OW	0.009 ± 0.001^{ab}	$_1 0.008\pm0.001^{ab}$	$_1 0.007\pm0.001^{a}$	$_1 0.009\pm0.001^{ab}$	0.009 ± 0.001^{ab}	0.010 ± 0.001^{b}
	K^+ISW	0.008 ± 0.001^{a}	$_2 0.014\pm0.001^{b}$	$_2 0.012\pm0.001^{bc}$	$_2 0.012\pm0.001^{b}$	0.009 ± 0.001^{ac}	0.013 ± 0.001^{b}
Day 28	OW	$_1 0.007\pm0.000^{a}$	0.007 ± 0.000^{a}	$_1 0.006\pm0.001^{a}$	0.009 ± 0.000^{ab}	0.007 ± 0.001^{ab}	$_1 0.010\pm0.001^{b}$
	K^+ISW	$_2 0.012\pm0.001^{a}$	0.008 ± 0.000^{b}	$_2 0.021\pm0.002^{c}$	0.009 ± 0.001^{a}	0.009 ± 0.001^{a}	$_2 0.016\pm0.003^{d}$
Day 42	OW	$_1 0.022\pm0.002^{a}$	$_1 0.017\pm0.002^{ab}$	$_2 0.021\pm0.001^{a}$	0.014 ± 0.002^{b}	$_1 0.015\pm0.001^{b}$	0.018 ± 0.001^{ab}
	K^+ISW	$_2 0.015\pm0.001^{a}$	$_2 0.010\pm0.001^{b}$	$_2 0.013\pm0.001^{a}$	0.014 ± 0.001^{a}	$_2 0.021\pm0.001^{c}$	0.022 ± 0.004^{c}
Day 56	OW	0.018 ± 0.003^{a}	0.012 ± 0.002^{b}	$_1 0.017\pm0.004^{a}$	0.016 ± 0.002^{ab}	0.015 ± 0.001^{ab}	0.019 ± 0.001^{a}
	K^+ISW	0.015 ± 0.002^{ab}	0.010 ± 0.001^{a}	$_2 0.010\pm0.001^{a}$	0.014 ± 0.000^{ab}	0.014 ± 0.001^{ab}	0.016 ± 0.002^{b}
Day 70	OW	0.023 ± 0.001^{a}	$_1 0.007\pm0.000^{b}$	0.009 ± 0.000^{b}	0.009 ± 0.002^{b}	$_1 0.009\pm0.001^{b}$	0.006 ± 0.001^{b}
	K^+ISW	0.020 ± 0.000^{ab}	$_2 0.026\pm0.002^{b}$	0.019 ± 0.004^{ab}	0.014 ± 0.002^{a}	$_2 0.042\pm0.0012^{c}$	0.009 ± 0.001^{a}
Day 84	OW	0.046 ± 0.003^{a}	$_1 0.013\pm0.001^{b}$	0.020 ± 0.001^{b}	0.015 ± 0.005^{b}	0.021 ± 0.000^{b}	0.010 ± 0.000^{b}
	K^+ISW	0.042 ± 0.004^{a}	$_2 0.029\pm0.003^{ab}$	0.027 ± 0.002^{b}	0.019 ± 0.001^{b}	0.033 ± 0.014	0.019 ± 0.004^{b}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at $P<0.05$ (t-test, n=4)

Table 6-6. The [NO_3^--N] (mg L^{-1}) in waters cultured *S. linearifolium* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	$_1$2.17±0.08^{a}	$_1$3.50±0.04^{b}	$_1$2.47±0.06^{c}	$_1$1.67±0.06^{d}	$_1$1.83±0.02^{e}	$_1$1.47±0.02^{f}
	K^+ISW	$_2$2.37±0.06^{ac}	$_2$2.27±0.02^{a}	$_2$1.73±0.08^{b}	$_2$2.30±0.07^{a}	$_2$2.47±0.02^{c}	$_2$2.27±0.05^{a}
Day 14	OW	2.46±0.29^{ac}	3.03±0.35^{a}	2.47±0.29^{a}	$_1$3.93±0.48^{b}	2.27±0.25^{ac}	1.81±0.08^{c}
	K^+ISW	1.87±0.10^{a}	2.60±0.11^{ab}	2.83±0.13^{ab}	$_2$2.07±0.10^{ab}	3.63±0.22^{b}	2.40±0.12^{ab}
Day 28	OW	$_1$2.83±0.14	2.47±0.21	2.30±0.11	$_1$2.53±0.25	$_1$2.53±0.27	$_1$2.60±0.15
	K^+ISW	$_2$1.93±0.18^{a}	2.17±0.25^{ac}	2.80±0.19^{b}	$_2$3.17±0.37^{bc}	$_2$3.47±0.24^{c}	$_2$3.73±0.13^{c}
Day 42	OW	2.67±0.18^{a}	$_1$4.17±0.65^{bc}	$_1$4.70±0.25^{b}	3.73±0.13^{bc}	$_1$3.20±0.12^{ac}	3.93±0.29^{bc}
	K^+ISW	3.20±0.15^{ac}	$_2$2.60±0.29^{a}	$_2$3.10±0.15^{ac}	3.23±0.29^{ac}	$_2$5.23±0.72^{b}	3.63±0.25^{c}
Day 56	OW	4.27±0.71^{ab}	3.50±0.52^{a}	$_1$5.17±0.15^{b}	4.30±0.59^{ab}	$_1$4.43±0.27^{ab}	5.17±0.35^{b}
	K^+ISW	3.83±0.25^{ac}	3.93±0.49^{ac}	$_2$3.27±0.12^{a}	3.30±0.25^{a}	$_2$6.63±0.98^{b}	5.00±0.33^{c}
Day 70	OW	$_1$2.53±0.16^{a}	$_1$2.30±0.08^{a}	2.63±0.17^{a}	2.93±0.18^{a}	2.83±0.12^{a}	$_1$4.63±0.66^{b}
	K^+ISW	$_2$4.83±0.58^{a}	$_2$4.90±1.13^{a}	3.33±0.08^{b}	4.23±0.41^{ab}	3.57±0.22^{ab}	$_2$6.57±0.56^{c}
Day 84	OW	2.23±0.52^{a}	2.63±0.17^{a}	$_1$5.20±0.67^{bc}	2.20±0.11^{a}	5.90±1.68^{b}	$_1$3.80±0.25^{ac}
	K^+ISW	2.70±0.65^{a}	3.03±0.23^{a}	$_2$2.20±0.07^{a}	2.67±0.66^{a}	5.93±1.02^{b}	$_2$6.70±0.62^{b}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at $P<0.05$ (t-test, n=4)

Table 6-7. The [TKN] (mg L^{-1}) in waters cultured *S. linearifolium* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	0.42 ± 0.03^{a}	${}_{1}1.07\pm0.02^{b}$	${}_{1}1.84\pm0.04^{c}$	${}_{1}2.33\pm0.02^{d}$	${}_{1}2.71\pm0.02^{e}$	${}_{1}3.01\pm0.06^{f}$
	K^+ISW	0.54 ± 0.07^{a}	${}_{2}1.80\pm0.02^{b}$	${}_{2}1.98\pm0.04^{c}$	${}_{2}2.17\pm0.03^{d}$	${}_{2}3.39\pm0.04^{e}$	${}_{2}3.60\pm0.10^{f}$
Day 14	OW	1.58 ± 0.53	${}_{1}1.32\pm0.41$	${}_{1}1.00\pm0.06$	${}_{1}1.38\pm0.34$	${}_{1}1.34\pm0.04$	1.42 ± 0.17
	K^+ISW	1.20 ± 0.05^{a}	${}_{2}2.32\pm0.10^{bc}$	${}_{2}2.67\pm0.22^{b}$	${}_{2}2.19\pm0.15^{bc}$	${}_{2}2.84\pm0.39^{b}$	1.73 ± 0.04^{ac}
Day 28	OW	${}_{1}0.47\pm0.28^{a}$	${}_{1}0.98\pm0.03^{bc}$	${}_{1}0.79\pm0.03^{ac}$	${}_{1}1.42\pm0.04^{d}$	${}_{1}1.24\pm0.07^{cd}$	${}_{1}1.59\pm0.07^{d}$
	K^+ISW	${}_{2}1.61\pm0.08^{a}$	${}_{2}2.87\pm0.10^{b}$	${}_{2}2.54\pm0.04^{b}$	${}_{2}5.14\pm0.22^{c}$	${}_{2}3.53\pm0.07^{d}$	${}_{2}3.90\pm0.19^{e}$
Day 42	OW	1.59 ± 0.18^{a}	${}_{1}2.15\pm0.12^{a}$	${}_{1}3.55\pm0.18^{b}$	4.16 ± 0.14^{b}	${}_{1}3.55\pm0.14^{b}$	${}_{1}3.88\pm0.14^{b}$
	K^+ISW	2.05 ± 0.09^{a}	${}_{2}4.48\pm0.30^{b}$	${}_{2}5.00\pm0.17^{b}$	4.62 ± 0.06^{b}	${}_{2}6.91\pm0.68^{c}$	${}_{2}6.12\pm0.36^{c}$
Day 56	OW	1.63 ± 0.09^{ac}	${}_{1}1.31\pm0.07^{a}$	${}_{1}2.66\pm0.21^{b}$	${}_{1}3.13\pm0.39^{b}$	${}_{1}2.05\pm0.24^{c}$	${}_{1}5.65\pm0.24^{d}$
	K^+ISW	1.63 ± 0.12^{a}	${}_{2}2.89\pm0.07^{b}$	${}_{2}3.60\pm0.23^{c}$	${}_{2}4.20\pm0.00^{d}$	${}_{2}4.53\pm0.14^{d}$	${}_{2}6.35\pm0.09^{e}$
Day 70	OW	1.91 ± 0.33^{a}	3.17 ± 0.65^{b}	4.02 ± 0.76^{b}	${}_{1}3.08\pm0.55^{ab}$	3.0821^{ab}	5.65 ± 0.21^{c}
	K^+ISW	2.47 ± 0.38^{a}	3.78 ± 0.56^{b}	4.39 ± 0.26^{b}	${}_{2}4.95\pm0.37^{bc}$	4.06 ± 0.41^{b}	5.74 ± 0.06^{c}
Day 84	OW	2.66 ± 1.50	2.87 ± 0.50	3.26 ± 0.23	3.71 ± 1.24	2.70 ± 1.10	4.81 ± 0.54
	K^+ISW	3.36 ± 0.78	3.40 ± 0.69	3.55 ± 1.19	3.62 ± 0.29	4.00 ± 0.45	4.73 ± 0.49

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at $P<0.05$ (t-test, n=4)

Table 6-8. The [NH_4-N] (mg L^{-1}) in waters cultured *S. linearifolium* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	Negligible^{a}	$_{1}$0.977±0.009^{b}	$_{1}$1.717±0.014^{c}	$_{1}$2.087±0.005^{d}	$_{1}$2.643±0.005^{e}	2.750±0.000^{f}
	K^+ISW	Negligible^{a}	$_{2}$1.003±0.005^{b}	$_{2}$1.617±0.009^{c}	$_{2}$1.987±0.010^{d}	$_{2}$2.607±0.006^{e}	2.750±0.000^{f}
Day 14	OW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	K^+ISW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Day 28	OW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	K^+ISW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Day 42	OW	Negligible^{a}	0.013±0.002^{a}	0.030±0.007^{ab}	0.058±0.022^{b}	$_{1}$0.003±0.002^{a}	0.057±0.017^{b}
	K^+ISW	Negligible^{a}	0.033±0.005^{b}	0.040±0.011^{b}	0.083±0.020^{c}	$_{2}$0.073±0.008^{c}	0.063±0.000^{c}
Day 56	OW	0.017±0.012^{ab}	0.015±0.002^{ab}	Negligible^{a}	$_{1}$0.017±0.005^{ab}	0.047±0.002^{b}	0.037±0.002^{ab}
	K^+ISW	0.003±0.002^{a}	$_{2}$Negligible^{a}	0.003±0.002^{a}	$_{2}$0.070±0.046^{b}	0.037±0.022^{ab}	0.007±0.005^{a}
Day 70	OW	$_{1}$Negligible^{a}	0.053±0.019^{ab}	0.103±0.043^{b}	0.093±0.032^{b}	0.033±0.005^{ab}	0.092±0.023^{b}
	K^+ISW	$_{2}$0.117±0.041^{a}	0.060±0.029^{ab}	0.067±0.012^{ab}	0.090±0.032^{a}	0.015±0.006^{b}	0.031±0.018^{b}
Day 84	OW	0.044±0.027^{a}	0.040±0.008^{a}	$_{1}$0.106±0.027^{ab}	$_{1}$0.023±0.014^{a}	0.200±0.074^{b}	0.097±0.017^{ab}
	K^+ISW	0.189±0.067^{ab}	0.173±0.065^{ab}	$_{2}$0.286±0.089^{a}	$_{2}$0.188±0.082^{ab}	0.090±0.032^{b}	0.203±0.005^{ab}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at $P<0.05$ (t-test, n=4)

Table 6-9. The [PO_4^{3-}-P] (mg L^{-1}) in waters cultured *S. linearifolium* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	1.00 ± 0.00^{a}	$_{1}2.57\pm0.05^{b}$	$_{1}2.50\pm0.12^{b}$	2.70 ± 0.07^{bc}	$_{1}2.47\pm0.02^{b}$	$_{1}2.83\pm0.02^{c}$
	K^+ISW	1.07 ± 0.02^{a}	$_{2}2.80\pm0.04^{b}$	$_{2}2.67\pm0.02^{bc}$	2.60 ± 0.00^{c}	$_{2}3.17\pm0.02^{d}$	$_{2}3.27\pm0.05^{d}$
Day 14	OW	1.18 ± 0.19	1.11 ± 0.03	1.00 ± 0.09	1.22 ± 0.06	1.03 ± 0.08	$_{1}0.96\pm0.07$
	K^+ISW	1.25 ± 0.10^{abc}	1.33 ± 0.07^{ac}	1.09 ± 0.08^{ab}	1.28 ± 0.05^{ac}	0.95 ± 0.07^{b}	$_{2}1.47\pm0.25^{c}$
Day 28	OW	$_{1}1.03\pm0.06^{ac}$	1.17 ± 0.10^{a}	1.03 ± 0.15^{ac}	$_{1}0.67\pm0.06^{b}$	0.77 ± 0.12^{bc}	$_{1}0.80\pm0.04^{bc}$
	K^+ISW	$_{2}1.43\pm0.12^{a}$	1.27 ± 0.02^{ab}	0.93 ± 0.12^{c}	$_{1}1.43\pm0.05^{a}$	1.00 ± 0.12^{bc}	$_{2}1.33\pm0.06^{a}$
Day 42	OW	0.97 ± 0.06^{a}	1.30 ± 0.23^{ad}	1.53 ± 0.02^{bd}	$_{1}1.73\pm0.06^{b}$	$_{1}1.00\pm0.15^{a}$	$_{1}2.13\pm0.12^{c}$
	K^+ISW	1.00 ± 0.12^{a}	1.13 ± 0.06^{a}	1.47 ± 0.25^{c}	$_{2}1.37\pm0.09^{bc}$	$_{2}1.67\pm0.02^{c}$	$_{2}1.07\pm0.05^{ab}$
Day 56	OW	$_{1}1.67\pm0.08^{ac}$	1.40 ± 0.12^{ab}	$_{1}1.47\pm0.14^{ab}$	$_{1}1.07\pm0.02^{b}$	1.67 ± 0.08^{ac}	$_{1}1.97\pm0.16^{c}$
	K^+ISW	$_{2}3.90\pm0.24^{a}$	1.20 ± 0.29^{b}	$_{2}0.73\pm0.13^{c}$	$_{2}1.93\pm0.14^{d}$	1.30 ± 0.18^{b}	$_{2}1.30\pm0.15^{b}$
Day 70	OW	0.53 ± 0.02^{a}	0.83 ± 0.09^{a}	0.63 ± 0.02^{a}	0.87 ± 0.13^{a}	2.43 ± 1.26^{b}	2.27 ± 0.12^{b}
	K^+ISW	1.20 ± 0.04^{ab}	0.73 ± 0.06^{a}	1.23 ± 0.06^{ab}	1.17 ± 0.37^{ab}	2.67 ± 0.69^{c}	2.00 ± 0.20^{bc}
Day 84	OW	1.37 ± 0.26^{ab}	1.43 ± 0.05^{ab}	1.73 ± 0.35^{ab}	1.63 ± 0.22^{ab}	$_{1}2.23\pm0.54^{a}$	1.33 ± 0.12^{b}
	K^+ISW	1.70 ± 0.11^{ab}	2.07 ± 0.10^{a}	1.30 ± 0.23^{ab}	1.77 ± 0.08^{ab}	$_{2}4.87\pm0.68^{c}$	1.07 ± 0.06^{b}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at $P<0.05$ (t-test, n=4)

Table 6-10. Pearson correlation of *S. linearifolium* biomass and quality parameters of waters enriched NH_4-N:PO_4^{3-}-P (N=48)

Dependent variable	pH	Temperature	NO_2^--N	NO_3^--N	PO_4^{3-}-P	NH_4-N	TKN
Biomass	-0.577**	-0.427**	-0.454**	-0.440**	-0.186**	0.237**	-0.582**
SGR	-0.300**	-0.386**	-0.339**	-0.339**	-0.289**	-0.344**	-0.539**

(**) – Correlation is significant at the 0.01 level (2-tailed); (*) - Correlation is significant at the 0.05 level (2-tailed) (P<0.05)

Table 6-11. Pearson correlation of *S. podacanthum* biomass and quality parameters of waters enriched NH_4-N:PO_4^{3-}-P (N=48)

Dependent variable	pH	Temperature	NO_2^--N	NO_3^--N	PO_4^{3-}-P	NH_4-N	TKN
Biomass	0.012	0.226**	-0.365**	-0.121*	-0.273**	-0.110*	-0.197**
SGR	-0.157**	0.163**	-0.234**	-0.115	-0.134*	-0.207**	-0.630**

(**) – Correlation is significant at the 0.01 level (2-tailed); (*) - Correlation is significant at the 0.05 level (2-tailed) (P<0.05)

Table 6-12. Fresh standing biomass (g) of *S. podacanthum* cultured in control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM) waters

Water	Time	Control	80:8	120:12	160:16	200:20	240:24
	Day 1	3.55±0.03	$_{12}$3.56±0.08	$_{1}$3.63±0.03	$_{1}$3.59±0.02	$_{12}$3.59±0.02	3.62±0.06
	Day 14	3.81±0.03^{ab}	$_{12}$3.97±0.13^{ab}	$_{1}$3.68±0.21^{ab}	$_{12}$4.15±0.17^{b}	$_{1}$3.61±0.28^{a}	4.08±0.24^{b}
	Day 28	3.97±0.30	$_{12}$3.96±0.36	$_{12}$4.34±0.41	$_{123}$4.58±0.27	$_{123}$3.86±0.51	4.59±0.26
OW	Day 42	3.88±0.36	$_{12}$3.97±0.36	$_{12}$4.83±0.46	$_{123}$4.87±0.34	$_{123}$4.08±0.66	$_{1}$4.78±0.34
	Day 56	3.48±0.47^{a}	$_{1}$4.39±0.46^{ab}	$_{2}$5.50±0.53^{b}	$_{23}$4.97±0.34^{ab}	$_{23}$4.64±0.43^{ab}	4.99±0.32^{ab}
	Day 70	3.25±0.44^{a}	$_{12}$3.75±0.16^{a}	$_{2}$5.55±0.90^{b}	$_{3}$5.22±0.52^{b}	$_{3}$4.91±0.335^{b}	$_{1}$4.63±0.50^{ab}
	Day 84	2.95±0.46^{a}	$_{2}$3.17±0.10^{ab}	$_{12}$4.72±0.51^{c}	$_{123}$4.54±0.40^{c}	$_{123}$4.28±0.40^{bc}	$_{1}$4.23±0.62^{bc}
	Day 1	$_{12}$3.58±0.08	$_{1}$3.54±0.13	$_{12}$3.54±0.11	$_{1}$3.57±0.08	3.57±0.09	$_{1}$3.58±0.03
	Day 14	$_{1}$3.84±0.11^{abc}	$_{1}$3.41±0.18^{a}	$_{12}$3.91±0.30^{bc}	$_{2}$4.56±0.35^{c}	3.25±0.26^{a}	$_{1}$3.95±0.22^{bc}
	Day 28	$_{1}$3.74±0.07^{ab}	$_{1}$3.40±0.16^{a}	$_{1}$4.14±0.29^{ab}	$_{2}$4.84±0.35^{b}	3.25±0.30^{a}	$_{1}$3.97±0.23^{ab}
K^+ISW	Day 42	$_{23}$3.58±0.22^{a}	$_{1}$3.12±0.32^{a}	$_{12}$3.77±0.31^{ab}	$_{2}$5.11±0.47^{b}	3.29±0.27^{a}	$_{12}$3.40±0.18^{ab}
	Day 56	$_{123}$3.52±0.37^{a}	$_{12}$2.87±0.36^{a}	$_{1}$3.91±0.36^{ab}	$_{2}$4.99±0.47^{b}	$_{2}$2.99±0.42^{a}	$_{1}$3.58±0.38^{ab}
	Day 70	$_{123}$3.07±0.11^{ab}	$_{12}$2.67±0.35^{a}	$_{12}$3.16±0.64^{ab}	$_{12}$4.31±0.22^{b}	$_{2}$2.38±0.85^{a}	$_{12}$3.25±0.17^{ab}
	Day 84	$_{3}$2.85±0.13^{ab}	$_{3}$2.31±0.25^{a}	$_{2}$2.68±0.52^{ab}	$_{1}$3.61±0.13^{b}	$_{2}$2.14±0.80^{a}	$_{2}$2.70±0.17^{ab}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column of one water-type sharing a common subscript are not significantly different (LSD-test; P>0.05; n=4)

Table 6-13. SGR (% d-1) of S. podacanthum cultured in control and five enriched levels of NH4-N:PO43--P (µM) waters

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1–14	OW	0.49±0.08	0.77±0.22	0.06±0.45	1.01±0.27	-0.03±0.54	0.83±0.43
	K^+ISW	0.49±0.22^{a}	-0.29±0.27^{ac}	0.66±0.64^{ab}	1.70±0.64^{b}	-0.74±0.53^{c}	0.66±0.45^{ab}
Day 1–28	OW	0.36±0.26	0.34±0.29	0.59±0.32	0.85±0.20	0.15±0.49	0.83±0.19
	K^+ISW	0.15±0.13^{ac}	-0.15±0.21^{ac}	0.54±0.18^{ab}	1.06±0.31^{b}	-0.38±0.30^{c}	0.35±0.23^{abc}
Day 1–42	OW	0.18±0.21	0.23±0.18	0.65±0.21	0.71±0.16	0.19±0.42	$_1$0.65±0.16^{a}
	K^+ISW	-0.01±0.12^{a}	-0.34±0.28^{a}	0.13±0.18^{a}	0.83±0.25^{b}	-0.21±0.15^{a}	$_2$-0.13±0.13^{a}
Day 1–56	OW	-0.08±0.22^{a}	$_1$0.34±0.19^{ab}	$_1$0.72±0.17^{b}	0.57±0.12^{b}	$_1$0.43±0.16^{ab}	$_1$0.57±0.11^{b}
	K^+ISW	-0.06±0.18^{ab}	$_2$-0.41±0.26^{a}	$_2$0.16±0.19^{bc}	0.57±0.18^{c}	$_2$-0.37±0.26^{ab}	$_2$-0.03±0.18^{ab}
Day 1–70	OW	-0.16±0.17^{a}	$_1$0.07±0.09^{ab}	$_1$0.54±0.19^{b}	0.50±0.16^{b}	$_1$0.42±0.10^{b}	$_1$0.32±0.16^{ab}
	K^+ISW	-0.22±0.02^{ab}	$_2$-0.42±0.21^{a}	$_2$-0.27±0.37^{a}	0.26±0.09^{b}	$_2$-0.19±0.15^{a}	$_2$-0.14±0.07^{ab}
Day 1–84	OW	-0.27±0.17^{a}	-0.14±0.12^{ac}	$_1$0.30±0.12^{b}	0.27±0.11^{bc}	$_1$0.20±0.11^{bc}	$_1$0.15±0.19^{abc}
	K^+ISW	-0.28±0.06^{ab}	-0.54±0.16^{a}	$_2$-0.43±0.30^{a}	0.01±0.06^{b}	$_2$-0.32±0.18^{ab}	$_2$-0.35±0.08^{ab}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column of one time period sharing a common subscript are not significantly different at P<0.05 (t-test, n=4)

Table 6-14. The [NO_2^--N] (mg L^{-1}) in waters cultured *S. podacanthum* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	0.011±0.001^{a}	0.007±0.000^{b}	0.006±0.000^{b}	0.007±0.000^{b}	0.007±0.000^{b}	0.006±0.000^{b}
	K^+ISW	0.012±0.000^{bc}	0.012±0.000^{bc}	0.014±0.000^{a}	0.011±0.002^{b}	0.015±0.001^{a}	0.007±0.001^{d}
Day 14	OW	0.011±0.001^{b}	0.007±0.001^{c}	0.007±0.001^{c}	0.012±0.001^{b}	0.028±0.001^{a}	0.008±0.001^{c}
	K^+ISW	0.008±0.001^{b}	0.015±0.001^{a}	0.010±0.000^{b}	0.013±0.000^{a}	0.010±0.001^{b}	0.008±0.000^{b}
Day 28	OW	0.007±0.000^{b}	0.007±0.000^{b}	0.007±0.001^{b}	0.009±0.000^{ab}	0.010±0.001^{a}	0.011±0.002^{a}
	K^+ISW	0.012±0.002^{b}	0.008±0.000^{d}	0.013±0.000^{b}	0.011±0.001^{bc}	0.009±0.001^{cd}	0.026±0.001^{a}
Day 42	OW	0.008±0.000	$_1$0.009±0.000	0.006±0.000	0.008±0.000	0.008±0.001	0.010±0.001
	K^+ISW	0.011±0.001^{b}	$_2$0.013±0.001^{b}	0.018±0.002^{a}	0.015±0.001^{a}	0.010±0.001^{b}	0.016±0.001^{a}
Day 56	OW	0.004±0.001^{bc}	$_1$0.007±0.002^{a}	0.004±0.001^{bc}	0.006±0.002^{ab}	0.003±0.001^{bc}	0.002±0.002^{c}
	K^+ISW	0.022±0.005^{b}	$_2$0.013±0.001^{c}	0.008±0.003^{d}	0.014±0.002^{c}	0.031±0.004^{a}	0.002±0.001^{e}
Day 70	OW	0.020±0.000^{a}	0.007±0.000^{b}	0.007±0.000^{b}	0.006±0.000^{b}	0.006±0.001^{b}	0.005±0.001^{b}
	K^+ISW	0.018±0.002^{b}	0.009±0.000^{d}	0.019±0.005^{b}	0.013±0.001^{c}	0.044±0.006^{a}	0.005±0.000^{e}
Day 84	OW	0.033±0.003^{a}	0.011±0.002^{c}	0.015±0.003^{bc}	0.006±0.002^{d}	0.020±0.005^{b}	0.007±0.000^{d}
	K^+ISW	0.038±0.002^{a}	0.026±0.002^{b}	0.016±0.001^{c}	0.014±0.002^{c}	0.041±0.003^{a}	0.013±0.001^{c}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at P<0.05 (t-test, n=4)

Table 6-15. The [NO_3^--N] (mg L^{-1}) in waters cultured *S. podacanthum* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	$_1$2.17±0.08^{a}	$_1$3.50±0.04^{b}	$_1$2.47±0.06^{c}	$_1$1.67±0.06^{d}	$_1$1.83±0.02^{e}	$_1$1.47±0.02^{f}
	K^+ISW	$_2$2.37±0.06^{ac}	$_2$2.27±0.02^{a}	$_2$1.73±0.08^{b}	$_2$2.30±0.07^{a}	$_2$2.47±0.02^{c}	$_2$2.27±0.05^{a}
Day 14	OW	2.07±0.13^{ab}	2.63±0.06^{bc}	$_1$1.90±0.11^{a}	$_1$2.90±0.23^{c}	$_1$2.37±0.15^{b}	$_1$1.83±0.10^{a}
	K^+ISW	1.87±0.10^{a}	2.60±0.11^{bc}	$_2$2.83±0.13^{c}	$_2$2.07±0.10^{ab}	$_2$3.63±0.22^{d}	$_2$2.40±0.12^{b}
Day 28	OW	$_1$2.10±0.37^{a}	1.93±0.10^{a}	2.33±0.06^{a}	2.23±0.19^{a}	$_1$1.77±0.06^{a}	$_1$2.90±0.19^{b}
	K^+ISW	$_2$1.47±0.05^{a}	2.33±0.12^{bc}	2.67±0.25^{c}	1.93±0.08^{ab}	$_2$3.43±0.06^{d}	$_2$3.90±0.13^{d}
Day 42	OW	1.53±0.19^{ab}	2.57±0.66^{c}	2.33±0.47^{bc}	$_1$1.60±0.23^{ab}	1.03±0.05^{a}	2.90±0.23^{c}
	K^+ISW	2.13±0.25^{b}	2.80±0.16^{b}	2.00±0.11^{b}	$_2$2.53±0.08^{b}	1.13±0.06^{a}	3.63±0.13^{c}
Day 56	OW	2.77±0.16^{b}	2.73±0.14^{b}	3.07±0.62^{b}	$_1$1.80±0.11^{a}	5.03±0.12^{c}	2.87±0.37^{b}
	K^+ISW	3.53±0.22^{bc}	2.47±0.13^{a}	3.93±0.13^{c}	$_2$3.97±0.63^{c}	5.30±0.49^{d}	2.70±0.15^{ab}
Day 70	OW	$_1$2.37±0.22^{b}	$_1$1.80±0.07^{a}	$_1$2.63±0.17^{b}	$_1$2.23±0.08^{ab}	2.73±0.08^{b}	$_1$1.97±0.12^{a}
	K^+ISW	$_2$4.20±0.37^{c}	$_2$3.50±0.08^{ab}	$_2$3.20±0.12^{b}	$_2$3.90±0.12^{bc}	2.90±0.27^{a}	$_2$3.13±0.16^{a}
Day 84	OW	1.85±0.39^{ab}	2.40±0.15^{b}	$_1$3.63±0.94^{bc}	1.77±0.10^{a}	4.83±1.25^{c}	3.47±0.33^{bc}
	K^+ISW	2.47±0.73^{ac}	2.80±0.33^{ac}	$_2$1.53±0.17^{a}	1.03±0.43^{a}	6.13±1.13^{b}	3.10± 0.08^{c}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at P<0.05 (t-test, n=4)

Table 6-16. The [TKN] (mg L^{-1}) in waters cultured *S. podacanthum* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	0.42 ± 0.03^{a}	${}_{1}1.07\pm0.02^{b}$	${}_{1}1.84\pm0.04^{c}$	${}_{1}2.33\pm0.02^{d}$	${}_{1}2.71\pm0.02^{e}$	${}_{1}3.01\pm0.06^{f}$
	K^+ISW	0.54 ± 0.07^{a}	${}_{2}1.80\pm0.02^{b}$	${}_{2}1.98\pm0.04^{c}$	${}_{2}2.17\pm0.03^{d}$	${}_{2}3.39\pm0.04^{e}$	${}_{2}3.60\pm0.10^{f}$
Day 14	OW	2.03 ± 0.06^{a}	${}_{1}2.45\pm0.14^{b}$	2.10 ± 0.06^{ab}	${}_{1}2.36\pm0.05^{ab}$	${}_{1}2.45\pm0.09^{b}$	${}_{1}3.15\pm0.19^{c}$
	K^+ISW	2.36 ± 0.23^{a}	${}_{2}3.55\pm0.07^{b}$	2.47 ± 0.23^{a}	${}_{2}3.13\pm0.06^{c}$	${}_{2}4.27\pm0.03^{d}$	${}_{2}3.74\pm0.09^{b}$
Day 28	OW	${}_{1}0.23\pm0.06^{a}$	${}_{1}1.12\pm0.10^{b}$	${}_{1}0.96\pm0.07^{b}$	${}_{1}1.17\pm0.16^{b}$	${}_{1}1.31\pm0.03^{bc}$	${}_{1}1.59\pm0.04^{c}$
	K^+ISW	${}_{2}1.70\pm0.15^{a}$	${}_{2}1.70\pm0.17^{a}$	${}_{2}2.75\pm0.17^{c}$	${}_{2}2.40\pm0.03^{b}$	${}_{2}3.10\pm0.12^{d}$	${}_{2}3.31\pm0.15^{e}$
Day 42	OW	${}_{1}1.82\pm0.21^{a}$	${}_{1}2.57\pm0.42^{a}$	${}_{1}2.15\pm0.29^{a}$	${}_{1}2.15\pm0.09^{a}$	${}_{1}4.02\pm0.23^{b}$	${}_{1}2.19\pm0.34^{a}$
	K^+ISW	${}_{2}3.74\pm0.09^{a}$	${}_{2}4.53\pm0.29^{ab}$	${}_{2}5.09\pm0.66^{c}$	${}_{2}4.30\pm0.14^{a}$	${}_{2}7.52\pm0.34^{d}$	${}_{2}5.14\pm0.37^{c}$
Day 56	OW	${}_{1}0.37\pm0.03^{a}$	${}_{1}2.29\pm0.44^{bc}$	${}_{1}1.59\pm0.09^{b}$	${}_{1}1.96\pm0.23^{bc}$	${}_{1}2.80\pm0.41^{c}$	${}_{1}4.02\pm0.23^{d}$
	K^+ISW	${}_{2}3.27\pm0.26^{a}$	${}_{2}3.31\pm0.23^{a}$	${}_{2}3.74\pm0.18^{a}$	${}_{2}4.76\pm0.06^{b}$	${}_{2}5.18\pm0.66^{b}$	${}_{2}6.54\pm0.43^{c}$
Day 70	OW	${}_{1}1.59\pm0.09^{ab}$	4.11 ± 0.34^{bc}	5.51 ± 0.69^{c}	${}_{1}1.54\pm0.21^{a}$	${}_{1}2.94\pm0.21^{b}$	${}_{1}0.61\pm0.17^{a}$
	K^+ISW	${}_{2}3.27\pm0.09^{a}$	4.25 ± 0.14^{ab}	5.56 ± 0.52^{b}	${}_{2}4.81\pm0.18^{b}$	${}_{2}4.11\pm0.41^{ab}$	${}_{2}4.76\pm1.45^{ab}$
Day 84	OW	${}_{1}1.88\pm0.46^{a}$	${}_{1}3.41\pm0.36^{bc}$	${}_{1}3.41\pm0.60^{bc}$	${}_{1}3.02\pm0.37^{ab}$	3.36 ± 0.55^{bc}	4.76 ± 0.66^{c}
	K^+ISW	${}_{2}4.61\pm0.49^{a}$	${}_{2}6.76\pm0.30^{b}$	${}_{2}5.26\pm0.73^{a}$	${}_{2}4.82\pm0.73^{a}$	4.25 ± 0.26^{a}	5.18 ± 0.21^{a}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at $P<0.05$ (t-test, n=4)

Table 6-17. The [NH_4-N] (mg L^{-1}) in waters cultured *S. podacanthum* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	Negligible^{a}	$_{1}$0.977±0.009^{b}	$_{1}$1.717±0.014^{c}	$_{1}$2.087±0.005^{d}	$_{1}$2.643±0.005^{e}	2.750±0.000^{f}
	K^+ISW	Negligible^{a}	$_{2}$1.003±0.005^{b}	$_{2}$1.617±0.009^{c}	$_{2}$1.987±0.010^{d}	$_{2}$2.607±0.006^{e}	2.750±0.000^{f}
Day 14	OW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	K^+ISW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Day 28	OW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	K^+ISW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Day 42	OW	0.017±0.002	0.007±0.002	0.010±0.007	$_{1}$0.010±0.007	$_{1}$0.030±0.021	0.033±0.017
	K^+ISW	0.010±0.004^{a}	0.040±0.028^{a}	0.080±0.018^{ab}	$_{2}$0.173±0.062^{bc}	$_{2}$0.193±0.110^{c}	0.033±0.009^{a}
Day 56	OW	Negligible^{a}	$_{1}$0.040±0.028^{b}	0.010±0.007^{a}	Negligible^{a}	Negligible^{a}	0.027±0.015^{ab}
	K^+ISW	Negligible^{a}	$_{2}$Negligible^{a}	Negligible^{a}	Negligible^{a}	0.020±0.014^{ab}	0.037±0.012^{b}
Day 70	OW	$_{1}$Negligible^{a}	0.063±0.023^{b}	$_{1}$0.133±0.046^{c}	$_{1}$0.093±0.023^{bc}	0.043±0.010^{b}	0.127±0.002^{c}
	K^+ISW	$_{2}$0.137±0.029^{b}	0.080±0.015^{ab}	$_{2}$0.047±0.006^{ab}	$_{2}$0.207±0.010^{c}	0.035±0.007^{a}	0.103±0.017^{b}
Day 84	OW	0.053±1.650	$_{1}$0.030±0.004	0.133±0.028	$_{1}$0.033±0.013	0.207±0.104	$_{1}$0.073±0.025
	K^+ISW	0.179±0.036	$_{2}$0.248±0.021	0.249±0.085	$_{2}$0.191±0.067	0.137±0.015	$_{2}$0.217±0.017

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at P<0.05 (t-test, n=4)

Table 6-18. The [PO_4^{3-}-P] (mg L^{-1}) in waters cultured *S. podacanthum* at control and five enriched levels of NH_4-N:PO_4^{3-}-P (µM)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	1.00±0.00^{a}	$_{1}$2.57±0.05^{bc}	$_{1}$2.50±0.12^{b}	2.70±0.07^{cd}	$_{1}$2.47±0.02^{b}	$_{1}$2.83±0.02^{d}
	K^+ISW	1.07±0.02^{a}	$_{2}$2.80±0.04^{c}	$_{2}$2.67±0.02^{cd}	2.60±0.00^{d}	$_{2}$3.17±0.02^{b}	$_{2}$3.27±0.05^{b}
Day 14	OW	$_{1}$1.03±0.02^{c}	$_{1}$1.17±0.02^{d}	$_{1}$1.10±0.04^{cd}	$_{1}$0.63±0.02^{a}	$_{1}$0.83±0.02^{b}	$_{1}$0.83±0.02^{b}
	K^+ISW	$_{2}$1.20±0.04^{a}	$_{2}$1.30±0.04^{b}	$_{2}$1.00±0.04^{c}	$_{2}$1.30±0.04^{b}	$_{2}$1.10±0.04^{d}	$_{2}$1.23±0.02^{a}
Day 28	OW	$_{1}$0.93±0.08^{ac}	1.10±0.07^{a}	0.97±0.13^{ac}	$_{1}$0.67±0.02^{bc}	$_{1}$0.60±0.11^{b}	$_{1}$0.83±0.02^{c}
	K^+ISW	$_{2}$1.17±0.02^{ac}	1.30±0.04^{a}	0.87±0.13^{b}	$_{2}$1.30±0.04^{a}	$_{2}$0.97±0.08^{bc}	$_{2}$1.23±0.02^{a}
Day 42	OW	0.30±0.11^{a}	0.33±0.02^{a}	$_{1}$0.37±0.13^{a}	$_{1}$0.33±0.08^{a}	$_{1}$0.57±0.02^{a}	$_{1}$1.23±0.06^{b}
	K^+ISW	0.50±0.22^{ab}	0.27±0.06^{a}	$_{2}$0.77±0.10^{bc}	$_{2}$0.83±0.02^{c}	$_{2}$1.03±0.02^{c}	$_{2}$1.53±$^{d0.05}$
Day 56	OW	0.60±0.11^{a}	0.90±0.12^{a}	0.97±0.08^{a}	0.53±0.06^{a}	$_{1}$0.77±0.18^{a}	0.57±0.10^{a}
	K^+ISW	1.03±0.17^{ab}	1.13±0.20^{ab}	1.07±0.18^{ab}	1.27±0.16^{ab}	$_{2}$1.10±0.45^{a}	0.77±0.02^{a}
Day 70	OW	0.47±0.02^{a}	0.73±0.08^{a}	$_{1}$0.43±0.05^{a}	0.60±0.11^{a}	$_{1}$0.50±0.04^{a}	0.43±0.08^{a}
	K^+ISW	1.13±0.02^{a}	0.63±0.02^{a}	$_{2}$1.37±0.17^{b}	0.87±0.33^{a}	$_{2}$2.53±0.63^{c}	0.57±0.02^{a}
Day 84	OW	1.17±0.33^{ab}	1.27±0.02^{ab}	1.43±0.34^{ab}	1.30±0.32^{ab}	$_{1}$1.87±0.46^{b}	1.07±0.17^{a}
	K^+ISW	1.43±0.05^{ab}	1.90±0.15^{b}	1.23±0.25^{ab}	1.43±0.06^{ab}	$_{2}$3.50±0.36^{c}	0.93±0.08^{a}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at a time sharing a common subscript are not the significantly different at P<0.05 (t-test, n=4)

6.3.3 Biomass and Growth Rate of *S. podacanthum*

Although the standing biomass of the *S. podacanthum* varied as the time progressed, different nutrient enrichments resulted in significant ($P<0.05$) differences in the growth of *S. podacanthum*. The standing biomass of *S. podacanthum* increased with increased nutrient supplementation concentrations. The ratio 160:16 resulted in a significantly ($P<0.05$) higher *S. podacanthum* standing biomass than the *S. podacanthum* exposed to all other nutrient concentrations throughout the trial.

In OW, *S. podacanthum* showed a significantly ($P<0.05$) higher biomass in OW_120, OW_160, OW_200 than the other nutrient concentrations in the second half of the trial period, and reached the maximum biomass at day 70, and then declined (Table 6-12). In K^+ISW, the *S. podacanthum* biomass was significantly higher in ISW_160 throughout the trial, and the biomass at the end was similar to that at the beginning. However, at all other nutrient concentrations, the biomass did not change during the first 70 days, and then significantly declined (Table 6-12).

Two water types (OW or K^+ISW) did not show any effect on the standing biomass of *S. podacanthum* in the first 28 days, but significantly ($P<0.05$) affected its biomass from day 42. Furthermore, higher concentrations of nutrients were significantly ($P<0.05$) affected the standing biomass of *S. podacanthum* (Table 6-11). From the day 56 until the end of the trial, the standing biomass of *S. podacanthum* was significantly higher in OW than in K^+ISW at high nutrient concentrations (120:12, 200:20, 240:24), except when enriched with NH_4-N:PO_4^{3-}-P 160:16 µM. No nutrient supplementation into culture medium and the ratio of 160:16, the standing biomass of *S. podacanthum* grown in OW showed no significant differences from that of *S. podacanthum* grown in K^+ISW.

The biomass of *S. podacanthum* was significantly correlated ($R^2>0.7$) with time (in fortnights) (Table 6-19), and was significantly ($P<0.05$) affected by water temperature, and the concentrations of NO_2^--N, NH_4-N, TKN and PO_4^{3-}-P in waters (Table 6-11).

Table 6-19. The regression correlation of the *S. podacanthum* biomass in mg (y) with the time in fortnight (x) at control and five additional nutrient levels of NH_4-N:PO_4^{3-}-P (µM) waters

Water	NH_4-N:PO_4^{3-}-P	Regression	R^2
OW	Control	$y = -7.89x^2 + 48.02x + 407.40$	$R^2 = 0.94$
	80:8	$y = -10.71x^2 + 80.38x + 367.69$	$R^2 = 0.77$
	120:12	$y = -10.53x^2 + 120.39x + 301.52$	$R^2 = 0.82$
	160:16	$y = -11.08x^2 + 112.51x + 337.68$	$R^2 = 0.94$
	200:20	$y = -3.66x^2 + 53.34x + 373.94$	$R^2 = 0.74$
	240:24	$y = -12.73x^2 + 116.69x + 336.53$	$R^2 = 0.97$
K^+ISW	Control	$y = -4.98x^2 + 11.94x + 422.08$	$R^2 = 0.93$
	80:8	$y = -2.98x^2 - 1.50x + 443.74$	$R^2 = 0.99$
	120:12	$y = -12.07x^2 + 77.30x + 377.8$	$R^2 = 0.93$
	160:16	$y = -20.80x^2 + 165.37x + 304.67$	$R^2 = 0.98$
	200:20	$y = -4.98x^2 + 11.94x + 422.08$	$R^2 = 0.93$
	240:24	$y = -7.14x^2 + 37.42x + 426.48$	$R^2 = 0.86$

(**) – Correlation is significant at the 0.01 level (2-tailed); (*) - Correlation is significant at the 0.05 level (2-tailed) ($P<0.05$)

The SGR of the *S. podacanthum* decreased towards the end of the trial and varied significantly ($P<0.5$) with the nutrient levels in two water types (Table 6-13). The SGR was significantly correlated with the water pH, temperature, and water quality parameters, with the exception of NO_3^--N (Table 6-11). The SGR ranged from negative values to 1.70% d^{-1}, recorded in the first 14 days in ISW_160. The *S. podacanthum* SGR was significantly ($P<0.01$) affected by the water types from day 42 onwards, except at the waters without nutrient supplementation and the ratio of 160:16.

At OW_120 and OW_240, the SGR of *S. podacanthum* remained positive during the entire trial. No effects of nutrient enrichments on SGR of *S. podacanthum* were observed in the first 42 days, however, from the day 42, the higher nutrient enrichment resulted in significantly ($P<0.05$) higher SGR.

A negative SGR of *S. podacanthum* was recorded in ISW_80, ISW_200 and ISW_240, as the trial progressed. In other nutrient levels in K^+ISW the SGR gradually decreased

from the beginning to the end of the trial. An exception SGR data were recorded at ISW_160, where highest ($P<0.05$) SGR among five nutrient concentrations over time were observed, and showed the only positive SGR of the *S. podacanthum* in K^+ISW during the whole trial (Table 6-13).

6.3.4 The Quality of Water Culturing *S. podacanthum*

The pH of the OW was similarly in five different nutrient concentrations and control during the trial. However, from the day 42 onwards, the pH in the ISW_240 was significantly ($P<0.05$) higher than that in all other supplementation levels in K^+ISW (Figure 6-2a).

The temperature of the culture media was similar in all the nutrient concentrations, ranging from 20 to 26^oC (Figure 6-2b).

At the commencement of the trial, the NO_2^--N, NO_3^--N, NH_4-N, TKN and PO_4^{3-}-P concentrations in raw ISW were similar in OW_0 and ISW_0. After two weeks, the concentrations of NO_2^--N, TKN and PO_4^{3-}-P significantly ($P<0.05$) increased while NO_3^--N and NH_4-N remained unchanged in raw ISW.

The [N] remained unchanged in the early stages of the trial and significantly ($P<0.05$) increased by the end of the trial in both water types. During the trial, [NO_2^--N] was similar at all the nutrient levels. Both NO_2^--N and NO_3^--N varied widely in K^+ISW, but in ISW_160, NO_2^--N was stable as the time progressed (Table 6-14), while NO_3^--N decreased significantly by the end of the trial (Table 6-15).

The NH_4-N and TKN concentrations significantly rose with increasing nutrient enrichment levels and were higher in K^+ISW than in OW. However, after releasing *S. podacanthum* into the water, [NH_4-N] was approximately negligible over the first 56 days, then increased to a maximum of 0.25 mg L^{-1}, which was lower than at the commencement of the trial (Table 6-16). Conversely, TKN decreased to a minimal value at day 28, and significantly ($P<0.05$) increased by the end of the trial (Table 6-17).

The [PO_4^{3-}-P] in both water types decreased significantly ($P<0.05$) during the trial compared to the beginning of the trial. It was higher in K^+ISW than OW at all nutrient supplementation concentrations greater than 80:8 (Table 6-18).

(a)

(b)

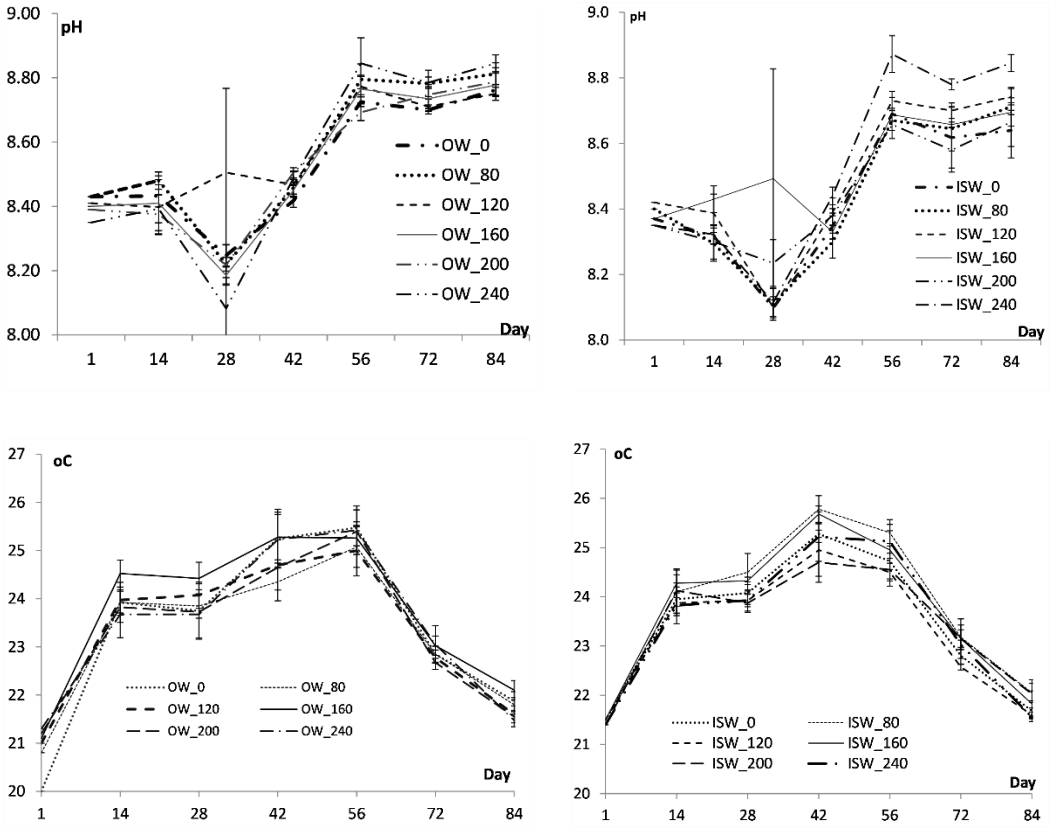

Figure 6-2. The pH (a) and temperature (b) of the nutrient enrichment waters culturing *S. podacanthum*

6.4 Discussion

Taking advantage of the short-seasonal growth of *Sargassum* (Martin-Smith, 1993), farming *Sargassum* spp. in salt-affected farms can provide several uses of additional seaweed crop, including by-product for cattle feed (Huisman, 2000). As nutrient requirements of *Sargassum* spp. in ISW have not yet been researched, the results of this study can be significant in improving technical feasibility of *Sargassum* spp. culture in ISW. The result of this study has shown that NH_4-N and PO_4^{3-}-P enrichment plays an important role for growing *Sargassum* spp. in K^+ISW under laboratory conditions.

K^+ is essential for the growth of plants (Blumwald *et al*., 2000; Talling, 2010), particularly for marine algae, as it is recognised as an important internal cation (Kirst, 1977) playing a role in protein and starch synthesis, and metabolic processes in living cells (Evans & Sorger, 1966). Moreover, K^+ balances the osmotic gradient of aquatic plant cells (Malhotra & Glass, 1995), and maintains the standard sodium to potassium ratio in plant cells (Blumwald *et al*., 2000). The [K^+] in ISW significantly affected the growth of both *Sargassum* species, particularly *S. linearifolium*, which reached an optimal growth in K^+ISW at a similar concentration of K^+ in OW (Bui, Luu, Fotedar, *et al*., 2017). Therefore, *Sargassum* spp. must be grown in K^+ISW at similar concentrations in OW at the same salinity, before adding nutrients - N and P, into ISW.

The range of N:P atomic ratio of *Sargassum* spp. is 20:1 to 38:1, while the average N:P for seaweed growth is from 10:1 to 30:1 (Atkinson & Smith, 1983), and the N:P (in moles) in OW is 37:1 on average (Downing, 1997). The nutrient supplementation NH_4-N:PO_4^{3-}-P ratio of 10:1 was adapted from similar research of Schaffelke and Klumpp (1998) and Schaffelke (1999), where the N and P demand for *S. baccularia* is from 2.9–15.0 and 0.10–0.68 µmol g^{-1} dry weight per day in August to December (Schaffelke & Klumpp, 1998). The *S. everve* grow faster in NH_4-N 200 µM than in 80 µM enriched OW (Liu *et al*., 2004), which were the basis level nutrient supplementation for this study. In the media where the K^+ISW was enriched with NH_4-N and PO_4^{3-}-P, the growth of both *Sargassum* spp. was significantly correlated with the nutrient concentrations but in different trend. The effect of nutrients within the range 120:12–160:16 µM resulted in higher and sustainable growth for *S. podacanthum* than all other nutrient levels.

In the short-term, nutrient enrichment in OW or K^+ISW resulted in no difference in the standing biomass of the two *Sargassum* spp. The effect of nutrient enrichment on *Sargassum* spp. was species dependent, shown by the significant difference between the standing biomass of the two *Sargassum* species. The effect of nutrients within the range 120:12–160:16 µM, was visible after one month of cultivation of *S. podacanthum* biomass than in other nutrient levels in K^+ISW, prevalent the effect of these nutrient levels on *S. podacanthum* growth. Whereas, the *S. linearifolium* biomass did not increase at any nutrient enriched level, and was severely reduced after one month in culture.

The growth of both *Sargassum* species in OW_0 and ISW_0, without nutrient addition showed similar life cycles in terms of growth patterns as any other sub-tropical and/or

temperate *Sargassum* spp., wherein their maximum growth occurred during the late winter and early spring (August–October), and then started to decline in November (Martin-Smith, 1994), similar to *S. linearifolium* (Bui, Luu, Fotedar, *et al*., 2017). The experiments lasted from September to December, and the growths of *S. podacanthum* and *S. linearifolium* significantly increased in the first 28 days, and then decreased from late October. In the nutrient enrichment condition, the growth stage of *S. podacanthum* lasted up to 70 days, similar to in the natural environment, which shows the significant effect of nutrients on the growth of *S. podacanthum* as this growth period was much longer than that in the K^+ fortification experiment (Section 5.3.2). This result demonstrated that the seasonal growth cycle of *S. podacanthum* in the laboratory condition was similar to the wild. However, *S. linearifolium* was sustained for 56 days in no or low nutrient enrichment levels, after which the biomass declined, similar to its growth pattern in the K^+ fortification experiment ((Bui, Luu, Fotedar, *et al*., 2017). These results demonstrated the need for NH_4-N:PO_4^{3-}-P enrichment for *S. podacanthum* growth, while *S. linearifolium* grew well in non-enriched waters.

The biomass of *S. podacanthum* was strongly correlated with the water temperature, which is similar to *S. polysystum, S. binderi* and *S. siliquosum* in the natural environment (May-lin & Ching-lee, 2013). The water temperature, with no significant differences among water types, was 20–25ºC, without any controlled mechanism in place. This temperature range reflected similar OW temperatures during this season (https://www.seatemperature.org/australia-pacific/australia/western-australia/, downloaded 23 Dec 2016). This is also a suitable temperature for the maximum growth of *Sargassum* spp. (Hanisak & Samuel, 1987). Although the pH was in a suitable range for seaweed growth (Lignell & Pedersén, 1989), pH was lowest at day 28 in control, and at the 80:8, 120:12 and 200:20 enriched levels in both water types for *S. podacanthum* and in K^+ISW for *S. linearifolium*, which coincided with the occurrence of *Sargassum* spp. mortality. In contrast, *S. podacanthum* grew well in ISW_160, where the pH was relatively stable over time.

The growth rates of *Sargassum* are species-specific (Hanisak & Samuel, 1987). The SGR of *S. horneri* (Turner) C. Agardh in the natural environment is 4.7% d^{-1} from August to December (Gao & Hua, 1997; Yamauchi, 1984). These were similar temperatures, but higher SGRs than found in our trial conditions. *S. baccularia* reaches twice its growth in a NH_4-N:PO_4^{3-}-P ratio of 10:1, in 3–5 µM NH_4-N, whereas the growth rate is reduced when NH_4-N and PO_4^{3-}-P are supplemented beyond these ranges (Schaffelke & Klumpp, 1998). The SGR of *S. baccularia* (Schaffelke &

Klumpp, 1998) is higher than that of *S. podacanthum*, when [NH_4-N] was lower but [PO_4^{3-}-P] was higher than the preferable range for the species, again highlighting the different nutrient requirements among various species of *Sargassum*. The SGR of *S. podacanthum* in OW in the first month, and in ISW_160 in the first 70 days was around 0.3% d^{-1}, which is similar to the SGR of the adult stage of *S. muticum* under natural OW for five months (Yamauchi, 1984), where the PO_4^{3-}-P was lower than this experiment and N (including NO_3^--N and NH_4-N) was similar. Conversely, *S. linearifolium* did not respond to the nutrient enrichment in either OW or K^+ISW, and presented a lower SGR than *S. podacanthum* in OW at all nutrient levels, and in K^+ISW at nutrient enrichment from 80:8 to 200:20 (Table 10-4). Due to the mortality that occurred in *S. linearifolium* cultured in all K^+ISW, and in OW at nutrient levels higher than 80:8, the SGR could not be calculated; therefore, the statistical comparison between SGRs of the two species could not be performed.

In addition to the weekly-supplementation of nutrients, the N and P in water were also produced by the decomposition process of dead *S. podacanthum* in some treatments. The soluble N and P concentrations in water are difficult to stabilise and measure as they are quickly cycled by living microbes (Downing, 1997). N and P have been consumed at different rates (Smith *et al.*, 1986), for example, at the same concentrations, NH_4-N uptake is faster than PO_4^{3-}-P (Wallentinus, 1984). *S. podacanthum* and bacteria in water quickly consumed the provided PO_4^{3-}-P and NH_4-N, resulting in a [PO_4^{3-}-P] level reduced to amounts found in natural OW throughout the trial, particularly in ISW_160, and NH_4-N quickly decreased to negligible levels after enrichment. The [PO_4^{3-}-P] in the cultured media was lowest from day 42 to day 70, also indicating a high biomass, in the form of a standing crop of *S. podacanthum* in both water types. In turn, towards the end of the trial, when a reduction of the *S. podacanthum* biomass was recorded, the PO_4^{3-}-P and NH_4-N supplements were not totally consumed and in turn, resulted in the increase of PO_4^{3-}-P and NH_4-N during this period. A sharp increase in PO_4^{3-}-P and NO_3^--N in ISW_200 towards the end of the trial showed a strong negative correlation with the highest reduction in the *S. podacanthum* biomass. The orthophosphate in Australian OW is 0.001–0.016 mg L^{-1} (Robards *et al*., 1994) and is much lower than the provided PO_4^{3-}-P concentration in water at the beginning of the trial. Similarly, NH_4-N was also highest at the trial commencement, and NH_4-N is the preferred source of N for seaweed over NO_3^--N (Liu *et al*., 2004).

Nitrogen becomes the limiting factor in the ecosystem when the N:P molar ratio is lower than 16:1 (Downing, 1997). The present study confirmed that the main factor for the highest standing biomass of *S. podacanthum* in real-time was limited by N, represented by NO_3^--N and NO_2^--N rather than NH_4-N, as seaweed can consume NO_3^--N instead of NH_4-N when NH_4-N is insufficient (Jie *et al.*, 2008) or is lower than 0.135 mg L^{-1} (Balode *et al.*, 1998). During the present trial, NH_4-N was usually negligible and [NO_3^--N] was always available at 2–4 mg L^{-1}, which met the requirements of *S. podacanthum*. The [NO_3^--N] in water was found to be significantly correlated with the *S. podacanthum* biomass, given that [NO_3^--N] and the biomass of *S. podacanthum* were both unchanged at all nutrient levels in the first half of the trial. The increase of [NO_3^--N] towards the end of the trial resulted in a reduction of *S. podacanthum* biomass in both water types. In ISW_160, [NO_3^--N] was stable over the first 56 days, and then increased, indicating that the *S. podacanthum* biomass decreased after reaching its maximum biomass at day 56.

The enrichment of NH_4-N and PO_4^{3-}-P from 120:12 to 200:20 in OW and 120:12 to 160:16 in K^+ISW in the culture of *S. podacanthum* resulted in a higher growth rate. It was clear from the trial that the nutrient levels lower or higher than the above mentioned concentrations reduced the growth of *S. podacanthum*, particularly in K^+ISW, and also caused mortality from the early stages of the culture period. This result supports the claims that high nutrient levels inhibit the growth of *S. baccularia* (Schaffelke & Klumpp, 1998) and *S. siliquosum* (Diaz-Pulido & McCook, 2005). The present study shows that ISW_160 retained the most suitable water for growing *S. podacanthum* in K^+ISW, when the standing biomass increased until the day 70, the highest among the K^+ISW waters, and the SGR of *S. podacanthum* in ISW_160 in the culture period was the only positive SGR among all nutrient supplementations K^+ISW.

6.5 Conclusions

The nutrient enrichment of 160:16 µM of NH_4-N:PO_4^{3-}-P, using NH_4Cl and NaH_2PO_4, in ISW which was fortified with K^+ at similar K^+ concentration in OW at the same salinity, results in a similar biomass and SGR of *S. podacanthum* cultured in OW. This nutrient level is the suitable water for growing *S. podacanthum* in K^+ISW. However, no nutrient supplementation is needed for *S. linearifolium* to grow in K^+ISW.

CHAPTER 7 [*]EFFECTS OF TEMPERATURE AND pH ON THE GROWTH OF THE *Sargassum linearifolium* AND *S. podacanthum* IN POTASSIUM-FORTIFIED INLAND SALINE WATER

7.1 Introduction

Australia has a significant inland saline water (ISW) resource (Allan *et al*., 2001; Nulsen, 1997; Timms, 2005). The wheat-belt area in Western Australia (WA), covering approximately 18 million hectares is the largest underground source of ISW (Doupé, Lymbery, & Starcevich, 2003; Lymbery *et al*., 2006) that could provide a source of water for inland marine aquaculture (Partridge, 2008). Targeting to the farm sustainability and environmental protection, the land management of nearly 30,000 farms in Australia has changed to prevent the expansion of salinization, 470,000 hectares of land were fenced and 210,000 km of levees, banks, drains for salinity management have been built (Trewin, 2002), providing an available water source for ISW aquaculture. Building onshore farms to culture seaweeds is cheaper than seaweed farms in the open sea (Borowitzka, 1997), as well as contributing to environmental protection by reducing the salinity contamination (Ogburn, 1997).

Sargassum, includes *S. naozhouense* and *S. fusiforme*, have been cultivated in many countries, such as Korea, Japan, and India, for human consumption (Bast, 2014; B. Wang *et al*., 2010). The *Sargassum* have been used commonly in Asia as a source of alginate and medicine for human (Wiltshire *et al*., 2015; Yende *et al*., 2014). For instance, *S. naozhouense* has been used as a source of food and drugs for traditional orientation treatments (Hur *et al*., 2008; J. Wang *et al*., 2010). *Sargassum* also provides a source of sargaquinoic acid, sargachromenol for neurite growth (Hur *et al*., 2008).

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Sargassum can also be used for agriculture as biochemical compounds for soil amendment, cattle food, fertilizer (Ara *et al*., 1997; Huisman, 2000).

Both *S. linearifolium* and *S. podacanthum* can be found in Western Australia including around Perth beaches (Womersley, 1987). In the ocean of South Australia, *S. linearifolium* was trialled in rope-culture which showed the low specific growth rate (Wiltshire *et al*., 2015), specially during summer months, when the temperature is from 28–32^{o}C (Martin-Smith, 1993; Wiltshire *et al*., 2015). However, little is known about the culture potential and the environmental requirements for these two species, particularly the environment around ISW conditions has not been investigated. Both species of *Sargassum* could be ideal species for culture as plenty of available ISW during the winter months, meet the requirements under 28^{o}C of the *S. linearifolium* (Martin-Smith, 1993).

At the same salinity, the ISW ionic profile in Australia is similar to the ocean water (OW) (Fielder *et al*., 2001; Prangnell & Fotedar, 2006a), but the potassium concentration ([K^+]) is lower (Boyd *et al*., 2007; Ingram *et al*., 2002), and varies (Nulsen, 1997; Nurmi *et al*., 1988). Hence, it is not feasible for marine shrimp, fish and mollucs to survive and grow without K^+ fortification, similar to K^+ levels to OW (Dinh, 2016; Doroudi *et al*., 2006; Fielder & Allan, 2003; Prangnell & Fotedar, 2006b). The *S. linearifolium* also needs K^+-fortified at similar K^+ concentration as in OW to sustain its growth in ISW (Bui, Luu, Fotedar, *et al*., 2017). In southwest WA, while the pH of OW is stable from 7.8–8.2, salinity from 35.5–36.5 (Hoang *et al*., 2016), and temperature of 22.0–32.0^{o}C (Martin-Smith, 1993), the pH, salinity and temperature of ISW in the wheat-belt of WA are generally varied by the depth and location of the groundwater (Nulsen, 1997; Nurmi *et al*., 1988; Taukulis & John, 2009). The pH varies from 3.9 to 9.7 in the wheat belt of WA (Nulsen, 1997; Taukulis & John, 2006), or 7.4 at 35 ppt in Broome (Lee, 1997; Taukulis & John, 2006). The pH of ISW is lower and unstable than OW (Lee, 1997). The salinity of inland water in WA varies from 0 to 320 ppt, and two-thirds of those areas has salinity of 5–40 ppt (Mazor & George, 1992), suitable for the growth of seaweed, including *Sargassum* (Hwang *et al*., 2006; Jie *et al*., 2008). The temperature of ISW in WA is from 6.3–28.1^{o}C with an average of 17.69^{o}C (Taukulis & John, 2009). The pH and temperature

are the two environmental factors that strongly influence the growth and heavy metal biosorption of *Sargassum* spp. (Davis *et al*., 2000). In OW, the chlorophyll fluorescence of *S. fusiforme* and *S. fulvellum* varies little over in the pH of 4–10 (Hwang *et al.*, 2015), and the pH 5–10 is suitable for *S. honeri* zygote germination (Ogawa, 1984). Similarly, the temperature is a vital factor affecting *Sargassum* growth (Uchida, 1993). The optimal growth temperature for *S. muticum* is at 25^{o}C (Hales & Fletcher, 1989), while *S. patents* preference is 20–30^{o}C (Endo *et al*., 2013).

ISW in WA is characterized by high changes in pH and temperature influenced by locations and seasons. In order to be use ISW for aquaculture and to reduce the adverse impact of salinization (Kolkovski, 2010), an attempt to grow the *Sargassum* in K^{+}-fortified ISW (K^{+}ISW) has been investigated. This study aims to evaluate the effects of temperature and pH on the growth of *S. linearifolium* and *S. podacanthum* and water quality in K^{+}ISW.

7.2 Materials and Methods

7.2.1 *Sargassum* spp. Collection

Sargassum linearifolium and *S. podacanthum* were hand-picked from Point Peron, WA (latitude 32^{o} 16.3'S, longitude 115^{o} 41.2'E), and then transported withn two hours in containers filled with OW to Curtin Aquatic Research Laboratory (CARL). At CARL, the species were rinsed with OW to remove all surface fouling, sediments, and epiphytic algae. Next, the *Sargassum* were acclimated for three days in aerated OW under indoor laboratory conditions (ambient room temperature, the light provided by plant white fluorescent lights of 90 µmol photon m^{-2} s^{-1} on a 14:10 hours light : dark cycle, one third of OW was exchanged everyday), and then treated according to the procedures of Schaffelke and Klumpp (1998) to clean the thalli followed by (1) discarding all visible macro-epiphytes, (2) wipping with soft tissue, (3) washing in filtered OW and then quickly washed in fresh water, (4) and puting into filtered OW for one day to recover.

The whole *Sargassum* thallus including holdfasts was chosen at the pre-selected weight of about 145g $fond^{-1}$, dried by paper towel, weighed (Model GX-4000, A&D

Company Limited, Tokyo, Japan), and then placed into tanks to get stocking densities of 0.8kg m^{-2}. The *Sargassum* thalli with similar height and weight were selected and their holdfasts were attached to gravel particles to keep them submerged in water.

7.2.2 Preparation of Inland Saline Water

The ISW at a salinity of 45 ppt was procured from a lake in Wannamal, WA (31°15″ S, 116°05″ E) and transported to CARL. The ISW was stored and aged in a reservoir of 10,000L for the duration of the experiment. The ISW was filtered through a 0.5µm glass fibre membrane, then diluted with filtered fresh water to get the 35 ppt water used in this experiment. The $[K^+]$ in ISW was fortified to a level of 100% of the $[K^+]$ in OW by adding potash of sulphate K_2SO_4 to receive cultured media K^+ISW. As the $[K^+]$ in OW and ISW at 35 ppt is 351.1 and 84.4 mg L^{-1} respectively; therefore, 642 mg L^{-1} K_2SO_4 was added into ISW to achieve the desired $[K^+]$ of ISW. The nitric acid HNO_3 was then added to water to reduce the pH to 5.5–6.5, and maintained at this pH level during the whole trial by adding HNO_3 daily at noon. During the experiment, the salinity of K^+ISW was maintained within a range of 34–35 ppt in all the experimental tanks by adding fresh water to compensate for any increases in salinity due to evaporation.

7.2.3 Experimental Setup

The experiments were conducted for 42 days using a total of 24 glass tanks of 54 L (60×30×30 cm), each holding 45 L of K^+ ISW. The treatments included two levels of pH (ambient of about 8 and lower at 5.5–6.5, of which the lower level is the natural acidity of ISW in many places (Partridge, Lymbery, & George, 2008), two water temperatures (ambient room temperature 21–22^{o}C and higher at 26–27^{o}C, which is the upper temperature level of ISW in WA (Taukulis & John, 2006), and two species of *Sargassum* (*S. linearifolium* and *S. podacanthum*). These eight treatments were randomly triplicated (Table 7-1). The tanks were aerated by two airstones in two sides of each tank and exposed to a plant white fluorescent lights of 90 µmol photon m^{-2} s^{-1} on a 14:10 hours light:dark cycle (Hanisak & Samuel, 1987). One submersible

automatic heater (Sonpar. Model: HA-200, Zhongshan, Guangdong, China) was used for a tank to maintain a higher temperature of 26–27^{o}C.

Table 7-1 pH and temperature of waters in eight treatments testing pH and temperature effects on the growth of *Sargassum* spp. in K^{+}ISW

Treatment	Species	pH (*)	Temperature (**)
T1	*S. linearifolium*	7.94 ± 0.01	21.67 ± 0.08
T2	*S. linearifolium*	6.12 ± 0.06	21.54 ± 0.08
T3	*S. linearifolium*	7.93 ± 0.00	26.67 ± 0.09
T4	*S. linearifolium*	6.30 ± 0.03	26.73 ± 0.06
T1	*S. podacanthum*	7.91 ± 0.02	21.73 ± 0.08
T2	*S. podacanthum*	6.04 ± 0.08	21.68 ± 0.12
T3	*S. podacanthum*	7.91 ± 0.04	26.71 ± 0.11
T4	*S. podacanthum*	6.02 ± 0.20	26.73 ± 0.04

(*) – No significant difference of the pH at the same levels (Ambient pH: T1 and T3; Lower pH: T2 and T4) (t-test, P>0.05, N=3)

(**) – No significant difference of the temperature at the same levels (Ambient temperature: T1 and T2; higher temperature: T3 and T4) (t-test, P>0.05, N=3)

7.2.4 Data Collection

Nitrogen (NO_3^--N, NO_2^--N, NH_4-N), and phosphorus (PO_4^{3-}-P) were measured every 14 days, using a Hach DR890 hand-held meter (Hach, Loveland, Colorado, USA). The Cadmium Reduction Method (Method 8171 and Method 8039) was used to measure NO_3^--N at low (0–5 mg L^{-1}) and higher concentrations. The Diazotization Method (Method 8507) was used to measure NO_2^--N at a lower range (0–0.350 mg L^{-1}), and the Ferrous Sulfate Method (method 8153) was used to measure NO_2^--N at a higher range (0–150 mg L^{-1}). The Salicylate Method (Method 8155; Method 10023) was used for NH_4-N at 0–0.05 mg L^{-1} and higher concentrations, and PO_4^{3-}-P was measured by the Amino Acid Method (Method 8178). Method 937.48 from the Official Method of the AOAC (Helrich, 1990) to analyze total Kjeldahl nitrogen

(TKN) in water using a Kjeltec Auto 1030 analyzer (Foss Tecator, Höganäs, Sweden) every 14 days.

Salinity and dissolved oxygen (DO) were recorded daily from 9:00–11:00 using a portable refractometer (RHS-10ATC, Xiamen Ming Xin Instrument, Xiamen, Fujian, China), and a DO meter (YSI model 58, Yellow Springs Instrument Co., Ohio, USA), respectively. The temperature was recorded hourly by data loggers (HOBO Pendant temperature/light Data Logger UA-002-08, UA-002-64). The pH was recorded daily at 9:00–11:00 and 13:00–15:00 using a pH meter (CyberScan pH 300, Eutech Instrument, Singapore). Once a fortnight, the pH and DO variations in a day was collected hourly.

The ionic profile of cultured medium was analyzed using Inductively Coupled Plasma (ICP) spectroscopy at CSBP Soil and Plant Laboratory, Bibra Lake, WA.

The fresh biomass of *Sargassum* spp. was measured every 14 days to calculate specific growth rate (SGR) by collecting the whole thalli in each tank by a small net and then dried by paper towels. The thalli were weighed using a scale (AW220, d=0.1 mg, Shimazu, Japan) and returned to their respective tanks.

The SGR of *Sargassum* was calculated as: $\mu_a=(\ln A_t-\ln A_o)\times 100/t$. Where: μ_a was the SGR (% d^{-1}); A_0 and A_t were the initial and final dried weights (mg) of the *Sargassum* in a fortnight; t=14 (days).

Samples of approximately 10% of the fresh *Sargassum* were dried at 60^{o}C for 72 hours to get stable dried weights. They were then grounded with a mortar and pestle to a fine powder, and stored in a freezer at $-18°C$ until the proximate composition was analyzed. The dried content of *Sargassum* was calculated by the ratio of the dried weight to fresh biomass. The ash content was determined by burning dried *Sargassum* at 550^{o}C for 30 minutes.

Tissue N was determined every 14 days according to the Official Method of the AOAC (Helrich, 1990) (method 937.48) by analyzing N using a Kjeltec Auto 1030 analyzer

(Foss Tecator, Höganäs, Sweden). The percentage of protein over the dried weight was calculated by multiplying the percent of N with a factor of 6.25.

At the commencement and day 28 of the experiment, the ionic composition of the *Sargassum* was analyzed using the prepared freeze fine powder by ICP spectroscopy at CSBP Soil and Plant Laboratory, Bibra Lake, WA. The total N and total C of *Sargassum* were also analyzed at the CSBP Soil and Plant Laboratory, Bibra Lake, WA.

7.2.5 Data Analysis

The SPSS for Windows version 24.0 was used to analyze data. Before applying parametric and non-parametric tests, the data were tested for normality and homoscedasticity as appropriate. Multivariate Analysis of Variance (MANOVA), pair samples t-test and Least Significant Difference post hoc tests were used to determine the significant differences at $P<0.05$ among the means of tested variables. Regresssion correlations were used to find out the significant relationships among variables. The one-way ANCOVA (analysis of covariance) was used to determine the significance difference between the treatments of the water quality parameters on the SGR of the seaweeds.

Percentage data were arcsine-transformed, and the homogeneity of variances confirmed with Cochran's test. Where the numeral data did not have a normal distribution and homogeneous variance, the Kruskal–Wallis (KW test) was used to verify the overall difference of all treatments, and data were transformed by log (x+10) before conducting MANOVA test.

7.3 Results

7.3.1 Biomass of the two *Sargassum* spp.

At the commencement of the experiment, the fresh biomass (approximately 145 g $tank^{-1}$) of the two *Sargassum* spp. was similar among the eight treatments. The pH and temperature significantly ($P<0.05$) affected *Sargassum* biomass in the first 28 days, and the pH and *Sargassum* species significantly ($P<0.05$) interacted at day 28 of the

trial. At the ambient temperature, the lower pH resulted in significantly ($P<0.05$) higher standing biomass of both species than the ambient pH of 7–8. The fresh standing biomass of both species at ambient temperature was significantly greater than at higher temperature over the trial period. The higher temperature resulted in a reduction of *S. podacanthum* and *S. linearifolium* biomass from the first and second week, respectively, followed by the total mortality by day 42. The *S. podacanthum* showed 100% motality in the ambient pH and higher temperatures during the day 14–28 of the experiment, whereas after the day 28, the *S. linearifolium* survived longer than *S podacanthum* at both pH levels. However, none of them could survive after 42 days under higher temperature levels.

The fresh standing biomass of *S. linearifolium* was significantly ($P<0.05$) higher than the *S. podacanthum* as the experiment progressed under ambient pH, and under ambient temperature. The standing biomass of both species was not affected by the higher temperature and lower pH during the second fortnight but was significantly different in the first 14 days.

There was no significant ($P>0.05$) interaction in the three-way interaction among species, pH and temperature on *Sargassum* SGR $F_{(2, 24)}=0.43$ at the first 14 days. Due to the total mortality in some tanks, the three-way ANOVA could not be performed after 28 days. The pH and temperature had significantly ($P<0.05$) interactive effects on the SGR of the Sargassum.

The SGR of the S. linearifolium was significantly ($P<0.05$) higher than the S. podacanthum in the first 14 days; however, due to the mortality at high temperature, the comparison between the two species could not be drawn. Only at the ambient temperature and ambient pH conditions, where the *Sargassum* spp. grew continuously, the *S. linearifolium* presented significantly ($P<0.05$) higher SGR than *S. podacanthum* over the experiment period. The SGRs of the two species were similar in other treatments as the experiment progressed (Table 7-2).

The effects of treatments on SGR of the *Sargassum* were only recorded at the first 14 days. By that time, the SGR of S. *linearifolium* was positive under the ambient temperature, which was significantly ($P<0.05$) higher than under higher temperature.

At ambient temperature, SGR of *S. podacanthum* in lower pH was significantly higher than in ambient pH.

7.3.2 Compositions of the *Sargassum* spp.

The dried weight of *Sargassum* was about 13% of the total fresh biomass at the commencement of the experiment and was similar in both species. The dried weight of *S. linearifolium* was significantly ($P < 0.05$) reduced at both higher temperature and lower pH. The dried weight of S. podacanthum remained unchanged in all treatments (Table 7-2).

The ash content of the S. linearifolium (37.06±0.49%) was significantly ($P<0.05$) lower than S. podacanthum (44.14±0.67%) at the commencement of the trial, but became similar during the rest of the experiment, except at ambient temperature and low pH in the second fortnight (Table 7-2). A significant ($P<0.05$) reduction in ash content over time occurred in all treatments, but to the greatest extent in lower pH and higher temperature. The energy of the Sargassum was approximate 10,356 ± 29.25 J g-1 and remained unchanged over the experiment period.

The protein contents of S. linearifolium and S. podacanthum at the commencement of the trial were similar (8.05±1.01 and 7.74±0.48%, respectively), and then significantly ($P<0.05$) increased as the experiment progressed. The ambient pH resulted in a higher ($P<0.05$) protein than the lower pH in *S. podacanthum,* and high temperature resulted in higher ($P<0.05$) protein in *S. linearifolium* than *S. podacanthum*.

The chemical composition of the *Sargassum* is presented in Table 7-3, of which, after one month of cultivation, the N content increased, the P reduced, and C either remained unchanged or increased. Overall, the C:N:P ratios were higher than at the commencement of the trial. The Cu contents in both *Sargassum* spp. reduced significantly after a month in cultivation, however the Zn was accumulated from the water which resulted in higher Zn concentration in *Sargassum* at day 28 than at the commencement of the trial.

Table 7-2. SGR, dried matter, ash and protein content of the *Sargassum* spp. cultured in K^+ISW at two levels of pH and temperature

Criteria	*S. lineariforlium*				*S. podacanthum*			
	21–22ºC		26–27ºC		21–22ºC		26–27ºC	
	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5
SGR (% d^{-1})								
Day 1-14	1.60±0.08[a]	$_1$1.56±1.09[a]	$_1$-1.26±0.51[b]	-0.10±0.23[ab]	-1.97±1.38[a]	0.37±0.44[b]	-3.52±0.000[a]	-1.51±0.49[ab]
Day 14-28	-0.39±0.59[a]	$_{1,2}$-0.90±1.08[a]	$_2$-4.94±0.38[a]	-4.08±0.37[a]	-9.23±6.39[a]	0.38±1.35[b]		-3.21±2.55[ab]
Day 28-42	-0.22±0.23[a]	$_2$-5.27±3.44[a]			-4.64±0.86[a]	-2.04±1.16[a]		
Dried matter (%)								
Day 1	13.31±0.80	$_1$13.31±0.80	$_1$13.31±0.80	$_1$13.31±0.80	$_{1,2}$12.98±0.19	$_1$12.98±0.19	$_1$12.98±0.19	$_1$12.98±0.19
Day 14	12.68±0.15[a]	$_2$10.33±0.54[b]	$_{1,2}$12.95±0.26[a]	$_{1,2}$11.06±0.52[b]	$_1$11.15±0.23	$_1$11.41±0.72	$_1$11.98±0.00	$_1$11.62±0.56
Day 28	13.23±0.27[a]	$_{1,2}$10.98±0.49[ab]	$_2$11.35±0.10[ab]	$_2$9.09±0.30[b]	$_{1,2}$12.62±1.24	$_1$11.20±1.36		$_1$12.89±2.03
Day 42	14.42±0.47	$_{1,2}$12.75±0.80			$_2$13.27±0.96	$_1$12.72±1.29		
Ash (%)								
Day 1	$_1$37.06±0.49	$_1$37.06±0.49	$_1$37.06±0.49	$_1$37.06±0.49	$_1$44.14±0.67	$_1$44.14±0.67	$_1$44.14±0.67	$_1$44.14±0.67
Day 14	$_2$30.77±0.44	$_2$32.11±0.12	$_2$30.79±0.43	$_1$32.10±3.30	$_2$35.25±2.95	$_2$35.73±0.54	$_2$34.44±0.00	$_2$34.07±1.48
Day 28	$_2$30.68±0.11[a]	$_3$26.98±0.69[bc]	$_2$29.51±1.64[ac]	$_2$24.20±0.98[b]	$_3$31.49±0.29[a]	$_3$32.94±1.22[a]		$_3$27.24±1.55[b]
Day 42	$_{1,2}$33.99±0.29	$_1$35.05±1.78			$_3$31.36±0.69[a]	$_2$38.11±0.83[b]		
Protein (%)								
Day 1	$_1$8.05±1.01	$_1$8.05±1.01	$_1$8.05±1.01	$_1$8.05±1.01	$_1$7.74±0.48	$_1$7.74±0.48	7.74±0.48	7.74±0.48
Day 14	$_2$9.75±0.54	$_2$10.76±0.26	$_2$10.79±0.50	$_{1,2}$9.98±0.16	$_2$10.45±0.28[a]	$_2$9.76±0.16[a]	7.65±0.00[b]	9.49±0.64[a]
Day 28	$_2$10.48±0.18	$_2$10.97±0.20	$_2$11.87±0.47	$_2$11.88±0.61	$_2$12.00±0.22[a]	$_3$11.40±0.71[a]		9.39±0.98[b]
Day 42	$_{1,2}$9.39±0.33	$_1$9.22±0.28			$_2$10.56±0.98[a]	$_{12}$8.48±0.26[b]		

Values (mean±SE) within a row in one species sharing a common superscript are not significantly different (LSD test; P>0.05; n=3). Values (mean±SE) within a column of one parameter sharing a common subscript are not significantly different (LSD test or t-test; P>0.05; n=3).

Table 7-3. The chemical compositions of the *Sargassum* spp. cultured in K^+ISW at two levels of pH and temperature by day 1 and day 28 of the experiment

Parameters	Unit	*S. linearifolium*					*S. podacanthum*			
		Day 1	Day 28				Day 1	Day 28		
			21–22ºC		26–27ºC			21–22ºC		26–27ºC
			pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5		pH 8	pH 5.5–6.5	pH 5.5–6.5
B	mg/kg	172.14	233.92	134.22	176.98	280.00	115.29	207.50	89.90	141.67
Ca	%	1.80	1.93	1.81	2.39	2.50	1.62	2.68	1.89	2.23
C	%	28.60	28.60	29.50	30.10	33.40	26.60	29.90	28.60	27.50
Cu	mg/kg	135.00	13.96	19.32	13.72	38.31	50.55	20.86	21.88	17.59
Fe	mg/kg	80.00	211.18	460.59	153.65	717.50	80.31	178.75	635.29	494.89
Mg	%	1.30	1.31	1.53	1.20	1.54	0.68	1.34	1.22	1.50
Mn	mg/kg	11.54	20.68	9.09	28.03	6.29	7.95	15.24	10.55	6.26
P	%	0.18	0.14	0.11	0.13	0.10	0.14	0.15	0.11	0.08
K	%	9.05	7.38	3.93	5.08	1.07	12.17	6.47	8.05	2.31
Na	%	1.84	2.45	7.15	2.51	3.44	2.39	2.85	3.70	6.69
S	%	1.67	1.66	1.77	1.35	1.87	1.12	1.49	1.61	1.40
Total N	%	1.43	1.48	1.67	1.73	1.86	1.31	1.77	1.59	1.25
Zn	mg/kg	65.00	468.90	392.21	444.83	510.00	29.08	755.13	497.72	370.88
C:N:P		159:8:1	204:11:1	268:15:1	232:13:1	334:19:1	190:9:1	199:12:1	260:14:1	344:16:1

Note: The total mortality of *S. podacanthum* in cultured in K^+ISW water at 26–27ºC, water pH of 8 at day 28 providing no samples for analysis

7.3.3 Water Quality

The ionic composition of water is provided in Table 7-4. After one month of cultivating *Sargassum*, the potassium and sodium ions were different from the beginning, and the heavy metals remained less than 0.05 mg L^{-1} except for Zn in water cultured *S. linearifolium* under low pH and low temperature and in water culture *S. podacanthum* under low pH and high temperature.

The water quality parameters, including NO_3^--N, NO_2^--N, NH_4-N, TKN and PO_4^{3-}-N showed no correlation with SGR of the *Sargassum.* The [NO_3^--N] at the lower pH was about 10–20 times higher than the ambient pH. The [NO_2^--N] increased significantly ($P<0.05$) as the experiment progressed, and the higher temperature resulted in higher nitrite (Table 7-5).

The NH_4-N was negligible in the first month and close to 0.1 mg L^{-1} at the completion of the trial. The TKN significantly ($P<0.05$) decreased at the lower pH. The PO_4^{3-}-P remained unchanged as the time progressed and presented no significant differences in various pH and temperatures; the exception being that in *S. linearifolium* where it was higher in low pH than ambient pH at the same temperature (Table 7-5).

The lower pH significantly ($P<0.05$) resulted in higher NO_3^--N, NO_2^--N TKN, and PO_4^{3-}-P concentrations in water than ambient pH due to the HNO_3 provided. However, no water quality parameter shown a significant effects on the SGR of the *Sargassum* (Table 7-6). The one-way ANCOVA results reproved the no significant ($P>0.05$) effect of NO_3^--N or NO_2^--N on the SGR of the seaweeds between two pH groups, neither nor among eight treatments (Table 7-7). The temperature presented no effect on these water quality parameters.

Table 7-4. Ionic profile (mg L^{-1}) of the K^+ISW cultured *Sargassum* spp. at two levels of pH and temperature by day 1 and day 28

Para-meters	Day 1	Day 28							
		S. lineariforlium				*S. podacanthum*			
		21–22ºC		26–27ºC		21–22ºC		26–27ºC	
		pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5
B	0.68	0.76	0.72	0.86	0.86	0.77	0.67	0.81	0.84
Ca	584	554	520	576	606	570	474	536	618
Cu	<0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Fe	<0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Mg	1565	1384	1304	1526	1540	1492	1229	1464	1680
Mn	<0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
P	0.07	< 0.05	< 0.05	0.09	0.11	< 0.05	< 0.05	< 0.05	0.05
K	353.3	351	361	369	366	359	347	364	359
Na	8574	7886	7282	8591	8838	7251	7141	8308	9452
S	769	763	716	824	818	765	791	780	816
Zn	<0.05	< 0.05	0.12	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.24

Table 7-5. Nitrogen metabolites and phostphates (mg L^{-1}) in K^+ISW cultured *Sargassum* spp. at two levels of pH and temperature

Para-meters	*S. lineariforlium*				*S. podacanthum*			
	21–22°C		26–27°C		21–22°C		26–27°C	
	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5
NO_2^--N								
Day 1	$_1$0.020±0.002^a	$_1$0.150±0.021^b	$_1$0.020±0.002^a	$_{12}$0.150±0.021^b	$_1$0.020±0.002^a	$_1$0.150±0.021^b	$_1$0.020±0.002^a	$_1$0.150±0.021^b
Day 14	$_2$0.005±0.002^a	$_1$0.018±0.005^{ab}	$_1$0.026±0.007^{ab}	$_1$0.055±0.015^b	$_1$0.016±0.003^a	$_1$0.013±0.004^a	$_2$0.081±0.026^b	$_1$0.115±0.025^b
Day 28	$_2$0.002±0.000^a	$_1$0.317±0.030^b	$_2$0.102±0.033^{ac}	$_2$0.166±0.073^c	$_1$0.015±0.003^a	$_1$0.184±0.098^b	$_3$0.375±0.000^c	$_2$0.375±0.000^c
Day 42	$_3$0.013±0.003^a	$_2$5.333±0.667^b			$_2$0.008±0.002^a	$_2$5.333±0.667^b		
NO_3^--N								
Day 1	$_1$2.53±0.12^a	27.67±0.44^b	$_1$2.53±0.12^a	27.67±0.44^b	$_1$2.53±0.12^a	$_1$27.67±0.44^b	$_1$2.53±0.12^a	27.67±0.44^b
Day 14	$_2$1.03±0.24^a	27.87±0.37^b	$_2$1.07±0.09^a	33.13±0.95^b	$_2$1.43±0.12^a	$_{12}$25.37±1.23^b	$_1$2.40±0.38^a	19.47±8.00^b
Day 28	$_3$0.27±0.18^a	28.53±2.47^b	$_1$2.40±0.15^c	25.17±1.13^b	$_2$1.07±0.12^a	$_2$23.47±1.05^b	$_2$3.47±0.55^c	25.67±0.35^b
Day 42	$_1$2.30±0.25^a	25.40±0.42^b			$_1$2.53±0.18^a	$_2$22.27±1.22^b		
NH_4-N								
Day 1	$_1$0.01±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.01±0.00	$_1$0.00±0.00	$_1$0.01±0.00	$_1$0.00±0.00
Day 14	$_1$0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	$_1$0.00±0.00	$_1$0.01±0.01	$_{12}$0.02±0.02
Day 28	$_2$0.04±0.03	0.00±0.00	0.02±0.02	0.01±0.01	0.00±0.00^a	$_2$0.11±0.03^b	0.$_2$08±0.00^{bc}	$_2$0.04±0.02^{ac}
Day 42	$_{12}$0.01±0.01^a	0.00±0.00^b			0.00±0.00^a	$_3$0.05±0.03^b		

Para-meters	*S. lineariforlium*				*S. podacanthum*			
	21–22°C		**26–27°C**		**21–22°C**		**26–27°C**	
	pH 8	**pH 5.5–6.5**	**pH 8**	**pH 5.5–6.5**	**pH 8**	**pH 5.5–6.5**	**pH 8**	**pH 5.5–6.5**
TKN								
Day 1	1.14 ± 0.16^{a}	$_{1}10.48\pm0.06^{b}$	$_{1}1.14\pm0.16^{a}$	$_{1}10.48\pm0.06^{b}$	1.14 ± 0.16^{a}	$_{1}10.48\pm0.06^{b}$	$_{1}1.14\pm0.16^{a}$	$_{1}10.48\pm0.06^{b}$
Day 14	1.40 ± 0.41^{a}	$_{1}9.85\pm0.12^{b}$	$_{12}1.42\pm0.21^{a}$	$_{1}8.68\pm1.28^{b}$	1.66 ± 0.17^{a}	$_{1}9.97\pm0.71^{b}$	$_{12}1.73\pm0.18^{a}$	$_{1}9.34\pm1.03^{b}$
Day 28	1.61 ± 0.35^{a}	$_{2}2.17\pm0.11^{ab}$	$_{2}1.77\pm0.20^{a}$	$_{2}3.67\pm1.22^{b}$	1.56 ± 0.45^{a}	$_{2}1.70\pm0.17^{a}$	$_{2}2.45^{a}$	$_{2}2.10\pm0.18^{a}$
Day 42	1.77 ± 0.19^{a}	$_{2}2.19\pm0.06^{a}$			1.91 ± 0.05^{a}	$_{2}1.77\pm0.25^{a}$		
PO_4^{3-}-P								
Day 1	$_{1}0.93\pm0.09$	$_{1}1.13\pm0.03$	0.93 ± 0.09	1.13 ± 0.03	$_{1}0.93\pm0.09$	1.13 ± 0.03	$_{1}0.93\pm0.09$	1.13 ± 0.03
Day 14	$_{2}0.30\pm0.06^{a}$	$_{1}0.67\pm0.12^{a}$	0.60 ± 0.26^{ab}	3.17 ± 2.45^{b}	$_{2}0.37\pm0.03$	0.83 ± 0.30	$_{2}1.57\pm0.29$	0.87 ± 0.18
Day 28	$_{1}1.00\pm0.06^{ac}$	$_{2}2.50\pm0.52^{b}$	0.93 ± 0.20^{a}	1.60 ± 0.29^{c}	$_{3}1.30\pm0.06$	1.00 ± 0.00	$_{12}1.13\pm0.07$	1.57 ± 0.03
Day 42	$_{1}0.93\pm0.19$	$_{1}0.73\pm0.03$			$_{1}0.83\pm0.07$	1.00 ± 0.06		

Values (mean±SE) within a row in one species sharing a common superscript are not significantly different (LSD test; P>0.05; n=3). Values (mean±SE) within a column sharing a common subscript are not significantly different (LSD test or t-test; P>0.05; n=3). (Data was transformed to log (x+10) before conducting ANOVA test)

Table 7-6. Pearson correlation of SGR (% d^{-1}) of the *Sargassum* spp. cultured in K^+ISW at two levels of pH and temperature, and water quality parameters

Criteria	NO_2^--N	NO_3^--N	PO_4^{-3}-P	NH_4-N	TKN
Pearson correlation	-0.120	0.145	-0.027	-0.052	0.235
Significant (2-tailed)	0.387	0.296	0.847	0.710	0.085
N	54	54	54	53	55

Table 7-7. The effect of nitrogen on the SGR of the *Sargassum* spp. cultured in K^+ISW between the two pH levels and among the eight treatments

Group	Source	Type III Sum of Squares	df	Mean Square	F	Signi-ficant	Partial Eta Squared
Two pH levels	NO_3^--N	0.00008	1	0.00008	0.050	0.825	0.001
	NO_2^--N	0.00300	1	0.00300	1.746	0.192	0.033
Eight treatments	NO_3^--N	0.00100	1	0.00100	0.293	0.591	0.006
	NO_2^--N	0.00500	1	0.00500	3.676	0.062	0.076

At high temperatures, the DO gradually increased from early afternoon to noon of the next day; whereas, at ambient temperature, DO was reduced at night and rose in the morning. During a day, the pH was normally increased in the morning, reached a peak at noon and decreased in the afternoon, lowest by 5.30 PM.

7.4 Discussion

Temperature and pH strongly influence the growth of *Sargassum* (Chen & Zou, 2014; Choi *et al*., 2009; Hwang *et al.*, 2015). The *Sargassum* growth rate is strongly affected by the variation of temperature (Endo *et al*., 2013; Uchida, 1993), and the effect of temperature within the tested range was stronger than the pH, shown by the significantly different SGR of *Sargassum* at different temperature levels. The temperature affects many aspects of the growth of seaweeds, such as the photosynthetic activity (Ding *et al*., 2013), ammonium and nitrogen uptake rate (Duke *et al.*, 1989; Hwang *et al*., 2004). The range of studied temperature was within a preferred range of 20–30ºC for *S. patens*, resulting in higher SGR (Endo *et al*., 2013). In the open sea, the *S. linearifolium* maximum biomass increases in May, when

temperature is about 22–24ºC, and reaches maximum wet weight and length in August to November when the temperature ranges from 24–28ºC and ceases in summer when temperature reaches over 29ºC (Martin-Smith, 1993). The temperature window in this experiment at 20–22ºC, given the higher growth rate of *Sargassum* than the higher temperature of 26–27ºC, is similar to the natural maximal growth rate condition. Both *Sargassum* species could not be sustained after a month at a high temperature of 26–27ºC in ISW. Similarly, the growth of young seedlings *S. henslowianum* reduced when temperature increased to 30ºC (Chen & Zou, 2014). The SGR of the *Sargassum* in this trial, were at adult stages, at 20–22ºC is higher than the adult stage of *S. muticum* (Yamauchi, 1984) but is lower than the juvenile *S. horneri* (Choi *et al*., 2009) and juvenile *S. muticum* (Hales & Fletcher, 1989) at 15ºC, presented the lower SGR of adults thalli and juvenile, which is similar to *S. horneri* (Choi *et al*., 2009). This implies a limitation of this study to lower temperatures, where more than 60% of WA inland saline ground water has the temperature lower than 20ºC (Taukulis & John, 2009).

The lack of changes in the dried weight, ash, and protein of the *S. linearifolium* as the trial progressed in the ambient temperature associating with the higher SGR than at higher temperature indicates the ambient room temperature 20–22ºC is preferred for the growth of *S. linearifolium* than a higher temperature. The dried weight and crude protein of *Sargassum* in this trial is similar with *Sargassum* spp. from Casas-Valdez *et al.* (2006) (89% and 8%, respectively), but protein is lower than *S. naozhouense* (11.2%) (Peng *et al*., 2013). Although the pH and temperature do not affect the protein of the *S. linearifolium*, the effect on protein is similar to *Porphyra* (Kim *et al.*, 2007), the *S. podacanthum* reduced protein shown a negatively affect by the high temperature and low pH. The protein level of the *Sargassum* increases significantly as the trial progressed. This indicates the protein of *Sargassum* under the laboratory conditions is better than in the wild, although no independent supplementary nutrients were provided.

Seaweed culture in ISW is expecting to be a potential means in the attempt to reduce the adverse effect of ISW in the agricultural farms (Borowitzka, 1997). The K^+ deficiency is in common in Australia and USA (Ingram *et al*., 2002) although the ionic profile of ISW can be similar to OW at the same salinity (Boyd & Thunjai, 2003;

Fielder *et al*., 2001). Therefore, ISW should be fortified with K^+ at similar or about 33–66% of the K^+ concentration in OW at the same salinity for proper growth of *S. linearifolium* and *L. catenata*, respectively (Bui, Luu, & Fotedar, 2017; Bui, Luu, Fotedar, *et al*., 2017). The K^+ plays a major role in the growth of algae and cannot be substituted by any other ion (Yarish *et al*., 1980). The K^+ is important in the photosynthesis of the marine diatom (Overnell, 1975), and higher plants through the mechanism of enzyme activation in protein synthesis (Checchetto *et al*., 2013). The low range of pH changes (within 0.5) do not affect the K^+ movement within cells (Tromballa, 1978); however, the two different pH levels at 8.0 and 6.0 may cause the differential movement of K^+, which in turn can affect the growth of *Sargassum*. As the K^+ movement at pH 10.0 is slower than at pH 6.5 (Tromballa, 1978), it is expected that in this trial, at the pH 6.0, the K^+ movement from the medium to the cell is faster than in the ambient pH of 8.0. This movement supports the photosynthesis of the *Sargassum*. In addition, the pH affects seaweed photosynthesis through the appearance of CO_2 or HCO_3. At low pH where a higher concentration of CO_2 is available, the affinity for inorganic carbon is greater than at high pH (Aizawa & Miyachi, 1986; Drechsler & Beer, 1991), which is proved by *Ulva rigida* thalli photosynthesis rate (Björk *et al*., 1992). Thus, providing a higher biomass of *Sargassum* at low pH than the ambient pH in a short-term. Under the pH and temperature effect, the biomass of the *Sagassum* has varied significantly as time progressed.

The SGR of *S. linearifolium* in the ambient pH and ambient temperature of this study was much lower than *S. linearifolium* (Bui, Luu, Fotedar, *et al*., 2017), although the environmental conditions and growing season (during different years) were similar showing the different growth feasibility of whole thalli (this study) and small piece (Bui, Luu, Fotedar, *et al*., 2017).

The ambient pH of 7.0–8.0 is suitable pH for long-term growth of *Sargassum* in ISW than the lower ones. This pH range is similar to the red seaweed *Gracilaria tikvahiae*, *G. secundata* and *G. manilaensis* needs for high production and maximum growth rate (Hidayat *et al*., 2015; Lignell & Pedersén, 1989; Skirrow, 1975). Their maximal growth rate is 1.3% d^{-1} (Hidayat *et al*., 2015), lower than *S. linearifolium* but higher than *S. podacanthum* at the ambient pH in this trial. The *S. linearifolium* biomass does

not significantly respond to the pH variation in the first month, but *S. podacanthum* biomass reduction rate was significantly slower in low pH than in ambient pH. The *S. linearifolium* showed a higher SGR than *S. podacanthum* at both pH levels, suggesting *S. linearifolium* is a potential pH adaptation species in a culture where the pH variation is wide. The pH also affects the ionic absorption by seaweed (Basha & Murthy, 2007) which peaks at pH 4.5 (Davis *et al*., 2000; Figueira *et al*., 1997). The *Sargassum* accumulated iron and zinc, particularly at low pH, but released the copper to the environment when copper in water is lower than 0.05 mg L^{-1}, which is a possible explanation for lower copper concentration in the *Sargassum* tissues at the day 28 than the commencement. It is a role as a biosorbent of *Sargassum* in terms of environmental protection from the heavy metal pollution (Davis *et al.*, 2003; Vijayaraghavan *et al.*, 2009).

Hydrochloric acid HCl was used in the preliminary experiment, however, it proved to be strong and reduced the water pH quickly and could not stabilize the pH. On the other hand, acetic acid CH_3COOH was too weak. Therefore, instead of HCl and CH_3COOH, HNO_3 was used to reduce pH which potentially could result in higher NO_3^--N and NO_2^--N concentrations than under the ambient pH treatments. However using statistical analysis, addition of HNO_3 has neither influence SGR of *Sargassum* spp. nor it influence the significant level pH and temperature on the SGR of *Sargassum* spp. Both the Pearson correlation and one-way ANCOVA presented no significant effect of NO_3^--N or NO_2^--N concentrations on the SGR of the *Sargassum* spp. Therefore using HNO_3 did not affect the outcomes of the experiment. The [NO_3^--N] was sufficient for *Sargassum* under the both low and ambient pH treatments, as *Sargassum* consumes NO_3^--N when NH_4-N is not available (Jie *et al*., 2008). As the N:P ratios under the low pH regime were much higher than the N:P ratios in the ambient pH, the nutrient consumption of the *Sargassum* was affected when the N:P ratio was high, resulting in higher biomass of the *Sargassum* in very short term.

The N:P ratio of the *Sargassum* in this study was much lower than the N:P of *S. echinocarpum* (Larned, 1998) and much lower than the C:N:P ratio for Australian *Sargassum* (Atkinson & Smith, 1983). The reason is the P content of *Sargassum* in this study was much higher whereas the C and N contents were similar. These can be

explained by the N:P in this study was lower than 30:1, the *Sargassum* growth is N-limited (Harrison & Hurd, 2001), and the surplus P was stored in *Sargassum* tissue.

7.5 Conclusions

The *S. linearifolium* and *S. podacanthum* grow faster in K^+ISW 35 ppt at pH 7.0–8.2 and temperature 20–22^oC than in lower pH and higher temperature, which are suitable for the growing season of *Sargassum* in the early summer, and the availability of ISW after the rainy season. The low pH negatively affects the growth of *Sargassum* and significantly affects the water quality and the chemical composition of *Sargassum*. Only *S. linearifolium* can grow in either low pH (5.5–6.5) or at the temperature of 26–27^oC in K^+ISW up to 28 days. A further study about the higher than the ambient pH 8 and temperature of 15^oC of K^+ISW effects on the growth feasibility of *Sargassum* spp. is recommended.

RED SEAWEED

CHAPTER 8 [*]THE CULTIVATION FEASIBILITY OF *Lomentaria catenata* UNDER LABORATORY CONDITIONS

8.1 Introduction

Of 5,000 red seaweed Rhodophyta species, 1,300 species are found in Australian waters (Huisman, 2000). Rhodymeniales, which contains three families and 38 genera, 17 genera have been recorded in Australia, of which three species of *Lomentaria* genus have been identified in Southern Australia, including *L. australis, L. pyramidalis, L. monochlamypdea* (Womersley, 1996). The *Lomentaria* thallus "erect or forming entangled clumps, much branched, with or without percurrent axes, branches terete or compressed, hollow, basally constricted with solid septa; holdfast discoid or hapteroid. Structure multiaxial, with a cluster of apical cells developing an inner cortex 2–3 cells thick and an outer cortex of small cells sometimes forming rosettes" (p. 34) with a life cycle of isomorphic gametophytes and tetrasporophytes (Womersley, 1996). The red seaweed can be used as a source of food, to extract agar, and producing fertilizers (Huisman, 2000). The *L. catenata* is distributed at New South Wales (Pushpamali *et al*., 2008), and this study is the first record in Western Australia. Little is known about the benefit of *L. catenata*, and there has been no record on growing *L. catenata* either in ocean water (OW) and or in inland saline water (ISW).

In Australia, ISW is available in natural rivers, lakes and aquifers (Nulsen, 1997). About 2.2 and 5.7 million hectares of land was salt-affected in 1996 and 2000, respectively (Nulsen, 1997; Timms, 2005), which is expected to be 17 million hectares in 2050 (Timms, 2005). Agricultural land, wildlife habitats and native vegetation are adversely affected due to ISW areas rising (Allan *et al*., 2008). ISW is available in reserves of underground water (Nulsen, 1997), which could provide a source of water for inland marine aquaculture (Partridge, 2008).

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Potassium (K^+) is crucial for algal growth (Talling, 2010), and it shares 1–2% of dry plant biomass (Evans & Sorger, 1966). K^+ is an important internal cation in algae (Kirst, 1977), and in the red algae *Chondrus crispus* and *Porphyra tenera*, it comprises 37 and 43%, respectively, of total internal cations (Rupérez, 2002). K^+ plays an important role in photosynthesis and respiration of the plant (Checchetto *et al*., 2013). With the presence of calcium, [K^+] of 230–350 mg L^{-1} at 15 ppt is suitable for the red seaweed *Caloglossa leprieurii* (Montagne) J. Agardh growth, but another red seaweed, *Bostrychia radicans* Montagne, prefers higher [K^+] at 400–500 mg L^{-1} at 25 ppt (Yarish *et al*., 1980). K^+ fortification for ISW to sustain the growth of marine species is needed (Dinh, 2016; Fielder & Allan, 2003; Mourad *et al*., 2012; Tantulo & Fotedar, 2006) when K^+-deficient ISW is common in Australia (Dinh & Fotedar, 2016; Prangnell & Fotedar, 2005; Tantulo & Fotedar, 2007). Studies on the effects of K^+ is important to determine the requirement of [K^+] for seaweed growth.

Ammonium (NH_4-N), the most common type of ammonia (NH_3) in OW (Burgess *et al*., 2003), and phosphate (PO_4^{3-}-P) are the preferred source of nitrogen (N) and phosphorus (P) for seaweed growth (Campbell, 2001; Kim *et al*., 2007; Ramus & Venable, 1987; Schaffelke & Klumpp, 1998). However, N and P in water do not always meet the algal demand (Robards *et al*., 1994). For higher seaweed growth, supplying NH_4-N is more efficient than nitrate (NO_3^--N) (Atkinson & Smith, 1983). In addition, the combination of NH_4-N and PO_4^{3-}-P have a positive effect on the growth of *Sargassum baccularia* than either NH_4-N or PO_4^{3-}-P alone (Schaffelke & Klumpp, 1998). As it is the first study on cultivating *L. catenata*, it is necessary to identify the needs of NH_4-N and PO_4^{3-}-P for optimal *L. catenata* growth.

Temperature strongly affects the growth of algae (Uchida, 1993). The temperature of ISW in Western Australia (WA) is approximately 18ºC, and varies around 6.3–28.1ºC (Taukulis & John, 2009). These temperatures levels are suitable for the growth of many red seaweeds. *Hypnea cervicornis* and *Gracilaria tikvahiae* prefer 20–25ºC for optimal growth (Bird *et al*., 1978; Ding *et al*., 2013), when *Hypnea musciformis* and *Gracilaria cornea* grow well in the Florida Keys at 15–25ºC (Dawes *et al.*, 1998; de Faveri *et al.*, 2015). At 15ºC, *Chondrus crispus* and *Furcellaria lumbricalis* reach their maximum growths (Bird *et al*., 1978). However, at temperature exceeding 30ºC, an

inferior growth of *Hypnea cervicornis* and *H. musciformis* is recorded (de Faveri *et al*., 2015; Ding *et al*., 2013).

Studies on seaweed culture in ISW in Australian is limited to *Gracilaria cliftonii* Withell, Miller and Kraft (Cordover, 2007; Kumar *et al.*, 2010), and *Sargassum linearifolium* (Bui, Luu, Fotedar, *et al*., 2017) even though there are abundant studies about seaweed growth, chemical and nutrient uptakes worldwide (Ahmad *et al*., 2011; Coutinho & Zingmark, 1993; Cruz-Suárez *et al*., 2010; Pérez-Mayorga *et al*., 2011; Perini & Bracken, 2014; Reef *et al*., 2012; Schaffelke & Klumpp, 1998). This study is the first attempt to grow *L. catenata* in the laboratory, testing the growth feasibility of *L. catenata* in OW and ISW, at different K^+ concentrations, nutrient and temperature levels, targeting on consuming the available ISW source to reduce adverse impacts of ISW on environment and agriculture.

8.2 Materials and Methods

8.2.1 Seaweed Collection

L. catenata was collected at Matilda Bay, Swan River, Western Australia (WA) (latitude 31º 97.9S, longitude 115º 82.2E). This species is currently identified by WA Herbarium as *L. catenata* Harvey 1857, a basionym of *Fushitsugiana catenata* (Guiry & Guiry, 2018). The *L. catenata* was transported in tanks holding ambient river salty water to Curtin Aquatic Research Laboratory (CARL) immediately after collection. The *L. catenata* were thoroughly cleaned in OW 30 ppt to remove all epibiotics.

Before using in experiments, the *L. catenata* was acclimated for one day in aerated OW 30 ppt at 22ºC in 114 L aquaria, under a downwelling photo-lux density of 120 µmol photon m^{-2} s^{-1} and a 14:10 h light:dark cycle (Hanisak & Samuel, 1987).

8.2.2 Experimental Setup

ISW had a salinity of 45 ppt and was procured from a lake at Wannamal, WA (31°15S, 116°05E). OW had a salinity of 35 ppt was procured at Hillary Habour (31º.83S, 115º.74E). They were both brought to CARL, and were stored and aged in separate 10,000 L reservoirs. All waters were filtered through a 0.5 µm glass fibre membrane

before being used in the experiments. OW and ISW were then diluted with filtered fresh water to achieve needed waters at 30 ppt.

A series of four experiments were conducted in order to determine suitable [K^+] levels for growing *L. catenata* in ISW, the growth feasibility of *L. catenata* in NH_4-N enriched water at suitable K^+-fortified ISW (K^+ISW), the effects of temperature on the growth of *L. catenata* in the NH_4-N enriched OW and suitable K^+ISW, and the effects of NH_4-N and PO_4^{3-}-P enrichment on the growth of *L. catenata* in OW and suitable K^+ISW.

Water salinity in all experiments was maintained at 30–31 ppt, similar to the salinity of Swan River where the *L. catenata* was collected, by adding filtered fresh water to compensate for evaporation. The tanks were exposed to light at 90 µmol photon m^{-2} s^{-1} on the surface and 22.5 µmol photon m^{-2} s^{-1} at the bottom.

Automatic heaters (Sonpar, HA-200, Zhongshan, Guangdong, China) were used to maintain temperatures levels at 25–26ºC or 21–22ºC.

8.2.2.1 *L. catenata* Growth in Potassium-fortified Inland Saline Water

A total of 20 glass beakers, with a capacity of 1.5 L, holding 1 L culture medium were used for 70 days in late winter. The experiment determined the growth rate of *L. catenata* in four replicates at three levels of K^+ISW with two controls of OW and ISW at 30 ppt in ambient room temperature. KCl was used to fortify ISW to approximately 100%, 66%, and 33% (termed as ISW100, ISW66, and ISW33, respectively) of [K^+] in OW at 30 ppt salinity. [K^+] at 30 ppt in OW and ISW was 313 and 77 mg L^{-1}, respectively. Therefore, 451, 248 and 50 mg L^{-1} of KCl were used to fortify ISW 30 ppt to achieve ISW100, ISW66, ISW33, respectively.

8.2.2.2 Effect of Ammonium Enrichment on the Growth of *L. catenata*

L. catenata was cultured in 24 glass tanks, including six treatments in four replicates, in 28 days in autumn. The six treatments were OW, ISW, ISW66 (ISW was fortified with K^+ by KCl at 66% of the [K^+] in OW at 30 ppt) as controls, and the 100 µM NH_4-

N weekly enriched OW, ISW, ISW66 waters by NH_4Cl termed as OW_NH_4, ISW_NH_4, and $ISW66\_NH_4$. Approximately 180 g of *L. catenata* was cultured in one tank holding 45 L water with aeration provided, in room temperature of 18–20°C.

The water salinity was maintained at 30–32 ppt by adding filtered fresh water for compensation of water evaporation.

8.2.2.3 Effects of Temperature on *L. catenata* Growth

The effects of temperature and NH_4-N enrichment on the growth of *L. catenata* were determined in two experiments.

The first experiment was conducted over four weeks in spring. Approximately 180 g $tank^{-1}$ of *L. catenata* was placed in a 45 L cultured medium. Total 24 tanks were used, combining of six treatments (two water types and three temperature levels) in four replicate, aeration provided. Three temperature regimes were 25–26°C, 21–22°C and 18–19°C. The two water types at salinity 30 ppt were used, including OW, and OW enriched with NH_4-N 100 µM by NH_4Cl, termed OW_NH_4.

The second experiment was conducted in 45 days in early summer. Total 24 beakers, included six treatments in four replication, were placed under two temperature regimes (25–26°C and 21–22°C) (which was achieved from the first temperature experiment). Three water types at salinity 30 ppt included OW, OW_NH_4, and ISW that was fortified with K^+ by KCl at 66% of the $[K^+]$ in OW at 30 ppt, termed as $ISW66\_NH_4$. The last two waters were weekly enriched with NH_4-N 100 µM by NH_4Cl. The *L. catenata* was selected by whole fond weight of approximately 3.5 g L^{-1}, cultivated in 1.5 L beakers holding 1 L of cultured medium and the beakers were placed in tank holding water. An automatic heater (Sonpar, HA-200, Zhongshan, Guangdong, China) was used in each tank to maintain the temperature.

8.2.2.4 Effects of Ammonium and Phosphate Enrichment on the Growth of *L. catenata* in Potassium-fortified Inland Saline Water

A total of 24 1.5 L beakers were used for eight treatments in three replicates for the experiment in 28 days in early summer. *L. catenata* was cultured at a density of 3.5 g L^{-1}. The beakers were placed randomly into tanks filled with water. One automatic

heater (Sonpar, HA-200, Zhongshan, Guangdong, China) and a pump (Grant Model GD 120, England) were used in each tank to maintain water temperature at 25–26ºC. The water salinity was kept constant at 30–31 ppt by adding filtered fresh water to compensate for evaporation.

Three levels of NH_4-N:PO_4^{3-}-P at ratio of 10:1 were supplied weekly for OW and ISW66, which was ISW fortified with K^+ by KCl at 66% of the [K^+] in OW at 30 ppt, by NH_4Cl and Na_2HPO_4 at 75:7.5, 150:15 and 300:30 µM termed as T2, T3, and T4, with the control treatment T1 was ambient OW and ISW66 at 30ppt.

8.2.3 Data Collection

8.2.3.1 Water Quality

The NH_4-N, NO_3^--N, NO_2^--N and PO_4^{3-}-P concentrations in water were determined fortnightly using a Hach DR890 hand-held meter (Hach, Loveland, Colorado, USA). The Cadmium Reduction Method (Method 8171 and Method 8039) was used to measure NO_3^--N. The Diazotization Method (Method 8507) and the Ferrous Sulfate Method (method 8153) were used to measure NO_2^--N. The Salicylate Method (Method 8155; Method 10023) was used for NH_4-N, and the Amino Acid Method (Method 8178) was used to measure PO_4^{3-}-P. Total Kjeldahl Nitrogen (TKN) in water was also determined every 14 days according to the Official Method of the AOAC (Helrich, 1990) (method 937.48) by analyzing N using a Kjeltec Auto 1030 analyzer (Foss Tecator, Höganäs, Sweden).

The pH and salinity were recorded daily at 9–11AM using a pH meter (CyberScan pH 300, Eutech Instrument, Singapore), and a portable refractometer (RHS-10ATC, Xiamen Ming Xin Instrument, Xiamen, Fujian, China), respectively.

Temperature was recorded hourly by data loggers (HOBO Pendant temperature/light Data Logger UA-002-08, UA-002-64).

8.2.3.2 *L. catenata* Growth

The weight of *L. catenata* was determined fortnightly, and at the termination of the experiment. All thalli were removed from the culture beakers/tanks by a small net and then dried using soft hand towels (Ahmad *et al*., 2011). The thalli were immediately transferred to a weighing scale (AW220, d=0.1 mg, Shimazu, Japan).

The specific growth rates (SGR) were calculated as: $\mu_a = (\ln A_t - \ln A_o) \times 100/t$.

Where: μ_a is the SGR of seaweed (% d^{-1}); A_t and A_0 are the weight (mg) or length (mm) at the current time (t, day), and the commencement of the experiment (0, day); t is the current time of the trial (days).

8.2.4 Data Analysis

All data were analysed using SPSS for Windows version 24.0. Data were tested for normality and homoscedasticity before applying parametric and non-parametric tests as appropriate. Analysis of variance (ANOVA), paired sample *t*-tests and least significant difference (LSD) post hoc tests were used to determine significant differences at $P<0.05$ among the means of variables (Mean±SE). Correlations were used to find out the significant relationships among variables. Where the data did not have normal distribution and homogeneous variance, the Kruskal-Wallis (KW) test was used to test the overall difference in all treatments. In the case of significant treatment effects, a Mann-Whitney test was applied to analyse the significant differences among the means of all variables.

8.3 Results

8.3.1 *L. catenata* Growth in Potassium-fortified Inland Saline Water

8.3.1.1 Biomass of *L. catenata*

L. catenata biomass remained unchanged in the first 56 days of the culture period, and a significant ($P<0.05$) reduction of the biomass was recorded in the last 14 days in OW, ISW and ISW100. Only ISW33 and ISW66 resulted in a significant ($P<0.05$) increase in the biomass during the culture period, by day 42 and day 14–42, respectively. After that, the biomass reduced quickly ($P<0.05$). ISW66 also resulted in the highest ($P<0.05$) biomass at day 28 among the five treatments (Table 8-1). On average, ISW66 resulted in higher biomass growth than other waters in the first 56 days.

Table 8-1. The biomass (g) of *L. catenata* in K^+ISW

Time	OW	ISW	ISW33	ISW66	ISW100
Day 1	$_{12}$3.30±0.62	$_{12}$3.32±0.40	$_{1}$3.30±0.47	$_{1}$3.31±0.65	$_{123}$3.28±0.58
Day 14	$_{1}$4.03±0.41^{ab}	$_{12}$3.56±0.15^{a}	$_{1}$3.70±0.12^{a}	$_{2}$4.47±1.88^{b}	$_{2}$3.97±0.30^{ab}
Day 28	$_{1}$3.51±0.23^{a}	$_{12}$3.63±0.12^{a}	$_{1}$3.84±0.25^{ab}	$_{2}$4.26±0.17^{b}	$_{123}$3.29±0.23^{a}
Day 42	$_{1}$3.83±0.39	$_{1}$3.91±0.28	$_{2}$4.51±0.28	$_{2}$4.49±0.35	$_{2}$3.75±0.27
Day 56	$_{1}$3.47±0.32^{ab}	$_{2}$3.01±0.42^{a}	$_{1}$3.79±0.24^{ab}	$_{12}$3.94±0.20^{b}	$_{2}$3.58±0.29^{ab}
Day 70	$_{2}$2.33±0.61^{ab}	$_{3}$1.57±0.36^{a}	$_{3}$2.51±0.18^{ab}	$_{3}$2.53±0.29^{ab}	$_{3}$2.86±0.22^{b}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; P>0.05; n=4).

Table 8-2. The SGR (% d^{-1}) of *L. catenata* in K^+ISW

Time	OW	ISW	ISW33	ISW66	ISW100
Fortnightly					
Day 1–14	$_{1}$1.44±0.20^{ab}	$_{1}$0.49±0.38^{a}	$_{1}$0.78±0.29^{a}	$_{1}$2.08±0.18^{b}	$_{1}$1.31±0.54^{ab}
Day 15–28	$_{2}$-1.12±.57^{ab}	$_{1}$0.15±0.41^{ac}	$_{1}$0.27±0.33^{c}	$_{23}$-0.32±0.30^{abc}	$_{2}$-1.45±0.44^{b}
Day 29–42	$_{12}$0.60±0.43	$_{1}$0.52±0.62	$_{2}$1.24±0.16	$_{2}$0.35±0.32	$_{1}$1.02±0.35
Day 43–56	$_{2}$-0.68±1.11	$_{2}$2.03±0.66	$_{3}$-1.26±0.26	$_{3}$-0.90±0.30	$_{2}$-0.35±0.30
Day 57–70	$_{3}$-4.09±2.34	$_{3}$-4.97±1.88	$_{4}$-2.95±0.39	$_{4}$-3.27±0.53	$_{3}$-1.59±0.38
Cumulative SGR					
Day 1–14	$_{1}$1.44±0.20^{ab}	$_{1}$0.49±0.38^{a}	$_{1}$0.78±0.29^{a}	$_{1}$2.08±0.18^{b}	$_{1}$1.31±0.54^{ab}
Day 1–28	$_{2}$0.20±0.21^{a}	$_{1}$0.31±0.13^{ab}	$_{1}$0.52±0.26^{ab}	$_{2}$0.89±0.13^{b}	$_{2}$-0.02±0.22^{b}
Day 1–42	$_{2}$0.32±0.25	$_{1}$0.37±0.16	$_{1}$0.73±0.16	$_{23}$0.71±0.19	$_{2}$0.31±0.17
Day 1–56	$_{2}$0.07±0.16^{ab}	$_{1}$-0.23±0.24^{a}	$_{1}$0.23±0.12^{ab}	$_{3}$0.30±0.07^{b}	$_{2}$0.14±0.15^{ab}
Day 1–70	$_{3}$-1.05±0.58	$_{2}$-1.28±0.31	$_{2}$-0.56±0.07	$_{4}$-0.83±0.13	$_{2}$-0.47±0.09

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; P>0.05; n=4).

In the first two fortnights, the SGR of *L. catenata* was significantly higher than the rest of the experimental periods in all waters. ISW66 resulted in the highest SGR in the first fortnight, but ISW33 gave a higher SGR in the following fortnight. The *L. catenata* presented a similar fortnightly SGR over the last three fortnights of the

experiment (Table 8-2). In the first 42 days of the culture period for growing *L. catenata*, either ISW66 or ISW33 gave higher biomass gains than other water sources.

8.3.1.2 The Quality of Potassium-fortified Inland Saline Water

The pH of cultured media was similar over the experimental period, except on day 14, when ISW66 resulted in the highest ($P<0.05$) pH among the five waters. As the experiment was conducted in ambient room temperature, it reflected daily temperature changes during the winter time. The temperature was significantly higher during the middle of the experiment, but the water temperature among the five treatments was similar as the experiment progressed (Table 8-3).

Table 8-3. The water pH and temperature in K^+ISW for culturing *L. catenata*

Time	OW	ISW	ISW33	ISW66	ISW100
pH					
Day 1	$_{1}$7.92±0.01	$_{1}$8.04±0.03	$_{1}$7.95±0.00	$_{1}$7.97±0.00	$_{1}$8.06±0.01
Day 14	$_{2}$8.46±0.04^{ab}	$_{2}$8.42±0.01^{a}	$_{2}$8.39±0.01^{a}	$_{2}$8.49±0.04^{b}	$_{2}$8.40±0.02^{ab}
Day 28	$_{2}$8.45±0.03	$_{2}$8.39±0.04	$_{2}$8.48±0.04	$_{2}$8.41±0.05	$_{2}$8.41±0.03
Day 42	$_{3}$8.82±0.07	$_{3}$8.71±0.04	$_{3}$8.71±0.06	$_{3}$8.72±0.02	$_{3}$8.72±0.05
Day 56	$_{3}$8.72±0.08	$_{4}$8.85±0.02	$_{4}$8.83±0.03	$_{3}$8.79±0.08	$_{4}$8.83±0.06
Day 70	$_{3}$8.70±0.02	8.92±0.26	8.72±0.02	8.79±0.08	8.67±0.04
Temperature (°C)					
Day 1	$_{1}$18.95±0.45	$_{1}$18.55±0.35	$_{1}$18.50±0.00	$_{1}$18.50±0.00	$_{1}$18.55±0.35
Day 14	$_{23}$20.35±0.09	$_{23}$20.33±0.06	$_{23}$20.35±0.10	$_{23}$20.43±0.09	$_{24}$20.30±0.06
Day 28	$_{3}$20.95±0.59	$_{23}$20.30±0.31	$_{23}$20.65±0.59	$_{3}$20.88±0.47	$_{3}$20.98±0.21
Day 42	$_{23}$20.60±0.15	$_{2}$20.63±0.11	$_{3}$20.85±0.10	$_{3}$20.80±0.15	$_{23}$20.73±0.14
Day 56	$_{12}$19.88±0.11	$_{3}$19.70±0.04	$_{24}$19.85±0.14	$_{2}$19.88±0.11	$_{4}$19.75±0.03
Day 70	$_{12}$19.68±0.09	$_{3}$19.53±0.06	$_{4}$19.65±0.10	$_{2}$19.68±0.17	$_{4}$19.53±0.14

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=4). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; $P>0.05$; n=4).

The ionic profile of three waters at the same salinity is almost identical in terms of heavy metals. However, the Ca^{2+} and S in ISW were higher than in OW, whereas K^{+} and Na^{+} in ISW are deficient than in OW (Table 8-4).

Table 8-4. The ionic profile (mg L^{-1}) of waters at 30 ppt

Parameters	Swan River	OW	ISW
Bo	3.01	3.60	0.59
Ca	272.4	320.9	530.8
Cl	15809.5		
Cu	<0.05	<0.05	<0.05
Fe	0.08	<0.05	<0.05
Mg	992.7	1015.0	1375.0
Mn	2.93	<0.05	<0.05
P	0.61	<0.05	<0.05
K	331.3	313.0	77.3
Na	8668	8803.0	7720
S	929.3	706.3	560.0
Zn	<0.05	<0.05	<0.05

Modified from Dinh (2016)

The N concentration in water varied differently at different points of the culture period. NH_4-N was negligible as the experiment progressed, whereas NO_2^--N decreased and NO_3^--N increased in all waters toward the end of the experiment. There was no significant difference of [NO_3^--N] among water types during the first 42 days of the culture period, whereas, at day 56 and day 70, ISW66 and ISW33, respectively, resulted in higher [NO_3^--N] than other waters. However, NO_3^--N showed no significant correlation with the biomass of *L. catenata*, but NO_2^--N did (Table 8-5).

PO_4^{3-}-P was significantly reduced during the middle of the experiment; however, it increased towards the end of the experiment, and showed a significant correlation with the biomass of *L. catenata*.

Table 8-5. The quality of water cultured *L. catenata* in K^+ISW experiment

Time	OW	ISW	ISW33	ISW66	ISW100
NO_2^--N					
Day 1	$_{12}$0.021±0.002	$_{1}$0.042±0.017	$_{12}$0.022±0.002	$_{13}$0.021±0.001	$_{1}$0.021±0.002
Day 14	$_{1}$0.063±0.033	$_{1}$0.038±0.005	$_{1}$0.038±0.014	$_{2}$0.040±0.008	$_{2}$0.040±0.000
Day 28	$_{12}$0.034±0.003^{ab}	$_{1}$0.041±0.004^{a}	$_{12}$0.028±0.007^{b}	$_{12}$0.028±0.005^{ab}	$_{3}$0.045±0.002^{a}
Day 42	$_{2}$0.005±0.000^{a}	$_{2}$0.009±0.001^{b}	$_{2}$0.006±0.000^{ac}	$_{3}$0.005±0.000^{a}	$_{4}$0.007±0.001^{c}
Day 56	$_{2}$0.006±0.000	$_{2}$0.006±0.000	$_{2}$0.007±0.001	$_{3}$0.007±0.001	$_{4}$0.007±0.001
Day 70	$_{1}$0.002±0.000^{a}	$_{2}$0.004±0.001^{bd}	$_{2}$0.007±0.000^{c}	$_{3}$0.004±0.001^{b}	$_{4}$0.006±0.000^{c}
NH_4-N					
Day 1	$_{1}$0.825±0.175^{a}	Neg.^{b}	Neg.^{b}	$_{1}$Neg.^{b}	Neg.^{b}
Day 14	$_{2}$Neg.	Neg.	Neg.	$_{1}$Neg.	Neg.
Day 28	$_{2}$0.003±0.003	Neg.	Neg.	$_{1}$0.010±0.004	Neg.
Day 42	$_{2}$Neg.^{a}	Neg.^{a}	Neg.^{a}	$_{2}$0.333±0.236^{b}	Neg.^{a}
Day 56	$_{2}$Neg.	Neg.	Neg.	$_{1}$Neg.	Neg.
Day 70	$_{2}$Neg.	Neg.	Neg.	$_{1}$Neg.	Neg.
NO_3^--N					
Day 1	$_{1}$1.23±0.13^{a}	2.10±0.22^{b}	2.05±0.22^{b}	2.03±0.15^{bc}	$_{13}$1.50±0.15^{ac}
Day 14	$_{23}$2.28±0.46	2.31±0.44	2.02±0.45	1.64±0.35	$_{1}$1.87±0.18
Day 28	$_{2}$2.69±0.29	2.23±0.09	2.03±0.13	2.10±0.43	$_{2}$2.37±0.09
Day 42	$_{13}$1.67±0.19	2.60±0.58	1.70±0.15	1.13±0.06	$_{1}$1.73±0.03
Day 56	$_{1}$1.18±0.10^{a}	2.88±0.80^{ab}	1.33±0.32^{ab}	3.60±1.08^{b}	$_{3}$1.13±0.13^{a}
Day 70	$_{1}$1.53±0.10^{a}	1.67±0.16^{a}	3.03±0.27^{b}	1.80±0.11^{c}	$_{2}$2.30±0.26^{bc}
PO_4^{3-}-P					
Day 1	1.55±0.12^{a}	$_{1}$1.68±0.05^{a}	$_{12}$2.08±0.11^{bc}	$_{1}$1.83±0.08^{ac}	$_{1}$1.65±0.10^{a}
Day 14	1.83±0.20	$_{1}$1.78±0.16	$_{13}$1.69±0.11	$_{1}$1.83±0.23	$_{1}$1.68±0.06
Day 28	1.30±0.06	$_{2}$1.17±0.13	$_{2}$1.23±0.20	$_{2}$1.23±0.08	$_{12}$1.23±0.10
Day 42	2.30±0.85	$_{2}$1.03±0.17	$_{23}$1.40±0.12	$_{3}$1.40±0.20	$_{2}$1.20±0.29
Day 56	1.50±0.15^{a}	$_{1}$1.73±0.06^{ab}	$_{1}$1.80±0.08^{b}	$_{2}$1.50±0.04^{a}	$_{12}$1.60±0.08^{ab}
Day 70	1.53±0.14^{a}	$_{1}$1.47±0.14^{a}	$_{4}$3.77±0.20^{b}	$_{4}$3.97±0.32^{b}	$_{3}$2.33±0.10^{c}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; P>0.05; n=4).

8.3.2 Effect of Ammonium Enrichment on the Growth of *L. catenata*

The temperature and pH of cultured medium was similar among the treatments over the cultured period (Table 8-6).

Table 8-6. pH and temperature in NH_4-N enriched water cultured *L. catenata*

Waters	NH_4-N (µM) addition	pH	Temp (^oC)
OW	0	8.10±0.02	19.00±0.01
OW_NH_4	100	8.07±0.02	19.10±0.01
ISW	0	7.95±0.03	18.99±0.01
ISW_NH_4	100	7.97±0.04	18.97±0.01
ISW66	0	8.21±0.02	18.93±0.01
ISW66_NH_4	100	8.19±0.02	18.95±0.01

NH_4-N did not affect the growth of *L. catenata* in OW, but it did show a significant effect on *L. catenata* growth in ISW. Both ISW_NH_4 and ISW66_NH_4 resulted in significantly higher biomass and SGR_w of *L. catenata* than ISW and ISW66, respectively. NH_4-N presented the highest effectiveness when used in ISW66; this resulted in higher biomass and SGR_w of *L. catenata* by the end of the experiment than OW_NH_4 and ISW_NH_4. However, a significant reduction was found in the biomass of *L. catenata* over the experimental period in all waters (Table 8-7).

8.3.3 Effects of Temperature on *L. catenata* Growth

In the first experiment with three levels of temperature were tested, the temperature significantly ($P<0.05$) affected the biomass and growth rate of *L. catenata* during the four weeks growing in the tanks. The ambient temperature of 18–19^{o}C resulted in the lowest *L. catenata* biomass and SGR_w in both OW and OW_NH_4. However in the OW_NH_4 water, the temperature of 25–26^{o}C gave a higher *L. catenata* biomass and SGR than at 21–22^{o}C (Table 8-8).

The pH and temperature of waters at the same temperature levels were similar over the experimental period (Table 8-9).

Table 8-7. Biomass (g) and SGR_w (% d^{-1}) of *L. catenata* in ambient and NH_4-N enriched OW, ISW, ISW66

Parameters	OW		ISW		ISW66	
	Ambient	NH_4-N	Ambient	NH_4-N	Ambient	NH_4-N
Biomass day 1	$_1$180.69±0.09	$_1$180.45±0.12	$_1$180.16±0.13	$_1$180.37±0.19	$_1$180.30±0.15	$_1$180.50±0.14
Biomass day 28	$_2$118.66±11.77^a	$_2$131.22±3.09^a	$_2$109.93±10.78^a	$_2$134.51±5.13^b	$_2$126.22±8.57^a	$_2$161.61±4.08^b
SGR_w	-1.48±0.33^a	-1.10±0.08^a	-1.74±0.35^a	-1.02±0.12^b	-1.24±0.23^a	-0.38±0.09^b

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=3). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (t test; P>0.05; n=3).

Table 8-8. Biomass and SGR_w (% d^{-1}) of *L. catenata* in OW and OW_NH_4 under three temperature levels

Parameters	25–26ºC		21–22ºC		18-19ºC	
	OW	OW_NH_4	OW	OW_NH_4	OW	OW_NH_4
Biomass day 1	$_1$180.44 ±0.23	$_1$180.15 ±0.43	$_1$180.16±0.13	$_1$180.50 ±0.27	$_1$180.69 ±0.09	$_1$180.45 ±0.12
Biomass day 28	$_2$152.73 ±1.36^a	$_2$150.99 ±3.16^a	$_2$156.21 ±2.36^a	$_2$113.97 ±2.48^b	$_2$118.66 ±11.77^a	$_2$131.22 ±3.09^a
SGR_w	-0.58±0.03^a	-0.61±0.07^a	-0.49±0.05^a	-1.59±0.07^b	-1.48±0.33^a	-1.10±0.48^a

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (t-test; P>0.05; n=4).

Table 8-9. pH and temperature of ammonium enriched OW cultured *L. catenata* under threee temperature levels

Waters	NH_4-N (µM) addition	pH	Temperature (^oC)
OW	0	8.14±0.04	25.07±0.01
OW_NH_4	100	8.16±0.02	25.31±0.00
OW	0	8.18±0.01	21.75±0.02
OW_NH_4	100	8.14±0.02	21.63±0.01
OW	0	8.10±0.02	19.00±0.01
OW_NH_4	100	8.07±0.02	19.10±0.01

In the second experiment where two temperature levels of 25–26ºC and 21–22ºC were used for three water types, OW, OW_NH_4, ISW66_NH_4, the pH and temperature of the three waters were similar at the same temperature levels (Table 8-10).

Table 8-10. pH and temperature of OW and K^+ISW at two levels of temperature

Factors	21–22ºC			25–26ºC		
	OW	OW_NH_4	ISW66_NH_4	OW	OW_NH_4	ISW66_NH_4
Tempera	21.64	21.64	21.69	25.78	25.67	25.50
-ture (^oC)	±0.13	±0.06	±0.16	±0.22	±0.03	±0.06
pH	8.61±0.03	8.74±0.03	8.71±0.03	8.47±0.05	8.49±0.05	8.45±0.04

The mortality of *L. catenata* started occurring on day 25. By day 45, there was no sign of living *L. catenata* in the beakers; therefore, the data of biomass and SGR_w were collected by day 25 of the experimental. At the 25–26ºC, both OW and OW_NH_4 resulted in a significant increase of biomass than at the beginning. However, these increases did not result in a significantly higher SGR_w of *L. catenata* than in ISW66_NH_4. On the other hand, the temperature showed no effect on the growth of *L. catenata* in all waters, where at the same temperature levels the three water sources resulted in a similar SGW_w. The length of the *L. catenata* showed no significant change over the culture period in all waters and temperatures (Table 8-11).

8.3.4 Effects of Ammonium and Phosphate Enrichment on the Growth of *L. catenata* in Potassium-fortified Inland Saline Water

In order to get enough fresh biomass to have adequate dried biomass of *L. catenata*, this experiment lasted for 25 days. By the end of the experiment, with no nutrient enrichment, ISW66 resulted in a significantly higher biomass and SGR_w of *L. catenata*, and $[NO_2^-\text{-N}]$ and $[PO_4^{3-}\text{-P}]$ than in OW; however, these were similar at other nutrient levels (Table 8-12, Table 8-13).

Nutrient enrichment did not significantly affect the growth of *L. catenata* in ISW66. The biomass, SGR_w and dried content of *L. catenata* were similar after 25 days of culture in three NH_4-N:PO_4^{3-}-P levels. In OW, the ratio of 75:7.5 resulted in the highest biomass and SGR, and the dried content of *L. catenata* cultured in 300:30 was lowest.

Although NH_4-N was provided weekly, NH_4-N levels in water were insignificant. By the beginning of the experiment, $[NO_3^-\text{-N}]$ in ISW66 was higher than in OW. However, by day 25, $[NO_3^-\text{-N}]$ in ISW66 was only higher than OW at the nutrient enriched of 150:15, and lower at 300:30. $[NO_2^-\text{-N}]$ was negligible in the lower nutrient enrichment levels at the beginning, and showed no significant difference among the nutrient levels as the experiment progressed. There was a significant reduction of $[PO_4^{3-}\text{-P}]$ during the experiment, and $[PO_4^{3-}\text{-P}]$ was significantly correlated with the biomass of the *L. catenata*.

Table 8-11. Biomass (g), length (mm) and SGR (% d^{-1}) of *L. catenata* cultured in OW, OW_NH_4 and ISW66_NH_4 at temperatures 21–22ºC and 25–26ºC

Criteria	21–22ºC			25–26ºC		
	OW	OW_NH_4	ISW66_NH_4	OW	OW_NH_4	ISW66_NH_4
Biomass day 1	3.49±0.07	3.49±0.26	3.53±0.07	$_1$3.20±0.13	$_1$3.23±0.12	3.60±0.14
Biomass day 25	4.53±0.50	4.89±0.77	5.01±0.70	$_2$4.71±0.49	$_2$4.19±0.29	4.39±0.43
SGR_W	1.01±0.57	1.40±0.25	1.32±0.56	1.59±0.16	1.08±0.08	0.80±0.17
Length day 1	10.88±0.52	13.50±1.10	13.60±0.39	11.32±0.66	12.43±1.49	12.83±0.60
Length day 25	11.98±0.30	13.13±1.20	14.28±0.47	11.67±0.67	13.00±1.53	13.00±0.64
SGR_L	0.41±0.10^a	-0.12±0.22^b	0.20±0.09^{ab}	0.13±0.09	0.19±0.02	0.05±0.03

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; P>0.05; n=4).

Table 8-12. Biomass (g), SGR_w (% d^{-1}) and dried content (%) of *L. catenata* cultured in three NH_4-N:PO_4^{3-}-P (µM) enriched levels

Criteria	OW				ISW66			
	Ambient	**75:7.5**	**150:15**	**300:30**	**Ambient**	**75:7.5**	**150:15**	**300:30**
Biomass								
Day 1	3.37±0.01	$_1$3.38±0.02	3.40±0.01	$_1$3.40±0.02	$_1$3.35±0.01	3.38±0.01	3.37±0.00	3.36±0.01
Day 25	3.30±0.28^a	$_2$4.28±0.12^b	3.87±0.36^a	$_2$4.10±0.13^a	$_2$4.21±0.19	4.14±0.50	3.65±0.29	3.75±0.17
SGR_W	-0.12±0.16^a	0.94±0.14^b	0.49±0.40^a	0.75±0.12^a	0.91±0.16^a	0.71±0.50^{ab}	0.29±0.32^b	0.43±0.18^b
Dried content								
Day 1	$_1$14.77±0.11	14.77±0.11	$_1$14.77±0.11	$_1$14.77±0.11	14.77±0.11	14.77±0.11	14.77±0.11	14.77±0.11
Day 25	$_2$16.04±0.64^a	14.51±0.56^{ab}	$_2$16.45±2.10^a	$_2$12.18±1.15^b	14.21±0.62	14.26±0.47	14.16±0.74	15.14±0.63

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=3) in one water. Values (mean±SE) within a column at one parameter sharing a common subscript are not significantly different (t-test; P>0.05; n=3).

Table 8-13. The quality of OW and ISW66 cultured *L. catenata* at three NH_4-N:PO_4^{3-}-P (µM) enriched levels

Criteria	OW				ISW66			
	Ambient	**75:7.5**	**150:15**	**300:30**	**Ambient**	**75:7.5**	**150:15**	**300:30**
NO_3^--N								
Day 1	$_1$0.97±0.03^a	1.47±0.03^{ab}	1.60±0.06^b	2.10±0.00^c	2.13±0.12^a	2.27±0.07^b	2.53±0.03^b	$_1$2.90±0.31^c
Day 25	$_2$1.17±0.03^a	1.13±0.30^{ab}	1.33±0.27^b	1.43±0.27^b	1.50±0.21	1.87±0.32	2.53±0.62	$_2$1.00±0.06
NO_2^--N								
Day 1	Neg.^{a}	1.00±0.00^b	0.33±0.00^{ab}	0.33±0.00^{ab}	Neg.	Neg.	0.33±0.00	0.33±0.00
Day 25	Neg.	0.09±0.08	0.01±0.01	0.12±0.05	0.42±0.41	0.01±0.00	0.16±0.16	0.06±0.03
PO_4^{3-}-P								
Day 1	$_1$2.17±0.09^a	$_1$2.53±0.29^a	$_1$2.97±0.09^a	$_1$3.93±0.20^b	2.23±0.09^a	2.73±0.09^{ab}	$_1$3.17±0.43^b	$_1$4.47±0.52^c
Day 25	$_2$1.30±0.10	$_2$1.03±0.09	$_2$1.23±0.12	$_2$1.23±0.13	3.17±0.94^a	2.00±0.40^b	$_2$1.73±0.03^b	$_2$1.73±0.28^b

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=3) in one water. Values (mean±SE) within a column at one parameter sharing a common subscript are not significantly different (t-test; P>0.05; n=3).

8.4 Discussion

This is the first study of culturing *L. catenata* in ISW. *L. catenata* showed the ability to be cultivated in ISW under special conditions of K^+ISW and seasonal temperatures.

Potassium fortification was needed for ISW to sustain the growth of *L. catenata*. The ISW66 resulted in higher *L. catenata* biomass than OW at the day 28, and the biomass of *L. catenata* was similar between these two waters. The growth of seaweed is significantly affected by [K^+], which plays an important role in photosynthesis and regulation of osmotic pressure of the seaweed cells (Checchetto *et al*., 2013; Kirst, 1977; Malhotra & Glass, 1995). The [K^+] in the seaweed cells should be between 100–200 mM for proper protein synthesis (Blumwald *et al*., 2000). Intracellular [K^+] is regulated by internal and external [K^+] exchange mechanisms, which are determined by external [K^+] (Blumwald *et al*., 2000; Tromballa, 1978). The osmotic gradient of aquatic plant cells is maintained by [K^+], and is facilitated by a suitable ratio between Na^+ and K^+ internally (Blumwald *et al*., 2000; Malhotra & Glass, 1995). Marine animals need the ISW to be fortified to 50–100% of [K^+] in OW at the same salinity to obtain sufficient [K^+] for a balanced osmo-regulation for a capacity to grow (Dinh, 2016; Fielder & Allan, 2003; Prangnell & Fotedar, 2006a, 2006b; Tantulo & Fotedar, 2006). Similarly, *L. catenata* also required a higher amount of [K^+] than in ambient ISW for cultivating. In this study, the concentration of K^+ of 103–206 mg L^{-1} (the Na:K ratio is 37:1–75:1) provided a higher biomass gain and SGR_w of *L. catenata* than higher or lower [K^+], and it is similar to the preferred Na:K for *Ulva* growth at 47:1 (Yamashita *et al*., 2009). This [K^+] range is lower than required by other red seaweeds *Caloglossa leprieurii* and *Bostrychia radicans* (Yarish *et al*., 1980). If the culture period was less than one month, ISW66 would be a better choice than ISW33. However, *L. catenata* should not be cultured longer than 42 days for a higher biomass gain.

Ammonium is preferred source of N for seaweed growth over NO_3^--N (Liu *et al*., 2004), which is why NH_4-N in water was negligible over the culture period, even in the waters supplied weekly with NH_4-N. The red seaweed *Gelidium amansii* grow faster at NH_4-N 80 µM than at 200 µM (Liu *et al*., 2004). However, in this study, the *L. catenata* showed no response in NH_4-N 100 µM in both OW and ISW66 in the tanks. This can be explained by the effect of the low temperature, since the ammonium-effect experiment was conducted at ambient room temperature in winter, when the

temperature was approximately 19ºC. This result was demonstrated in the temperature-effect experiment, where the reduction rate of *L. catenata* cultured in 18–19ºC was higher than other two higher temperature levels. As the *L. catenata* cultured in tanks holding OW and OW_NH_4 showed different responses to the 21–22ºC and 25–26ºC temperatures, the second experiment was conducted in beakers at these two temperature levels. In addition, ISW66_NH_4 provided the lowest reduction SGR in the NH_4-N-effect experiment, was also tested. A similar SGR_w was found for *L. catenata* cultured in one water source at two temperature levels and cultured in four different water sources at one temperature level, and this revealed that the suitable temperature for *L. catenata* cultured in captivity was 21–26ºC. This preferred temperature range was similar to the green seaweeds *Ulva curvata* (Malta *et al*., 1999), *Ulva lactuca* (Van Khoi & Fotedar, 2011), and *Ulva pertusa* (Liu & Dong, 2001), and the red seaweed *Hypnea cervicornis* J Agardh (Ding *et al*., 2013), but was higher than the need of the red seaweeds *Phycodrys rubens* and *Membranoptera alata* (Lüning, 1984).

Contrary to the negative SGR found in *L. catenata* cultured in all temperature conditions in tanks, the *L. catenata* cultured in beakers at 21–26ºC in the temperature effect experiment and K^+-fortification effect experiment at 18.5–21ºC resulted in a positive SGR_w, revealing the scale of growing *L. catenata*. This can only be explained by the different seasons of sampling. The *L. catenata* were collected from the field 2–3 days before the beginning of each experiment, reflecting the seasonal growth of *L. catenata* at different stages. The experiment conducted in the tanks were from the middle of winter to the end of autumn, whereas the beaker experiments were in early winter and late autumn to early summer. Observations in the field in early summer showed that the *L. catenata* grew quickly and the canopy was largest. Furthermore, the *L. catenata* standing crop decreased gradually by the end of summer, and reappeared in the spring. This is similar to the *L. catenata* seasonal growth in Japan (Lee, 1978), although it is conducted in different hemispheres.

At 21–22ºC, the length of *L. catenata* cultured in OW_NH_4 were reduced, resulting from apical cell breakage; however, the biomass gain was positive, indicating weight growth of the *L. catenata*. The similarity of the SGR_w and SGR_L of the *L. catenata* cultured in ISW66_NH_4 and the sources of OW showed the ability of *L. catenata* to grow in ISW66_NH_4.

Although NH_4-N was necessary for *L. catenata* growth in ISW66, the combination of NH_4-N and PO_4^{3-}-P did not result in higher biomass than using only NH_4-N. In addition to the weekly supplied NH_4-N and PO_4^{3-}-P, N and P in water were also produced by the decomposition of *L. catenata*. NH_4-N combines with PO_4^{3-}-P result in a higher growth rate of *Sargassum baccularia* than single nutrient sources (Schaffelke & Klumpp, 1998). The living microbes in water sequence the soluble N and P quickly, therefore N and P concentrations are not stable, then difficult to measure (Downing, 1997). Seaweeds uptake NH_4-N is faster than PO_4^{3-}-P when they are available in water at similar concentrations (Wallentinus, 1984). Consequently, NH_4-N levels in water were nigligible as the trial progressed, NO_3^--N levels were reduced over the culture period, and [PO_4^{3-}-P] were lower at the termination of the experiment than at the beginning in the NH_4-N:PO_4^{3-}-P effect trial, showing *L. catenata* growth.

In OW, the NH_4-N:PO_4^{3-}-P ratio at 75:7.5 µM resulted in the highest SGR and a significant increase of biomass at the end of the experiment compared with the beginning. These nutrient concentrations were similar to those needed by the red seaweed *Gelidium amansii* (Liu *et al*., 2004). However, in ISW66, NH_4-N:PO_4^{3-}-P enrichment showed no effect on the growth of *L. catenata*, since water not enriched with nutrients resulted in a significant gain of biomass over the culture period. This result verified those of the previous experiment, where ISW66_NH_4 gained a similar SGR of *L. catenata* to OW and OW_NH_4 at 21–26ºC.

8.5 Conclusions

This study identifies the suitable environmental and chemical parameters to grow *L. catenata* under laboratory conditions with temperature levels of 21–26ºC, salinity level of 30–31 ppt and supplied NH_4-N concentrations of no greater than 100 µM in both OW and K^+ISW. The K^+ fortification for ISW is needed at 33–66% of [K^+] in OW at the same salinity for higher *L. catenata* biomass gain in the culture period of no longer than 42 days.

GREEN SEAWEED

CHAPTER 9 CULTIVATION FEASIBILITIES OF *Ulva lactuca* IN INLAND SALINE WATER

9.1 Introduction

Ulva and *Enterromorpha* are the two most widespread genera of the order Ulvales (Sze, 1998), and they should be considered as one genera (Graham *et al*., 2009; Hayden *et al*., 2003; Kraft *et al*., 2010). There are 140 species and 135 species, respectively, in the *Ulva* and *Enteromorpha* genera: of these, 50 and 35 species are identified respectively (Hayden *et al*., 2003). However, Kraft *et al*. (2010) and Loughnane *et al*. (2008) state that *Ulva* genera should include 127 species, of which 6 species are found in Southern Australia (Kraft *et al*., 2010).

Ulva spp. are cosmopolitan species, widely distributed in different environments (Morrison & Storrie, 2010) and are naturally distributed in Europe (Koeman & van den Hoek, 1981; Loughnane *et al*., 2008; Malta *et al*., 1999), Japan (Hiraoka & Oka, 2008), Australia (Kraft *et al*., 2010), America (Sousa *et al*., 2007) and in inland water in Poland (Messyasz & Rybak, 2009). In Australia, *Ulva* spp. are naturally distributed in Western Australia (WA), New South Wales and Tasmania at depths of up to five meters (Kraft *et al*., 2010; Womersley, 1984).

Ulva is the most commonly used seaweeds, including human food, medicine, conditioners for soil, manure and water cleaner (Lahaye *et al*., 1994; Lindsey Zemke-White & Ohno, 1999; Sze, 1998). The polysaccharides extracted from *Ulva* can be used in industries and for human (Lahaye & Axelos, 1993; Lahaye *et al*., 1994; Lindsey Zemke-White & Ohno, 1999; Ray & Lahaye, 1995) and the lipids in *Ulva* can be converted to biodiesel (Petrus & Noordermeer, 2006). *U. lactuca* contains similar protein and energy as lucerne hay and can be used as cattle-feed (Ventura & Castañón, 1998). *Ulva* can accumulate trace metals in polluted water (Ho, 1990; Mamboya *et al*., 2009). In addition, *Ulva* can be integrated cultured with other species to clean the water discharge and increase yield of both species (Neori *et al*., 1996; Van Khoi & Fotedar, 2011).

Ulva has been studied widely in ocean water (OW), in terms of taxonomy (Heesch *et al*., 2009; Koeman & van den Hoek, 1981; Kraft *et al*., 2010; Loughnane *et al*., 2008;

Malta *et al*., 1999; Phillips, 1988), growth rate (Ahmad *et al*., 2011; de Casabianca *et al*., 2002), seasonal variation (Duke *et al.*, 1987; Villares *et al.*, 2002), nutrient uptakes (Ale *et al*., 2011; Pérez-Mayorga *et al*., 2011), pollution removal (Bartoli *et al*., 2005; Blackmore, 1998; Burgess *et al*., 2003; Ho, 1990), photosynthesis (Axelsson *et al*., 1995; Beer & Israel, 1990; Larsson *et al.*, 1997), life cycle (Bendoricchio *et al.*, 1994), and chemical composition and nutrition (Aguilera-Morales *et al.*, 2005; Lahaye & Jegou, 1993; Ray & Lahaye, 1995; Robic *et al.*, 2008; Ventura & Castañón, 1998; Yaich *et al*., 2011). Particularly, the bicarbonate uptake of *Ulva* (Axelsson *et al*., 1995; Björk *et al.*, 1993; Drechsler *et al.*, 1993) and in the integrated culture of *Ulva* with other marine species have been studied broadly (Cruz-Suárez *et al*., 2010; Neori *et al*., 1996; Wang *et al.*, 2007; Yokoyama & Ishihi, 2010).

Salinity is the most important parameter significantly affecting growth, photosynthesis, chlorophyll-a content, spore biomass and heavy metal absorption of *Ulva* (Choi *et al*., 2010; Kamer & Fong, 2001; Mamboya *et al*., 2009; Scherner *et al*., 2013; Sousa *et al*., 2007). The salinity requirement is species specific. *U. lactuca* and *U. ridiga* can withstand large salinity ranges (Friedlander, 1992; Zavodnik, 1975). *U. curvata* and *U. scandinavica* grow well in salinity level of 17–34 ppt, but *U. rigida* tolerates lower salinity levels, from 4 to 34 ppt (Koeman & van den Hoek, 1981). Therefore, re-testing the salinity requirement for local *U. lactuca* is needed for its aquaculture practice.

Ulva prefers NH_4-N (Ahmad *et al*., 2011), and requires 3.6 µM supplied continuously for maximal growth in winter (Campbell, 2001), and the growth of *Ulva* is significantly correlated with NH_4-N uptake (Ramus & Venable, 1987). The presence of phosphate (PO_4^{3-}-P) in cultured media of providing NH_4-N increases the *U. reticulata* NH_4-N uptake capacity (Ahmad *et al*., 2011). The local OW contains [PO_4^{3-}-P] more than 1 mg L^{-1} (Bui, Luu, Fotedar, *et al*., 2017), which is sufficient for *Ulva* requirement (Ahmad *et al*., 2011; Campbell, 2001), therefore the source of nutrient for *Ulva* culture just need to focus on NH_4-N.

Furthermore, agricultural land and farms are showing signs of higher salinity in Western Australia (WA) and Australia (Trewin, 2002), which negatively affects agriculture and other industries (Allan *et al*., 2001; Allan *et al*., 2008; Borowitzka,

1997; Cordover, 2007; NLWRA, 2001). In order to prevent salinization, hundred thousand kilometers of banks have been built around Australia farms (Trewin, 2002), providing an available water resources to culture seaweed. However, attempt to culture seaweed in inland saline water (ISW) is still limited.

Of the seaweed species, *U. lactuca* is a possible candidate to culture in ISW as a source of feed for abalone (Borowitzka, 1997). *Ulva* can be integrated with marine fish *Oreochromis spilurus* (Al-Hafedh *et al*., 2012), western king prawn (*Penaeus latisulcatus*) (Van Khoi & Fotedar, 2011) and abalone (Robertson-Andersson *et al*., 2009), and these marine species, as well as many other marine speices, have been successfully cultured in ISW in Australia (Doroudi *et al*., 2006; Fielder *et al*., 2001; Fotedar *et al*., 2008; Ingram *et al*., 2002; Partridge, Lymbery, & George, 2008; Partridge *et al*., 2006). The effects of salinity and temperature on the growth of *Ulva* have also been studied but the results are species dependent (Choi *et al*., 2010; Duke *et al*., 1989; Xia *et al*., 2004). In addition, the temperature of ISW is varied widely, from 6.3–28.1ºC (Taukulis & John, 2009). Finding out the suitable temperature for local *U. lactuca* cultivation in ISW is necessary to develop its aquaculture potential.

K^+ deficiency in Australian ISW is common (Fielder *et al*., 2001; Nulsen, 1997; Saoud *et al*., 2003), so the ISW needs to be fortified to around 50–100% of K^+ concentrations in ocean water (OW) to cultivate fish (Fielder & Allan, 2003; Fielder *et al*., 2001), and shrimp (Shakeeb Ur *et al*., 2005; Tantulo & Fotedar, 2006). The presence of potassium (K^+) in water is crucial for algal growth (Talling, 2010), without any exception to *Ulva*. The ratio of Na:K of 47 provides the best growth rate for *U. ohnoi*, which cannot grow in the low Na:K ratio of 2 (Yamashita *et al*., 2009). *Ulva* has the ability to accumulate K^+, so it can be present in 3.2% of the *Ulva* dry weight, 20 times higher than its concentration in ocean water (OW) (Yamashita *et al*., 2009). The Na:K of ISW in WA is about 100:1 at salinity 30–35 ppt (Dinh, 2016), much higher than the prefer Na:K ratio of *U. ohnoi* (Yamashita *et al*., 2009), therefore it is necessary to test the K^+ need of *U. lactuca* in order to develop its aquaculture practice.

This study was divided into two sections. The first one was aimed to find a suitable salinity, stocking density and nutrient requirements for *U. lactuca* in OW. This section was further used as a base line research in section 2 to cultivate the same species in ISW and K^+-fortified ISW (K^+ISW).

9.2 Materials and Methods

The whole fond of *U. lactuca* including holdfast was collected as described in Chapter 3. The water preparation, data collection and data analysis were followed as in Chapter 3.

9.2.1 *U. lactuca* Culture in Ocean Water

The feasibility of *U. lactuca* cultivation in OW was determined in two different trials.

9.2.1.1 Effect of Salinity on the Growth of *U. lactuca*

This trial was conducted in a fortnight in early autumn. Whole thalli of *U. lactuca* at approximately 36 g each was attached to gravel to submerge whole fond in water and placed into 54 L glass tanks holding 45 L of water. Three salinity levels of 30, 35 and 40 ppt formed the three studied treatments to be tested in triplicates. OW at 35 ppt was exposed to sunlight to obtain 40 ppt of OW due to evaporation. OW at 35 ppt was diluted with filtered fresh water to achieve 30 ppt. The tanks were placed under the ambient room temperature of 18–24ºC, under the lights of 120 µmol photon m^{-2} s^{-1} provided by white fluorescent in a cycle of 14:10 h light:dark (Kraemer & Yarish, 1999).

9.2.1.2 Effects of Stocking Densities and Ammonium Enrichment on the Growth of *U. lactuca*

This trial was conducted for 14 days in winter using OW at 30 ppt by diluting filtered OW at 35ppt with the filtered fresh water. A total of 12 treatments, in triplicates, combining four *U. lactuca* stocking densities of 0.2, 0.4, 0.8 and 1.6 kg m^{-2} at thee water types, which were two NH_4-N weekly enriched levels of 28 and 56 µM supplied by NH_4Cl (Campbell, 2001) and a control of ambient OW. The whole fond of *U. lactuca* at the same development stage of 36, 72, 144 and 288 g were placed in glass tanks of 54 L (60×30×30 cm) holding 45 L of OW. The tanks was placed under the light of 120 µmol photon m^{-2} s^{-1} white fluorescent in a cycle of 14:10 h light:dark

(Kraemer & Yarish, 1999). The temperature was maintained at 24–26ºC by automatic heaters (Sonpar, HA-200, Zhongshan, Guangdong, China).

9.2.2 Feasibility of Cultivating *U. lactuca* in Inland Saline Water

ISW was diluted with filtered freshwater to achieve 30–31 ppt. Two independent trials were set up.

9.2.2.1 Effect of Temperature on the Growth of *U. lactuca*

Whole thalli of *U. lactuca*, at approximately 36 g $tank^{-1}$, were placed into 54 L glass tanks holding 45 L of water at 30–31 ppt, and was conducted in a fortnight in early autumn. Eight treatments included four water types and two temperature levels in three replicates each were conducted, requiring 24 tanks. Four water types were OW and ISW, and were weekly enriched with NH_4-N 56 µM by adding NH_4Cl. These water types were termed as OW_NH_4 and ISW_NH_4. The other two water types were ambient OW and ISW. All tanks were exposed to two temperature levels; high temperature of 25–26ºC and ambient temperature 20–21ºC. The high temperature of 25–26ºC was maintained by using automatic heaters (Sonpar, HA-200, Zhongshan, Guangdong, China).

9.2.2.2 Effects of Potassium Fortification on the Growth of *U. lactuca* in Inland Saline Water

Approximately 36 g *U. lactuca* whole thalli were cultured in one tank during 42 days in early winter. Total of 15 glass tanks of 54 L were used, each tank held 45 L of water, included five treatments in triplicate. Three levels of K^+ISW were used at 33, 66 and 100% of the [K^+] of OW at 30 ppt termed as ISW33, ISW66 and ISW100, and the control of ambient ISW termed as ISW0, and OW. The cultured media were weekly supplied NH_4Cl at [NH_4-N] 56 µM. Automatic heaters (Sonpar, HA-200, Zhongshan, Guangdong, China) was used to maintain the cultured media temperature at 24–26ºC.

9.3 Results

9.3.1 *U. lactuca* Culture in Ocean Water

9.3.1.1 Effect of Salinity on the Growth of *U. lactuca*

Salinity had a significant (P<0.05) effect on the growth of *U. lactuca* in OW, where seaweed biomass at all treatments declined by the end of the experiment. The salinity level of 40 ppt resulted in the highest reduction of biomass, and the lowest SGR among the three salinities. On day 14th, the biomass and SGR of *U. lactuca* at 30 and 35 ppt were similar, but significantly (P<0.05) higher than at 40 ppt (Table 9-1).

Table 9-1. The biomass (g) and SGR (% d^{-1}) of *U. lactuca* in three salinity levels in OW

Criteria	30 ppt	35 ppt	40 ppt
Biomass day 1	36.21±0.21	36.44±0.04	$_1$36.19±0.17
Biomass day 14	30.50±1.46^a	26.62±2.17^a	$_2$13.78±0.53^b
SGR	-1.24±0.34^a	-2.31±0.63^a	-6.90±0.27^b

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=3). Values of biomass (mean±SE) within a column sharing a common subscript are not significantly different (t-test; P>0.05; n=3).

Table 9-2. The ionic profile (mg L^{-1}) of natural waters at different salinities

Parameters	Swan River 31ppt	OW 30 ppt	OW 35 ppt	OW 40 ptt
Bo	3.01	3.60	3.95	4.32
Ca	272.4	320.9	371.6	406.3
Cl	15809.5		19679.5	22095.3
Cu	<0.05	<0.05	<0.05	<0.05
Fe	0.08	<0.05	<0.05	<0.05
Mg	992.7	1015.0	1168.0	1302.0
Mn	2.93	<0.05	<0.05	<0.05
P	0.61	<0.05	<0.05	<0.05
K	331.3	313.0	351.1	410.1
Na	8668	8803.0	10010	11040
S	929.3	706.3	805.4	924.7
Zn	<0.05	<0.05	<0.05	<0.05

(Modified from Dinh (2016)

Table 9-3. Biomass (g) and SGR (% d^{-1}) of *U. lactuca* at four stocking densities (kg m^{-2}) and three levels of NH_4-N (µM)

Criteria	0.2 kg m^{-2}			0.4 kg m^{-2}			0.8 kg m^{-2}			1.6 kg m^{-2}		
	0	**28**	**56**	**0**	**28**	**56**	**0**	**28**	**56**	**0**	**28**	**56**
Biomass day 1	36.08± 0.03	36.13± 0.04	$_1$36.14± 0.03	$_1$72.24± 0.04	$_1$72.22± 0.15	$_1$72.22± 0.07	$_1$144.25 ±0.03	$_1$144.22 ±0.08	$_1$144.25 ±0.09	$_1$288.24 ±0.09	$_1$288.54 ±0.03	$_1$288.23 ±0.16
Biomass day 14	36.83± 0.37^a	37.27± 1.25^a	$_2$41.17± 1.20^b	$_2$56.15± 3.41	$_2$54.01± 2.68	$_2$61.56± 4.02	$_2$130.5± 4.73^a	$_2$137.04 ±2.80^a	$_2$115.69 ±5.61^b	$_2$253.14 ±5.83	$_2$256.71 ±5.42	$_2$230.53 ±32.69
SGR	0.16± 0.07^a	0.24± 0.26^a	1.08± 0.25^b	-1.61± 0.37	-1.84± 0.30	-1.04± 0.40	-0.64± 0.23^a	-0.32± 0.12^a	-1.40± 0.32^b	-0.82± 0.14	-0.73± 0.13	-1.64± 1.06

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; P>0.05; n=4).

Table 9-4. Biomass (g) and SGR (% d^{-1}) of *U. lactuca* under two temperature levels

Criteria	25–26ºC				20–21ºC			
	OW	**OW_NH_4**	**ISW**	**ISW_NH_4**	**OW**	**OW_NH_4**	**ISW**	**ISW_NH_4**
Biomass								
Day 1	36.13±0.03^a	36.21±0.01^a	36.23±0.10^a	36.14±0.08^a	$_1$36.16±0.01^a	$_1$36.08±0.04^a	$_1$36.13±0.10^a	$_1$36.20±0.06^a
Day 14	37.35±0.45^{ab}	39.41±1.24^a	33.83±2.79^b	37.39±1.76^{ab}	$_2$23.98±0.51^a	$_2$21.21±0.85^a	$_2$22.44±0.62^a	$_2$25.46±1.50^a
SGR	0.24±0.08^{ab}	0.60±0.22^a	-0.54±0.61^b	0.23±0.36^{ab}	0.13±20^a	-2.94±0.15^a	-3.81±0.29^a	-3.41±0.21^a

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values of biomass (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; P>0.05; n=4).

9.3.1.2 Effects of Stocking Densities and Ammonium Enrichment on the Growth of *U. lactuca*

The SGR of *U. lactuca* responded differently ($P<0.05$) depending on the density and the amount of NH_4-N provided. By the end of the experiment, the SGR was similar in all NH_4-N levels at densities of 0.4 and 1.6 kg m^{-2}. However, SGR in 56 µM NH_4-N was significantly ($P<0.05$) higher than the other two NH_4-N levels at 0.2 kg m^{-2}, but was lower in 0.8 kg m^{-2}. A significant reduction of biomass was recorded over the experimental period at all stocking density except at the lowest one. At 0.2 kg m^{-2}, the biomass of *U. lactuca* remained the same apart from a significant ($P<0.05$) increase in the NH_4-N level of 56 µM. The higher biomass found at 56 µM NH_4-N at density of 0.2 kg m^{-2} resulted in a highest SGR (Table 9-3), and this stocking density and NH_4-N level were selected for further studies.

9.3.2 Feasibility of Cultivating *U. lactuca* in Inland Saline Water

9.3.2.1 Effect of Temperature on the Growth of *U. lactuc*a

Temperature had a significant ($P<0.05$) effect on biomass growth and SGR of *U. lactuca*. In one water type, the 25–26ºC resulted in significantly higher biomass at the end of the experiment and higher SGR than the 20–21ºC with an exception of OW. No effect of water or NH_4-N was found on the biomass and SGR of *U. lactuca* at 20–21ºC, however, at the 25–26ºC the OW_NH_4 resulted in higher biomass and SGR than ISW (Table 9-4).

NH_4-N concentrations were negligible in all water types, and there was no significant difference among water types and temperature levels. At 25–26ºC, the highest NO_3^--N and PO_4^{3-}-P levels were found in ISW_NH_4. However, at 20–21ºC, only NO_2^--N showed a high concentration in ISW_NH_4.

The pH, temperature, dissolved oxygen (DO) and salinity were similar among the treatments at the same temperature level. In ambient temperature levels of 18.0–22.3ºC, the pH was in the range 7.80–8.58, DO was 6.62–8.95 mg L^{-1}. At higher temperature levels of 24.1–26.6ºC, pH was 7.94–8.26, and DO was 5.50–6.00 mg L^{-1} (Table 9-5).

Table 9-5. The environmental factors of the temperature effect experiment

Criteria	OW	OW_NH_4	ISW	ISW_NH_4
Ambient room treatment				
Temperature (^oC)	20.58±0.26	20.51±0.26	20.42±0.26	20.46±0.25
pH	8.20±0.06	8.23±0.05	8.24±0.05	8.22±0.05
DO (mg L^{-1})	6.95±0.10	6.90±0.08	6.91±0.08	6.92±0.07
High temperature treatment				
Temperature (^oC)	25.12±0.38	25.17±0.40	25.12±0.38	25.18±0.39
pH	8.08±0.04	8.08±0.03	8.08±0.04	8.08±0.03
DO (mg L^{-1})	5.73±0.06	5.73±0.06	5.74±0.06	5.74±0.06

9.3.2.2 Effects of Potassium Fortification on the Growth of *U. lactuca* in Inland Saline Water

U. lactuca responded differently ($P<0.05$) to the K^+ fortification in the NH_4-N 56 µM enriched ISW; a higher ($P<0.05$) biomass was found in the ISW33 than other waters during the experiment. Except ISW33 and ISW66, a significant reduction was found in *U. lactuca* biomass in all water types (Table 9-6). As a result, the SGR of *U. lactuca* in ISW33 and ISW66 was significantly higher than in OW and ISW100 by the termination of the experiment. From day 14, the *U. lactuca* biomass was reduced gradually, resulting in a similar SGR for *U. lactuca* among the lower K^+ water media (ISW33 and ISW66) and higher K^+ (OW and ISW100) (Table 9-7).

Table 9-6. Standing biomass (g) of *U. lactuca* in K^+ fortification ISW

Time	OW	ISW0	ISW33	ISW66	ISW100
Day 1	$_{12}$36.36±0.22	$_{1}$36.38±0.16	36.49±0.14	36.29±0.17	$_{12}$36.48±0.07
Day 14	$_{2}$37.28±0.14	$_{1}$37.21±0.71	37.83±0.25	36.70±0.09	$_{1}$37.28±0.51
Day 28	$_{1}$34.76±0.66^{a}	$_{2}$34.38±0.48^{a}	36.89±0.68^{b}	34.87±0.13^{a}	$_{23}$34.93±0.77^{a}
Day 42	$_{3}$32.90±0.91^{a}	$_{3}$32.55±0.70^{a}	37.01±1.37^{b}	33.30±2.12^{ab}	$_{3}$33.53±0.70^{ab}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=3). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; $P>0.05$; n=3).

Table 9-7. SGR (% d^{-1}) and dried content (%) of *U. lactuca* in K^+ fortification ISW

	Time	OW	ISW0	ISW33	ISW66	ISW100
S	Day 1–14	0.18 ±0.07	0.16±0.11	0.26±0.12	0.08±0.06	0.17±0.15
G	Day 1–28	-0.16±0.09^{ab}	-0.20±0.04^{a}	0.04±0.11^{b}	-0.14±0.02^{ab}	-0.16±0.02^{ab}
R	Day 1–42	-0.24±0.08^{a}	-0.27±0.06^{ac}	0.03±0.09^{b}	-0.22±0.16^{bc}	-0.20±0.05^{a}
Dried content		21.25±2.45	20.37±1.04	17.21±0.95	19.52±0.61	17.49±0.37

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=3)

Table 9-8. The quality parameters of K^+ISW cultured *U. lactuca*

Par.	Time	OW	ISW0	ISW33	ISW66	ISW100
NH_4-N	Day 1	1.00±.00^{a}	2.00±1.00^{b}	1.00±.00^{a}	1.00±.00^{a}	1.50±0.50^{b}
	Day 7	2.55±2.45^{b}	1.00±0.00^{a}	0.50±0.50^{a}	Neg.^{a}	Neg.^{a}
	Day 14	Neg.	Neg.	Neg.	Neg.	Neg.
	Day 28	Neg.	Neg.	Neg.	Neg.	Neg.
	Day 42	Neg.	Neg.	Neg.	Neg.	Neg.
NO_2^--N	Day 1	$_1$0.05±0.01	$_1$0.03±0.00	$_1$0.03±0.00	$_1$0.03±0.00	$_1$0.03±0.01
	Day 7	$_2$Neg.	$_1$0.50±0.50	$_1$0.50±0.50	$_1$0.50±0.50	$_1$0.50±0.14
	Day 14	$_3$1.00±0.00^{ab}	$_1$0.50±0.50^{ab}	$_1$0.50±0.50^{ab}	$_{12}$1.00±0.00^{ab}	$_2$1.50±0.50^{b}
	Day 28	$_3$1.00±0.00	$_2$1.00±0.00	$_1$0.50±0.00	$_{12}$1.00±0.00	$_{12}$1.00±0.00
	Day 42	$_3$0.67±0.33	$_2$1.33±0.88	$_2$1.00±0.00	$_2$1.33±0.33	$_{12}$1.00±0.58
NO_3^--N	Day 1	$_1$0.70±0.10^{b}	$_1$1.40±0.00^{a}	$_{12}$2.45±0.75^{a}	$_1$1.95±0.45^{a}	$_{12}$2.10±0.30^{a}
	Day 7	$_2$1.85±0.55^{b}	$_1$1.85±0.25^{b}	$_{12}$2.10±0.00^{b}	$_1$1.85±0.15^{b}	$_1$1.85±0.05^{b}
	Day 14	$_3$3.25±0.55^{ab}	$_2$4.70±0.40^{b}	$_2$3.50±1.30^{ab}	$_2$4.05±1.45^{ab}	$_2$3.45±0.85^{ab}
	Day 28	$_3$3.70±1.40	$_2$5.15±0.95	$_{12}$2.95±0.55	$_2$3.35±0.45	$_2$4.95±2.65
	Day 42	$_3$2.87±0.32^{ab}	$_3$3.50±0.30^{ab}	$_2$3.80±0.85^{b}	$_2$3.40±1.06^{ab}	$_2$3.90±0.55^{b}
PO_4^{3-}-P	Day 1	$_1$0.80±.20^{b}	$_1$1.10±0.00^{d}	$_1$1.30±0.00^{bcd}	$_1$1.30±0.00^{bcd}	$_1$1.50±0.40^{cd}
	Day 7	$_1$0.55±0.25^{a}	$_1$0.95±0.15^{ab}	$_1$1.30±0.00^{b}	$_1$1.20±0.40^{ab}	$_1$1.35±0.15^{b}
	Day 14	$_{12}$1.20±0.00^{a}	$_2$1.65±0.05^{a}	$_2$1.60±0.10^{a}	$_1$1.95±0.25^{ab}	$_2$2.45±0.55^{b}
	Day 28	$_2$1.50±0.00^{a}	$_3$2.15±0.25^{b}	$_2$1.85±0.35^{ab}	$_1$1.90±0.10^{ab}	$_1$1.80±0.10^{ab}
	Day 42	$_2$1.50±0.21^{a}	$_2$1.70±0.15^{ab}	$_2$1.70±0.12^{ab}	$_1$1.77±0.15^{ab}	$_1$1.83±0.28^{ab}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=3). Values (mean±SE) within a column at one time sharing a common subscript are not significantly different (LSD test; P>0.05; n=3). Par. means parameters. Neg. means negligible.

There was no significant effect of K^+ fortification on the dried biomass of the *U. lactuca*, presenting no significant difference of *U. lactuca* dried biomass among different water types (Table 9-7).

Although the water was enriched with NH_4-N, its concentration was negligible during the experimental period, whereas except for in OW, NO_2^--N, NO_3^--N and PO_4^{3-}-P increased toward the end of the experiment (Table 9-8).

9.4 Discussion

Ulva is one of the most common genera in green seaweeds, providing a source of food and extraction of chemicals for human needs, as well as products for agriculture (Lahaye *et al*., 1994; Lindsey Zemke-White & Ohno, 1999; Sze, 1998). Although *U. lactuca* prefers the open sea water with salinity level of 34–40 ppt for optimal growth (Friedlander, 1992), the ambient salinity of Swan River at the time of *U. lactuca* collection was 30–31 ppt, similar to the optimal salinity for *Ulva* spp. growth (Choi *et al*., 2010; Malta *et al*., 1999). *Ulva* can be cultured in a wide range of salinity levels (5–40 ppt) (Choi *et al*., 2010; Koeman & van den Hoek, 1981), and this study indicated that local *U. lactuca* preferred 31–35 ppt for higher salinity.

Optimal stocking density for *U. lactuca* Linnaeus cultured in outdoor conditions is 0.8 kg wet weight m^{-2}, within the suitable range of 0.13–4.50 kg wet weight m^{-2} (Lapointe & Tenore, 1981). In the current study, the only positive SGR of *U. lactuca* at 0.2 kg m^{-2} presented a potential stocking density for growing *U. lactuca* in captivity, whereas there was no significant difference in the negative SGR of *U. lactuca* cultured at higher stocking densities. This is similar to *U. lactuca* cultured in integrated with western king prawn (*Penaeus latisulcatus* Kishinouye, 1986), where the lowest stocking density of *U. lactuca* of 0.25 kg m^{-2} results in the highest SGR in the first week, and the biomass decreases up to day 42 of the culture period (Van Khoi & Fotedar, 2011). As a negative SGR was presented in higher stocking density, the effect of NH_4-N enrichment was not clear, except at 0.8 kg m^{-2}, which is the optimal stocking density for *U. fasciata* at a temperature of 14±1^oC (Lapointe & Tenore, 1981). The higher NH_4-N concentrations resulted in a greater biomass reduction rate. In order to eliminate the effect of light, a fixed saturating light intensity of 120 µm photon m^{-2} s^{-1} (Kraemer & Yarish, 1999) was chosen for application in all experiments. Since the light is similar for all stocking density, for higher stocking density less light was available for *U. lactuca* photosynthesis, and nutrient competition occurred, which may be the main effects on the negative growth of *U. lactuca*.

Ulva prefers NH_4-N as a source of nitrogen, and the presence of PO_4^{3-}-P stimulates nitrogen uptake (Ahmad *et al*., 2011). [PO_4^{3-}-P] in OW was approximate 1 mg L^{-1} (Bui, Luu, Fotedar, *et al*., 2017), which met *Ulva* demands (Ahmad *et al*., 2011; Campbell, 2001), and it was therefore not supplied. When NH_4-N is not sufficient, *Ulva* consumes NO_3^--N, and the concentration of NO_3^--N and PO_4^{3-}-P in water (Bui, Luu, Fotedar, *et al*., 2017) were sufficient for *Ulva* needs (Ahmad *et al*., 2011). NH_4-N showed a significant effect on the growth of *U. lactuca* at the stocking density of 0.2 kg m^{-2}, whereas the highest NH_4-N level resulted in the highest SGR. The [NH_4-N] for *Ulva* sp. optimal growth is 28 µM provided continuously at 15ºC, pH 8.3 (Campbell, 2001) was the value applied in the NH_4-N enriched experiment. In this study, the weekly supplied NH_4-N was consumed immediately after releasing, which caused the [NH_4-N] in cultured media to be negligible during the cultured period. This can be explained by nutrient uptake of *U. lactuca* (Kim *et al*., 2007).

This study showed that the suitable temperature for *U. lactuca* growing in captivity was 25ºC. The temperature effect was also achieved from the previous trials. In the trial of effect of stocking density and ammonium enrich, OW_NH_4 56 µM resulted in highest and positive SGR of *U. lactuca* during the first 14 days, where the temperature was maintained at 24–26ºC. However, in trial of effects of salinity, when the ambient room temperature of 18–24ºC was set up and the variation of day/night temperature was larger, *U. lactuca* died during the first 14 days. In ISW, *U. lactuca* showed a significantly higher SGR at 25–26ºC than at 20–21ºC. This result is similar to *U. curvata* (Malta *et al*., 1999) and the temperature condition for *U. lactuca* in an integrated system with prawn (Van Khoi & Fotedar, 2011), and *U. pertusa* (Liu & Dong, 2001). The temperature of 25ºC is higher than *U. rigida* temperature requirement (de Casabianca *et al*., 2002), and different from *U. lactuca* in the Netherlands (Malta *et al*., 1999). The high temperature level is similar to the summer sea surface temperature in WA (https://www.seatemperature.org/australia-pacific/australia/), when it is the growing season of *Ulva* (Ramus, 1978; Vermaat & Sand-Jensen, 1987; Yoshida *et al*., 2015).

This study demonstrated that the [K^+] in water is correlated to *U. lactuca* growth, since *U. lactuca* died in ambient ISW from the first week, but was sustained for longer in higher [K^+] ISWs, similar to the demand of other marine species culturing in ISW (Dinh, 2016; Fielder & Allan, 2003; Tantulo & Fotedar, 2007). *U. lactuca* in this study

required 33% of [K^+] in OW as only ISW33 resulted in positive SGR of *U. lactuca*. Similarly to *U. ohnoi* which prefers the Na:K at 47:1 (Yamashita *et al*., 2009), the ratios of Na:K in OW and ISW100 were 27:1 and 25:1, respectively, which resulted in lower SGR for *U. lactuca* than the ISW33, which provided a higher ratio of Na:K of 77:1 for *U. lactuca* growth in ISW33. The higher external K^+ may damage the cell membrane (Peterson *et al*., 1995), although the intracellular Na:K of *Ulva* is not affected by the ratio of Na:K in cultured medium (Yamashita *et al*., 2009). The biomass presented were collected before the termination of K^+ effect trial, in reality, the K^+ fortification effect lasted until total mortality of *U. lactuca* happened at all tanks, therefore, no *U. lactuca* tissue remained for proximate composition analysis, which was a limitation of this study.

The SGR of *U. lactuca* in this study was lower than *U. rotundata* and *U. intestinalis* (Hernández *et al.*, 2002), and *U. pertusa* cultured in OW under laboratory conditions (Kim & Han, 1999; Liu & Dong, 2001), but was similar to *U. lactuca* in Denmark (0.044–0.199% d^{-1}) (Geertz-Hansen *et al.*, 1993). The difference amongst these was due to the fact that *U. lactuca* used in these experiments were mature blades, where the thalli were over 10 cm, and these blades grow more slowly than young blades (Kraemer & Yarish, 1999). Moreover, the *U. lactuca* in this study was not freely floating in water with aeration provided, another factor that may affect the growth of *U. lactuca*, as floating *U. pertusa* grows in the ocean (Hiraoka *et al.*, 2004; Yoshida *et al*., 2015).

9.5 Conclusions

Salinity levels of 30–35 ppt, stocking density of 0.2 kg m^{-2} and nutrient enrichment of NH_4-N at 56 µM are suitable for culturing *U. lactuca* in OW, and they are recommended to evaluate the growth feasibility of *U. lactuca* in ISW.

In ISW, *U. lactuca* develop better under temperature of 25–26ºC than the lower one of 21–22ºC. K^+ fortification of 33% of [K^+] as in OW at the same salinity is required for *U. lactuca* cultured in ISW. *U. lactuca* is unable to grow in captivity longer than 42 days in either OW or K^+ISW, so a short culture season is recommended.

CHAPTER 10 GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

Culturing seaweed in inland saline water (ISW) is expected to be one way to reduce the adverse impacts of salinisation on agriculture (Borowitzka, 1997) to generate additional income for farmers. This is the first study to culture native or naturally distributed seaweed species in Western Australia (WA), under different environmental parameters, prevalent under ISW environment. Comparisons of the culture feasibilities of four targeted species including one green, two brown and one red seaweeds are discussed in this chapter to highlight their requirements forthe selected environmental variales.

10.1 Seasonal Effects on Growth of Seaweeds

The seasons significantly influence the growth of the seaweeds under similar experimental conditions. The SGR of *U. lactuca* during the first 14 days in the ocean water (OW) enriched 56 µM ammonium (NH_4-N) (OW_NH_4) was significant ($P<0.5$) difference between trial "effect of temperature on growth of *U. lactuca*" (0.60±0.22) conducting in early autumn (Table 9.4) and trial "effect of potassium (K^+) fortification on growth of *U. lactuca*" conducting in early winter (0.18±0.07) (Table 9-7). Although the environmental factors (temperature, light) of the trials were the same, the natural growth of blades of *U. lactuca* collected from the field were different depending on the time of trials. *U. lactuca* also responded differently to the ammonium enrichment depend on seasons: in the trial effect of stocking density and NH_4-N enrichment, conducted during winter, the NH_4-N 56 µM gave a significantly higher SGR of *U. lactuca* than ambient OW (Table 9.3). However, in the trial of effect of temperature on *U. lactuca* growth, conducted in early autumn, there was no NH_4-N enrichment effect on the growth of *U. lactuca* (Table 9.4).

The seasonal effect showed more clearly in case of *L. catenata*. When *L. catenata* was cultured in late winter, regarding the similar conditions of the K^+ fortification, *L. catenata* showed positive growth (Chapter 8) than when it was cultured in the early winter (Chapter 4). Early winter significantly affected the growth of *L. catenata* since *L. catenata* died in all water types from the first 14 days, including ISW66, where it showed the highest SGR during 56 days of culture in late winter (Table 10-1). According to the observations from Matilda Bay, Swan River, WA, the growth season for *L. catenata* is from spring to early summer, which is similar to Japanese *L. catenata* (Lee, 1978).

Similarly, *Sargassum* was also affected by the seasons. Under the same K^+ levels and environmental factors, *S. linearifolium* was cultured in early summer that resulted in a significantly lower SGR than corresponding numbers in midwinter or early spring (Table 10-2). *S. linearifolium's* growing season in the wild is from August to November (Martin-Smith, 1993) that proved the growth of *Sargassum* under laboratory conditions is similar to that in the wild.

The stagnant growth of *S. linearifolium* in the non-enriched OW and K^+ISW during the period from 29-42th day (Chapter 6) again proved that the seasonal growth of *S. linearifolium* under the laboratory conditions is similar to that under the outdoor natural conditions, where the biomass growth season ends by October (Martin-Smith, 1994). The seasons did not show a significant effect on the *S. podacanthum* growth that demonstrated a similar growth in winter and summer seasons. However, the biomass of *S. podacanthum* increased in very early summer and then decreased afterward, reflecting the growth of *S. podacanthum* was similar to *S. linearifolium* (Table 10-2).

Table 10-1. Seasonal effects on *L. catenata* growth in the OW and K^+ISW

SGR	Season	OW	ISW0	ISW33	ISW66	ISW100
Day 1–14	Early winter (Chapter 4)	$_1$-0.87±0.23^a	$_1$-1.19±0.86^a	$_1$-1.48±0.23^a	$_1$-1.28±0.30^a	$_1$-1.83±0.69^a
	Late winter (Chapter 8)	$_2$1.44±0.20^{ab}	$_2$0.49±0.38^a	$_2$0.78±0.29^a	$_2$2.08±0.18^b	$_2$1.31±0.54^{ab}
Day 1–28	Early winter (Chapter 4)	$_1$-0.87±0.23^a	$_1$-1.00±0.34^a	$_1$-1.53±0.23^a	$_1$-0.97±0.14^a	$_1$-1.43±0.49^a
	Late winter (Chapter 8)	$_2$0.20±0.21^a	$_2$0.31±0.13^{ab}	$_2$0.52±0.26^{ab}	$_2$0.89±0.13^b	$_2$0.02±0.22^b

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column of one time period sharing a common subscript are not significantly different at P<0.05 (t-test, n=4)

Table 10-2. SGR (% d^{-1}) of *Sargassum* spp. in different seasons

SGR	*S. linearifolium*				*S. podacanthum*			
	OW		ISW100		OW		ISW100	
	Winter	Early summer	Winter	Early summer	Winter	Early summer	Winter	Early summer
Day 1–14	1.92±0.12	1.44±0.41	2.57±0.66^a	0.12±0.66^b	-0.16±0.43^a	0.49±0.08^b	0.97±0.41	0.49±0.22
Day 1–28	2.29±0.18	0.57±0.16	1.78±0.05^a	-0.16±0.76^b	-0.47±0.71	0.36±0.26	-0.46±0.47	0.15±0.13
Day 1–42	2.02±0.10^a	-0.29±0.17^b	1.34±0.14^a	-0.47±0.46^b	-0.32±0.54	0.18±0.21	-0.17±0.33	-0.01±0.12
Day 1–56	1.50±0.03^a	-0.51±0.10^b	1.03±0.14^a	-0.58±0.32^b	-0.26±0.41	-0.08±0.22	-0.17±0.24	-0.06±0.18
Day 1–70	1.07±0.03^a	-1.01±0.20^b	0.81±0.09^a	-1.21±0.28^b		-0.16±0.17		-0.22±0.02
Day 1–84	1.03±0.08^a	-1.18±0.10^b	0.52±0.09^c			-0.27±0.17		-0.28±0.06

Values (mean±SE) within a row in the same water of one species sharing a common superscript are not significantly different (t-test; P>0.05; n=4).

10.2 Feasibility of Cultivating the Four Different Species in the Inland Saline Water: Comparative Analysis of Potassium Fortification, Temperature and Nutrient Effects

Potassium (K^+) is important for the growth of algae, which plays a crucial role in photosynthesis (Checchetto *et al*., 2013) and protein synthesis (Blumwald *et al*., 2000), and cannot be replaced by any other cations (Yarish *et al*., 1980). This study proves that the growth of all the studied seaweed species was adversely affected by the K^+ deficiency in the ISW, similar to other marine animal species (Dinh, 2016; Fielder & Allan, 2003; Prangnell & Fotedar, 2006b; Tantulo & Fotedar, 2006), when all the studied seaweed species died in the non-fortified ISW during the first 14 days.

External [K^+] determines the internal and external cell gradient that allows Na^+ and K^+ exchange (Blumwald, 2000). In crustaceans, the normal ratio between Na^+ and K^+ is regulated by the Na^+/K^+ ATPase enzyme (Burton, 1995; Roer & Dillaman, 1993). Similarly, this is also true for higher plants and seaweeds specieswhere the gradient is regulated by the internal exchange mechanism between Na^+ and K^+ (Blumwald *et al*., 2000). The different ionic gradient is crucial for nutrient transport within cells (Blumwald *et al*., 2000). Since the [K^+] in the ambient ISW in our trials were too low to support the differential gradient, leading to inhibition of the nutrient transport into cells for protein synthesis, resulting in lower survival and growth in ISW. The low [K^+] in ISW also inhibits the normal physiological processes of cultured algae. For instance, the marine diatom *Phaeodactylum tricornutum* releases K^+ from its cell when exposed to a free K^+ solution and looses its photosynthetic capacity (Overnell, 1975). Similarly, lower [K^+] in the cultured medium decreases *Bostrychia radicans* and *Caloglossa leprieurii* intracellular [K^+] (Yarish *et al*., 1980).

The K^+ is actively and passively uptaken in both the low and high [K^+] media, hence the K^+ can be accumulated and maintained at high concentrations in cells, while Na^+ can be extruded and kept at lower concentrations (Tromballa, 1978). In a lower K^+ medium, the intercellular K^+ decreases (Zimmermann & Steudle, 1971), and Na^+ is accumulated from the medium (Nuccitelli & Jaffe, 1976), the tissue K^+ is reduced and

unable to assist the seaweed growth. A rapid decline in the diatom *Asterionella formosa* growth rate occured in a culture medium after a proportional depletion of [K^+] (Jaworski *et al.*, 2003; Talling, 2010). The demand of energy for K^+ uptake resulted in the poor survival and growth of seaweed in the ambient ISW in *S. linearifolium* until day 56th, and *S. podacanthum, L. catenata, U. lactuca* in the first 14 days of the culture. The longer survival time of *S. linearifolium* in the ISW100 with a positive cumulative specific growth rate (SGR) until day 84th, without the nutrient supplement, showed its relative higher ability to grow in the K^+ISW than *L. catenata* and *U. lactuca* wherein the growth was limited until the day 42th (Table 10-3).

The need of ther K^+ in water for the seaweed growth varies by species to species (Yarish *et al*., 1980), similar to higher plants where the K^+ deficiency impacts are species specific (Sale & Campbell, 1987). Brown seaweed *Sargassum* required a similar concentration of K^+ in OW to sustain its growth, whereas red seaweed *L. catenata* and green seaweed *Ulva lactuca* required lower [K^+], from 33 to 66% in OW at the same salinity. This different requirements of K^+ in ISW of the seaweeds can be explained by the differences of the impact of the K^+ on the photosynthesis and protein synthesis of the seaweeds (Blumwald *et al*., 2000; Checchetto *et al*., 2013). The physiological mechanisms of seaweeds, in terms of their dependency on K^+ as a macronutrient to support their growth, is comparable and similar to higher plants. The K^+ deficiency significantly reduce the chlorophyll a and b formation in cotton *Gosypium hirsutum* (Onanuga *et al*., 2012), maize (*Zea Mays* L.) (Zhao *et al*., 2016), and causes chlorophyll break-down in *Ananas comos*us L. (Sideris & Young, 1945). The K^+ activates enzymes for protein and carbohydrates synthesis (Checchetto *et al*., 2013), and affects the CO_2 assimilation rate of *Carya cathayensis* leaves (Jin *et al*., 2011). All the seaweeds have a chloroplast envelope, but only green and red algae have chloroplast eukaryotic (Sze, 1998). The red, green and brown seaweeds have chlorophyll as the principal photosynthetic pigments, in addition, the red seaweed has phycoerythrobilin, and the green seaweed has chlorophyll a, whereas the brow seaweed has chlorophyll c_1, c_2 and fucoxanthin (Sze, 1998). Those differences in photosynthesis pigments of the brown seaweed from the green and red seaweeds may lead to a higher need of [K^+] in ISW for the growth of *S. linearifolium* than *U. lactuca*

and *L. catenata*, where the similarity in chloroplast eukaryotic of the red and green seaweeds may result in a similar need for K^+ in ISW of *U. lactuca* and *L. catenata*. The low K^+ supply results in a low K^+ content and low chlorophyll a+b which leads to a lower CO_2 assimilation rate and a low total soluble protein content in leaves of *Carya cathayensis* (Jin *et al*., 2011).

The previous experiments have revealed that the different effects of low [K^+] in ISW on the survival and growth of seaweed cultured in different [K^+] levels in ISW (Bui, Luu, & Fotedar, 2017; Bui, Luu, Fotedar, *et al*., 2017) and as described in Chapter 9. The K^+ effect could be species dependent. There was a higher SGR of *S. linearifolium* than *L. catenata* and *U. lactuca* during all culture periods, as well as a longer survival time of *S. linearifolium* in ISW100. Whereas a lower SGR of *S. linearifolium* than *L. catenata* and *U. lactuca* was recorded in lower [K^+] ISWs (ISW0 and ISW33). The role of the K^+ in the growth of seaweeds is important for supporting the survival of these three seaweed species, and has a complexity in terms of its relation and the compounded effect to other cations, which requires further investigation.

L. catenata presented a higher SGR than *U. lactuca* under similar conditions of K^+ fortification in the first 35 days of the cultured period. During that period, a positive SGR of *L. catenata* proved having higher sustainable growth feasibility than a negative SGR of *U. lactuca* in all the K^+ISW levels. *U. lactuca* responded to K^+ISW in a similar way to *S. podacanthum*, where the positive SGR of *S. podacanthum* only presented in the ISW100 in the first fortnight, and then a negative SGR remained over the rest of the cultured period in all other K^+ISW waters. The study also proved that *S. podacanthum* needs nutrient supplementation to be grown in the K^+ISW (Table 6-13), as K^+ISW cannot sustain a positive growth rate of *S. podacanthum* after 14 days (Table 5-7), whereas *S. linearifolium* showed a similar response in the ISW as *L. catenata*, whereby these two species grew for up to 56 days in the ISW100.

Of the four seaweed species that were cultured in K^+ISW, *S. linearifolium* sustained longest in the ISW, and showed the highest growth rate (mg d^{-1} L^{-1}). Under the optimal K^+ISW (ISW100), *S. linearifolium* increased its biomass until day 56^{th} and the growth rate gradually decreased over time, as in the OW (Figure 10-1 and Figure 10-5). *L.*

catenata exhibited positive growth up to day 42th in ISW33 and reached its highest growth rate at day 42th then declined (Figure 10-3). K^+ played a more important role in the growth of *S. podacanthum* than *S. linearifolium*, as *S. podacanthum* died in the K^+ deficiency conditions sooner than *S. linearifolium* (Figure 10-2, Figure 10-4) and the growth rate in ISW100 of *S. podacanthum* was significantly lower than *S. linearifloium* in the first 28 days (Figure 10-5).

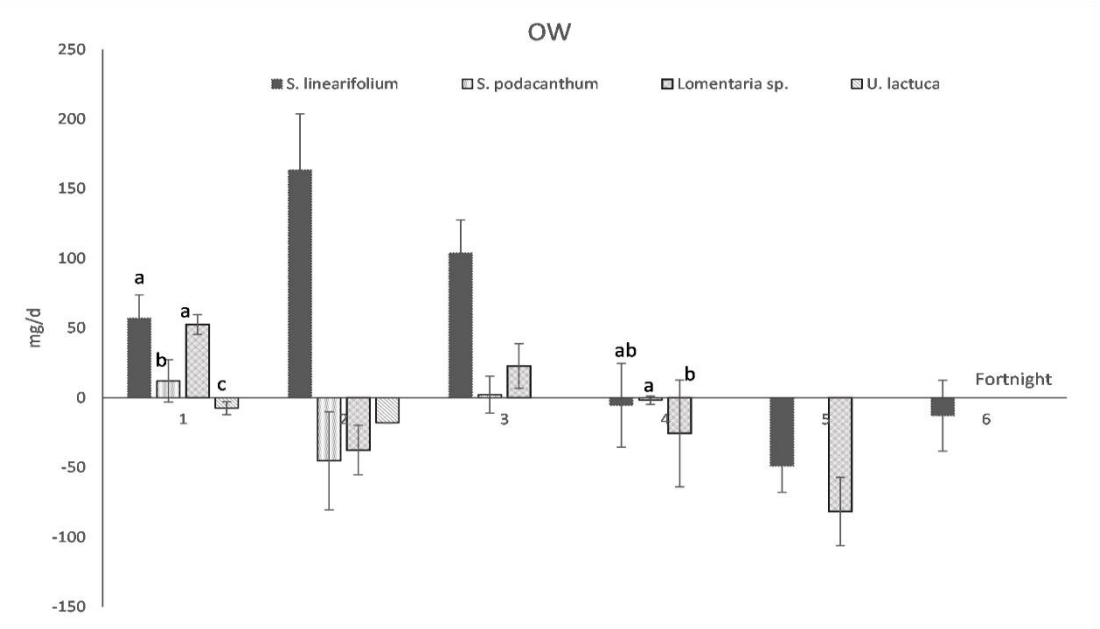

Figure 10-1. Growth rate (mg d^{-1} L^{-1}) of the four seaweed species in OW

Values (mean±SE) in bars sharing a common letter in a fortnight are not significantly different (LSD test; P>0.05)

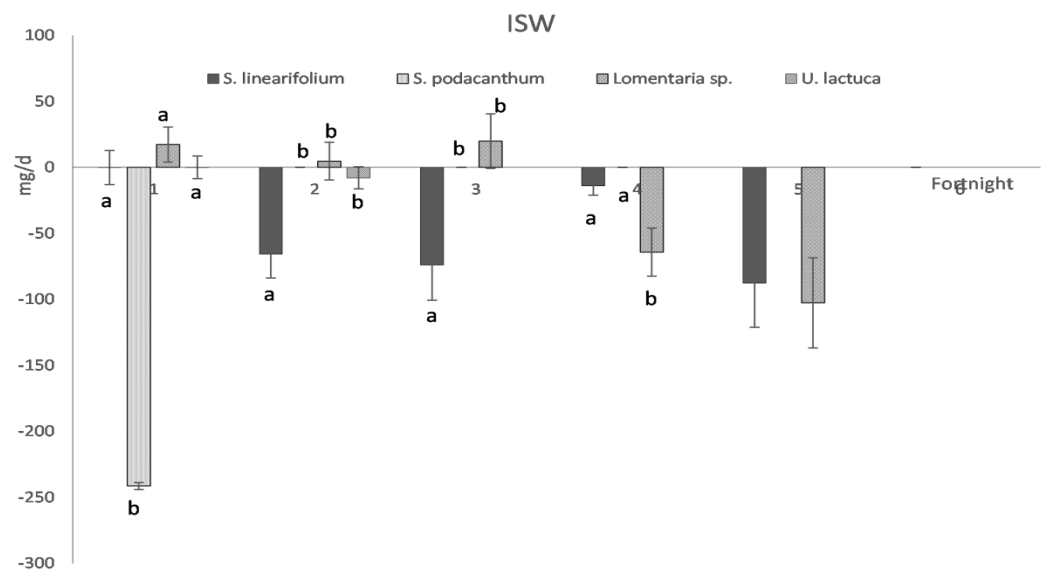

Figure 10-2. Growth rate (mg d^{-1} L^{-1}) of the four seaweed species in ISW

Values (mean±SE) in bars sharing a common letter at a fortnight are not significantly different (LSD test; P>0.05)

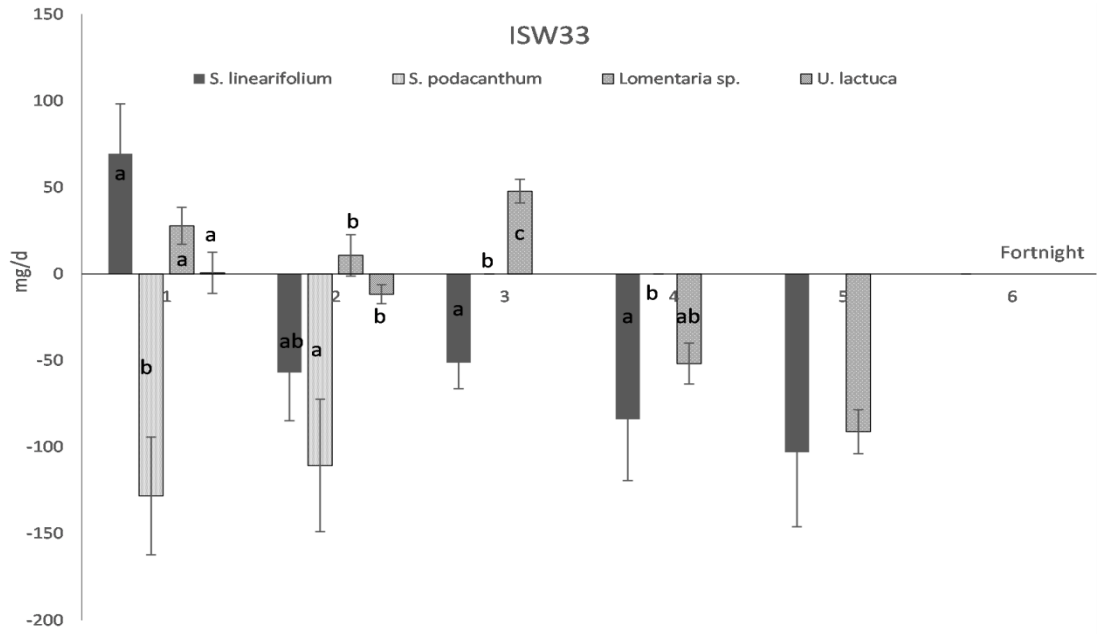

Figure 10-3. Growth rate (mg d^{-1} L^{-1}) of the four seaweed species in ISW33

Values (mean±SE) in bars sharing a common letter at a fortnight are not significantly different (LSD test; P>0.05)

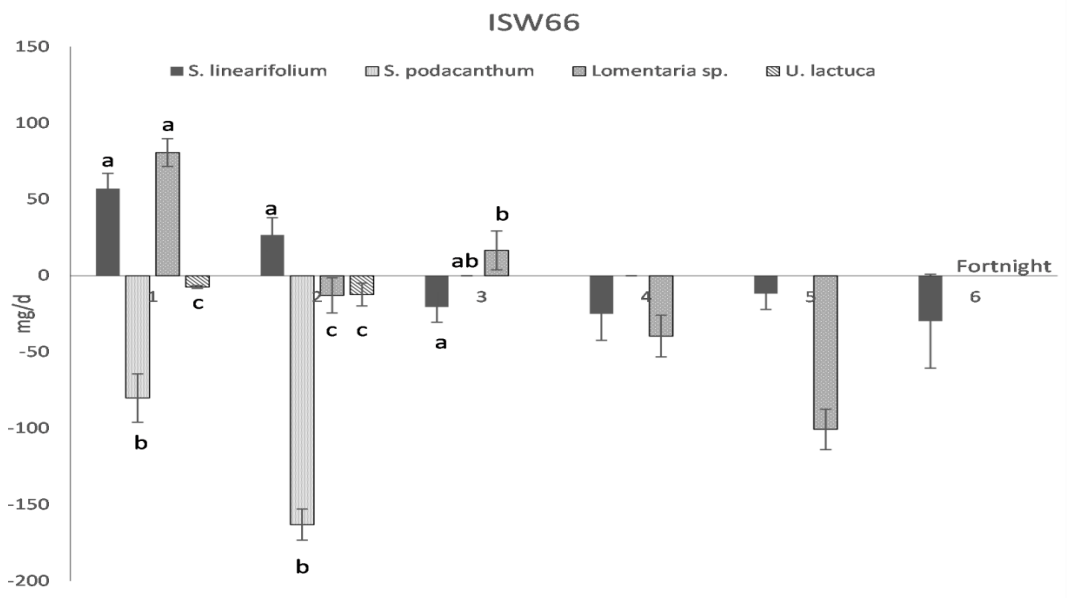

Figure 10-4. Growth rate (mg d^{-1} L^{-1}) of the four seaweed species in ISW66

Values (mean±SE) in bars sharing a common letter at a fortnight are not significantly different (LSD test; P>0.05)

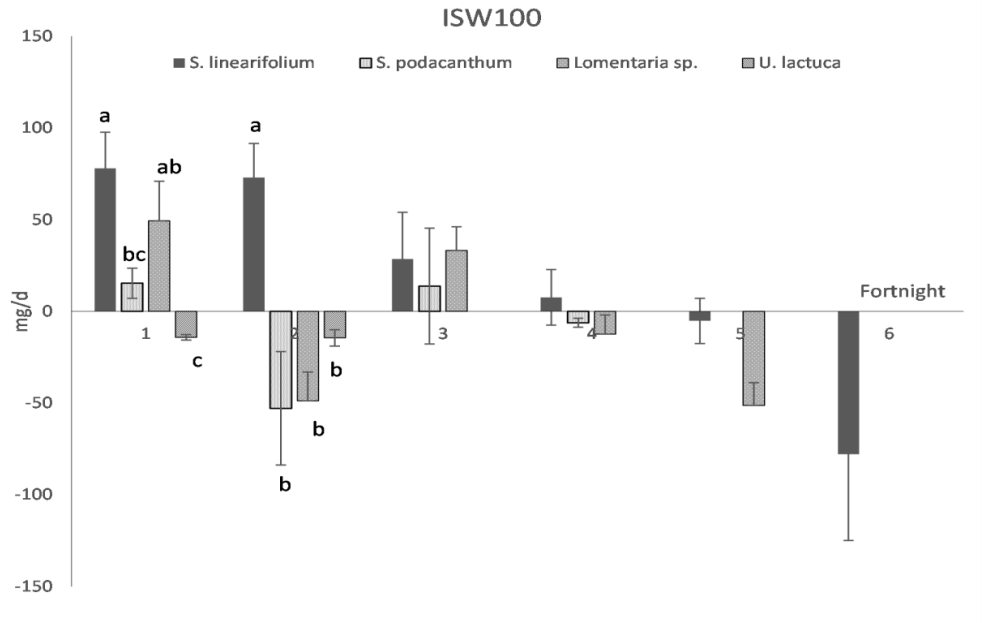

Figure 10-5. Growth rate (mg d^{-1} L^{-1}) of the four seaweed species in ISW100

Values (mean±SE) in bars sharing a common letter at a fortnight are not significantly different (LSD test; P>0.05)

Table 10-3. Comparison of cummulative SGR (% d^{-1}) of the selected seaweed species culturing in OW and K^+ISW

Time	Species	OW	ISW0	ISW33	ISW66	ISW100
Day 1–28	*S. linearifolium*	$_{1}2.29\pm0.18^{a}$	$_{1}-1.22\pm0.50^{b}$	$_{1}0.19\pm0.14^{c}$	$_{1}1.06\pm0.06^{d}$	$_{1}1.78\pm0.05^{ad}$
	S. podacanthum	$_{2}-0.47\pm0.71$				$_{2}-0.46\pm0.47$
	L. catenata	$_{3}0.20\pm0.21^{a}$	$_{2}0.31\pm0.13^{ab}$	$_{1}0.52\pm0.26^{ab}$	$_{1}0.89\pm0.13^{b}$	$_{3}-0.02\pm0.22^{b}$
	U. lactuca	$_{23}-0.16\pm0.09^{a}$	$_{2}-0.20\pm0.04^{ab}$	$_{1}0.03\pm0.06^{b}$	$_{2}-0.14\pm0.02^{ab}$	$_{2}-0.16\pm0.09^{a}$
Day 1–42	*S. linearifolium*	$_{1}2.02\pm0.10^{a}$	$_{1}-3.78\pm2.33^{b}$	-0.46 ± 0.17^{a}	0.55 ± 0.10^{a}	$_{1}1.34\pm0.14^{a}$
	S. podacanthum	$_{2}-0.32\pm0.54$				$_{2}-0.17\pm0.33$
	L. catenata	$_{12}0.32\pm0.25$	$_{2}0.37\pm0.16$	0.73 ± 0.16	0.71 ± 0.19	$_{12}0.31\pm0.17$
	U. lactuca	$_{2}-0.24\pm0.08^{a}$	$_{2}-0.27\pm0.06^{ac}$	0.03 ± 0.09^{b}	-0.22 ± 0.16^{bc}	$_{2}-0.20\pm0.05^{a}$
Day 1–56	*S. linearifolium*	$_{1}1.50\pm0.03^{a}$	-3.15 ± 1.80^{b}	-2.95 ± 1.99^{b}	0.24 ± 0.19^{ab}	$_{1}1.03\pm0.14^{a}$
	S. podacanthum	$_{2}-0.26\pm0.41$				$_{2}-0.17\pm0.24$
	L. catenata	$_{2}0.07\pm0.16^{ab}$	-0.23 ± 0.24^{a}	0.23 ± 0.12^{ab}	0.30 ± 0.07^{b}	$_{2}0.14\pm0.15^{ab}$
Day 1–70	*S. linearifolium*	$_{1}1.07\pm0.03^{a}$			$_{1}0.14\pm0.12^{b}$	$_{1}0.81\pm0.09^{c}$
	L. catenata	$_{2}-1.05\pm0.58$	-1.28 ± 0.31	-0.56 ± 0.07	$_{2}-0.83\pm0.13$	$_{2}-0.47\pm0.09$

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; $P>0.05$; n=4). Values (mean±SE) within a column of one time period sharing a common subscript are not significantly different at $P<0.05$ (t-test, n=4)

Table 10-4. Comparison of SGR (% d^{-1}) between two *Sargassum* species in NH_4-N:PO_4^{3-}-P (µM) enriched K^+ISW

Time	Species	Control	80:8	120:12	160:16	200:20	240:24
Day 1–14	SL	0.12±0.66	0.09±0.37	0.47±0.26	0.44±0.40	-0.01±0.38	0.97±0.48
	SP	0.49±0.22^{a}	-0.29±0.27^{ac}	0.66±0.64^{ab}	1.70±0.64^{b}	-0.74±0.53^{c}	0.66±0.45^{ab}
Day 1–28	SL	-0.16±0.76^{a}	-2.69±1.60^{b}	$_1$-0.37±0.17^{ab}	-0.39±0.26^{ab}	-1.33±0.40^{ab}	-0.41±0.25^{ab}
	SP	0.15±0.13^{ac}	-0.15±0.21^{ac}	$_2$0.54±0.18^{ab}	1.06±0.31^{b}	-0.38±0.30^{c}	0.35±0.23^{abc}
Day 1–42	SL	-0.47±0.46^{a}	$_1$-1.68±0.45^{b}	$_1$-1.43±0.14^{b}	$_1$-1.54±0.35^{b}	$_1$-2.01±0.17^{b}	-0.72±0.24^{ab}
	SP	-0.01±0.12^{a}	$_2$-0.34±0.28^{a}	$_2$0.13±0.18^{a}	$_2$0.83±0.25^{b}	$_2$-0.21±0.15^{a}	$_2$-0.13±0.13^{a}
Day 1–56	SL	-0.58±0.32^{a}	-1.35±0.36^{b}	-	-1.42±0.67^{b}	-	-1.07±0.10
	SP	-0.06±0.18^{ab}	-0.41±0.26^{a}	0.16±0.19^{bc}	0.57±0.18^{c}	-0.37±0.26^{ab}	-0.03±0.18^{ab}
Day 1–70	SL	-1.21±0.28	-	-	-	-	-
	SP	-0.22±0.02^{ab}	-0.42±0.21^{a}	-0.27±0.37^{a}	0.26±0.09^{b}	-0.19±0.15^{a}	-0.14±0.07^{ab}

Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column of one time period sharing a common subscript are not significantly different at P<0.05 (t-test, n=4)
SL: *S. linearifoium*; SP – *S. podacanthum.*

The water quality of the nutrient-enriched media of *S. linearifolium* and *S. podacanthum* was similar in terms of nitrite (NO_2^--N) in both OW and K^+ISW. However, the nitrate (NO_3^--N) and PO_4^{3-}-P concentrations in waters culturing *S. podacanthum* were lower than in waters culturing *S. linearifolium*, and were positively correlated to the decrease in the *Sargassum*, whereas higher NO_3^--N and PO_4^{3-}-P concentrations were linked to the higher mortality rate of the *Sargassum* (Table 6-10, Table 6-11). In the case of *U. lactuca*, this conclusion was again proved when a significant increase of [NO_3^--N] in the K^+ISW culturing *U. lactuca* at the termination of the experiment simulated the negative SGR of *U. lactuca* (Table 9-8).

The need for NH_4-N and PO_4^{3-}-P was species-dependent, and *U. lactuca* and *L. catenata* did not need the supplement of NH_4-N and PO_4^{3-}-P for their higher growth rates. *U. lactuca* grew faster at the NH_4-N 56 µM, which was similar to the conclusion of Campbell (2001).

L. catenata also responded similarly to all enriched nutrient levels, without a requirement of PO_4^{3-}-P supplementation in K^+ISW. For *L. catenata* better growth, no greater than a concentration of NH_4-N 100 µM weekly supplementation was recommended. As this is the first indoor study on *L. catenata* culture, these preliminary results are useful and serve as a baseline for future research.

Sargassum responded differently to the nutrient enrichment, represented by NH_4-N and PO_4^{3-}-P supplementation. *S. linearifolium* did not response to the nutrient enrichment. *S. linearifolium* biomass decreased in all the nutrient-enriched waters toward the termination of the cultured period, and received no significant influence from the nutrient supplementation. In addition, no *S. linearifolium* biomass gain was recorded over the experiment period, except for ambient OW during the first 14 days. However, *S. podacanthum* grew fastest at 160:16 µM NH_4-N:PO_4^{3-}-P, where the SGR of *S. podacanthum* showed no significant difference from K^+ISW and OW. In other nutrient levels, the *S. podacanthum* grew faster in OW than in K^+ISW. Generally, *S. podacanthum* responded better than *S. linearifolium* to the nutrient enrichment, resulting in higher SGR at all nutrient-enriched levels in both OW and K^+ISW (Table 10-4). In addition to the K^+ fortification to ISW, *S. podacanthum* required NH_4-

N:PO_4^{3-}-P 160:16 µM for higher growth, whereas *S. linearifolium* grew well in non-enriched K^+ISW. However, *S. baccularia* required NH_4-N:PO_4^{3-}-P from 3:0.3 to 5:0.5 µM, which was continuously provided in the cultured media (Schaffelke & Klumpp, 1998), and this [PO_4^{3-}-P] was lower than the [PO_4^{3-}-P] in the cultured media for *S. linearfolium* and *S. podacanthum* (Table 5-4, Table 5-12).

In addition to the salinity, pH and temperature are important to the growth and nutrient uptake of seaweeds (Ding *et al*., 2013; Endo *et al.*, 2013; Hidayat *et al*., 2015; Hwang *et al*., 2015; Lignell & Pedersén, 1989). The temperature affects the growth of seaweeds through the effects on the photosynthetic activity (Ding *et al*., 2013) and the nutrient uptake (Duke *et al*., 1989; Hwang *et al*., 2004). Each seaweed species prefers a range of temperature and pH for the optimal growth, including different species within the same family. The ambient pH of ISW at about 8 was suitable for all the studied seaweeds. Whereas the temperature preference is species-specific. For instance, *U. lactuca* from this study preferred 25–26ºC for a higher growth rate. The temperature preference of *U. lactuca* in this study was similar to *U. curvata* (de Casabianca *et al*., 2002), whereas *U. rigida* reached a higher growth rate at 17ºC (de Casabianca *et al*., 2002). A temperature of 20–22ºC was preferred for *Sargassum* spp. growth, which was higher than the requirement for *S. thunbergia*, which reached its maximal growth rate at 15ºC (Choi *et al*., 2009; Yamauchi, 1984). The preferred temperature range is 20–30ºC for *S. patens* (Endo *et al*., 2013), 22–28ºC for *S. linearifolium* (Martin-Smith, 1993). The *S. linearifolium* could not survive in temperature higher than 29ºC (Martin-Smith, 1993). Similarly, young seedlings of *S. henslowianum* showed reduced growth at temperature of 30ºC (Chen & Zou, 2014). Although temperature was not studied in Chapter 4, Section 4.2.2, wide water temperature fluctuations were considered a factor that affected the growth of two *Sargassum* spp. Under the outdoor conditions, as a consequence of the large variation of temperature (13–38ºC), the *Sargassum* spp. could grow in the period of no longer than 42 days. In the nutrient enriched experiments (Chapter 6), the temperature trend of the cultured media was different between the two *Sargassum* species. The cultured media of *S. podacanthum* had the temperature increased during the middle of the experiments. A high temperature of 25ºC for *S. podacanthum* was maintained for 28

days, from day 43th to 70th, and was then reduced to the preferred range of *Sargassum* spp. at the end of the trial, similar to the commencement. However, this short-term high temperature of the cultured media did not negatively affect the growth of *S. podacanthum* (Table 6-11). Under the effect of high temperature for a longer period, which remained at about 25^{o}C from the day 28th to the end of the experiment, the high temperature did negatively affect the SGR of *S. linearifolium* (Table 6-4), which could be one of the causes of the deceased *S. linearifolium* from day 56 of the cultured period. These results again proved that the *Sargassum* can sustain a short period (of about 28 days) under a high temperature of 25–26^{o}C (see Conclusion in Chapter 7). Only *L. catenata* could stand a broad range of temperature, from 20–26^{o}C, without any significant difference of *L. catenata* SGR in this range of temperature.

In general, the seaweed species were cultured at the same stocking density of 0.2 kg m^{-2} (equivalent to 0.8 kg m^{-3}). The green seaweed *U. lactuca* and red seaweed *L. catenata* preferred the salinity of 30–32 ppt and the K^+ fortification for ISW at the 33–66% of the [K^+] in OW. These seaweed species grew faster in the nutrient enriched 33–66% K^+ISW, at the level of weekly enriched NH_4-N 56 µM and 100 µM for *U. lactuca* and *L. catenata*, respectively. Whereas the *S. podacanthum* required a weekly supplementation of a combination of NH_4-N and PO_4^{3-}-P at 160:16 µM in 100% K^+ISW. However, *S. linearifolium* did not require any nutrient supplementation in 100% K^+ISW (Table 10-5).

Table 10-5. Selected physical and chemical conditions where the seaweeds achieved the higher SGR (% d^{-1})

Criteria	***L. catenata***	***U. lactuca***	***S. linearifolium***	***S. podacanthum***
Salinity (ppt)	30–31	30–31	34–35	34–35
Temperature (^{o}C)	21–22	25–26	20–22	20–22
Nutrient enrichment (µM)	NH_4-N 100	NH_4-N 56	Non	NH_4-N:PO_4^{3-}-P 160:16
K^+ fortification (% of [K^+] in OW)	66	33	100	100

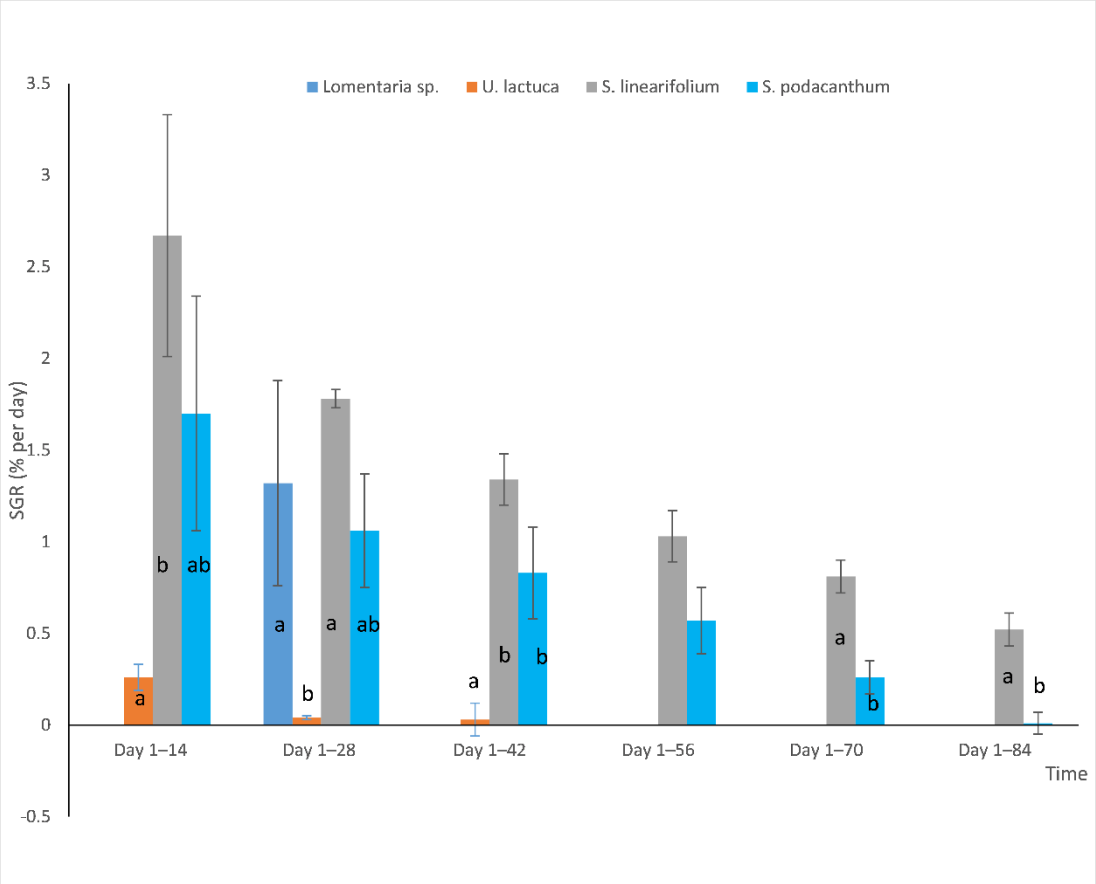

Figure 10-6. The SGR (% d^{-1}) of the four seaweed species under their preferable conditions (SGR values (mean±SE) within a time frame sharing a common letter are not significantly different (LSD test or t-test; P>0.05; n=3)

The *S. linearifolium* presented the most suitable candidate to grow in K^+ISW, following by *S. podacanthum*, and *L. catenata* (Figure 10-6), respectively. In the K^+ISW at the OW-equivalence [K^+], at favourite temperature of 20–22ºC, without nutrient enrichment, the SGR of the *S. linearifolium* in 84 days was higher than the SGR of *S. podacanthum* under favourable NH_4-N:PO_4^{3-}-P 160:16 µM enrichment in K^+ISW. The SGRs of the two *Sargassum* species were similar to the SGR of *L. catenata* in the first month of the cultured period, and higher than the SGR of *U. lactuca* in the period of 42 days (Figure 10-6). In this study, the brown seaweed survived longer in the K^+ISW than the red and green seaweeds, in addition to the higher SGR, the *Sargassum* spp. presented as a potential candidate to grow in ISW in WA. Although the green seaweed *U. lactuca* was more common than the three other seaweed species, towards its tolerance to K^+ISW was shortest.

10.3 Conclusions

This study presented the first attempt to culture seaweeds in ISW in WA. Particularly, this has been the first study to culture *L. catenata* indoors.

The K^+ deficiency in ISW results in the mortality of seaweeds during the early period of the cultivation and hence, the K^+ fortification either by the grade KCl or the potash of sulphate K_2SO_4 is necessary to sustain the growth. However, the need for the K^+ is species-dependent. The red seaweed *Grateloupia suspectinata* and brown seaweed *Cystophora subfacinata* presented lower toletance in ISW and K^+ISW than other species of red seaweed and brown seaweed, such as *L. catenata* and *Sargassum*, respectively. Therefore *G. suspectinata* and *C. subfacinata* were not studied further. The green seaweed *U. lactuca* and the red seaweed *L. catenata* prefer the [K^+] in ISW equivalent to 33–66% of the [K^+] of OW at a similar salinity. However, *Sargassum* spp. requires a higher concentration of the K^+, at a level similar to its concentration in OW at the same salinity. Among the six candidates of seaweed species selected to be tested for their culture potential in ISW, *Sargassum* spp. presented the highest SGR and longest survival time in K^+ISW.

It is possible to grow seaweed species in K^+ISW of WA at the ambient pH and temperature ranging from 20–26^{o}C. The temperature requirement for seaweed culture depends on the species. For example, *U. lactuca* prefers a temperature of 24–26^{o}C to grow and *Sargassum* spp. grows faster at 20–22^{o}C, whereas a temperature of 21–26^{o}C is suitable for the *L. catenata* growth.

Seaweed species are able to grow in K^+ISW in a salinity similar or a lower salinity than OW. As the *Sargassum* was collected from the open sea, where salinity was 35 ppt, salinity was not a factor for its growth. However, *U. lactuca* and *L. catenata* were collected from a river at a lower salinity, and this study proved that the salinity of 30–35 ppt and 30–31 ppt were also suitable for *U. lactuca* and *L. catenata* culture, respectively.

Ammonium chloride and sodium dihydrogen phosphate can be used as nutrients to sufficiently provide NH_4-N and PO_4^{3-}-P for seaweed growth. A supplemented NH_4-N concentration of no greater than 100 µM for *L. catenata* and 56 µM for *U. lactuca* are

required. *L. catenata* and *U. lactuca* do not demand additional PO_4^{3-}-P in K^+ISW. However, *S. podacanthum* rrequires a combination of NH_4-N:PO_4^{3-}-P 160:16 µM in K^+ISW, whereas the ambient nutrient concentration is enough for *S. linearifolium* in K^+ISW.

The results in this study confirmed that seaweed growth is subjected to a seasonality. Early summer is growing season of *L. catenata* and *Sargassum* spp. A culture period of no longer than 42 days and 56 days are sufficient for *L. catenata*, and *Sargassum* spp., respectively, to grow.

10.4 Limitations of the Study and Future Research Recommendations

In the light of the following limitations of the present study and recommendations of the future research, it is envisaged that a few locally available seaweed species, around Perth metropolitical region, do have technical possibility to be cultured in K^+ISW. However, broad recommendation of the study would be to validate and demonstrate the outcomes of the present research under a commercial envorinment, where bioeconomic of the seaweed production is included.

The mortality of seaweeds toward the termination of the experiments resulted in a lack of adequate of sample quantities to conduct a proximate composition analysis. The nutrient and K^+ uptake by seaweed could not be determined due to this limitation. No comparison of the K^+ variation in the culture medium and seaweed tissues could be made, therefore the role of the K^+ in the cells could not be further investigated. Although NH_4-N and PO_4^{3-}-P were supplemented, NH_4-N and PO_4^{3-}-P uptake could not be measured, which was a limitation of this study.

The compounded effect of the K^+ to other cations in water, such as Na^+, and H^+, which are related to the Na^+/K^+ exchange and H^+ATPase activities at different levels of the K^+. A comprehensive understanding and detailing of the process of interactions among the cations in an intercelullar context, in relation to the effect of the K^+ deficiency ISW in seaweed culture, requires further investigation.

Further study on the K^+ and nutrient uptake of the seaweeds cultured in ISW is needed to assimilate the Na^+/K^+ roles, as well as clarify the mechanism of nutrient efflux and influx in seaweed tissues that may affect the seaweed growth. These require large-scale experiments to receive enough seaweed samples at each stage of the cultured period.

The study of salinity, pH and temperature are crucial for seaweed culture, particularly in a new cultured media such as the ISW. However, a salinity lower than 30 ppt has not been tested so far, whereas the *U. lactuca* may grow in a salinity of 5–40 ppt in the OW (Choi *et al*., 2010; Koeman & van den Hoek, 1981), and can also grow in an integrated system at 25 ppt salinity of the OW (Van Khoi & Fotedar, 2011). The growth of seaweeds should be further studied at a salinity range from 10–25 ppt as the salinity of the ISW is lower during the winter season. An ambient pH of ISW in this study was 7.0 to 8.4 that is considered the most suitable pH for all the targeted species. However, a very low pH under 5.5 or a higher pH above 9 can also be studied, to investigate whether the seaweeds can sustain their growth in nature where the ISW pH varies widely. A higher temperature than 26^{o}C and a lower temperature at about 15^{o}C are suggested to study for the application of growing seaweeds in the ISW in fields, where those parameters fluctuate widely.

Therefore, it is also recommended that more salinity, temperature, pH, nutrient and ionic profiles should be studied under K^+ISW environment in order to develop culture ranges of the selected seaweed species. It is also recommended that ionic composition of the selected seaweed species be conducted with the ionic profiles of the ISW.

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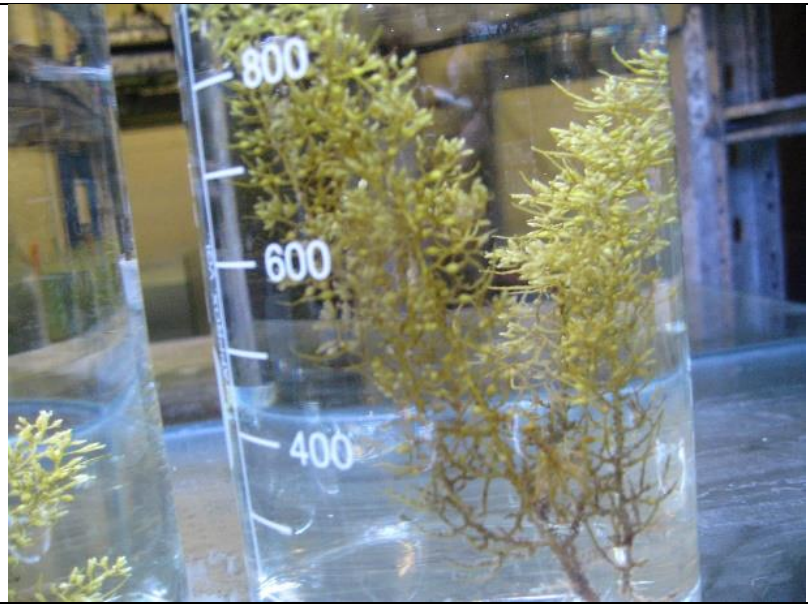
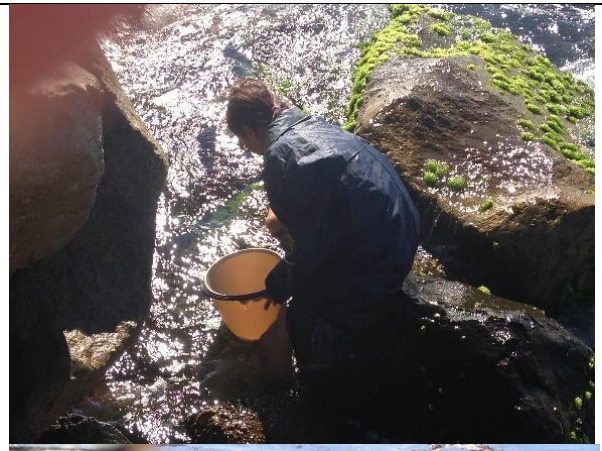
APPENDICES

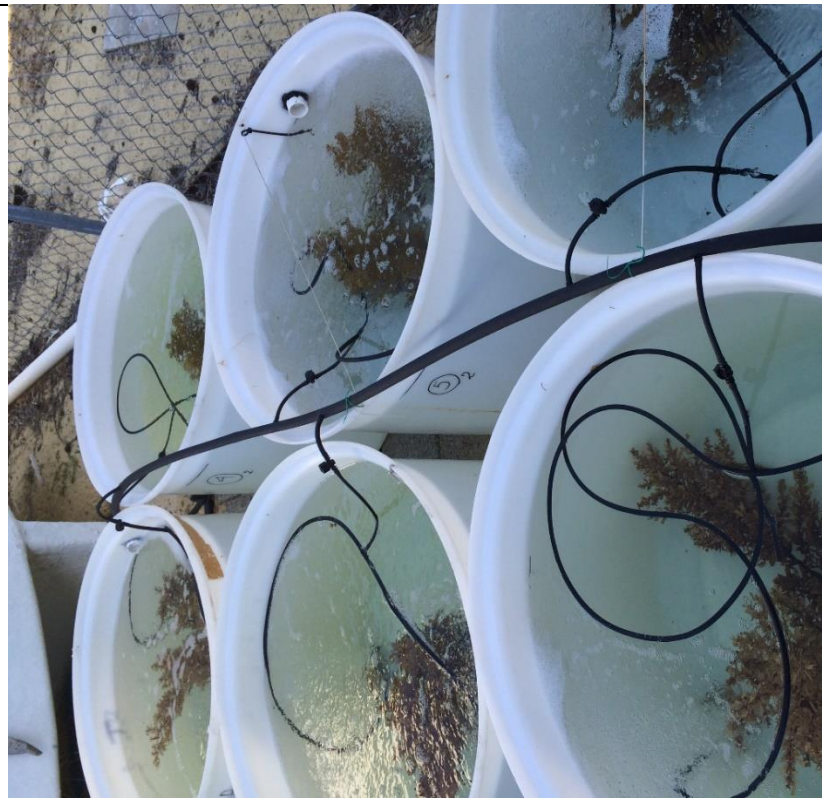
Appendix 1. Seaweed Collections and Experiments

Species	Seaweeds Collection	Experiments
Cystophora subfacinata		

Species	Seaweeds Collection	Experiments
Ulva lactuca		
Grateloupia suspectinata		

Species	Seaweeds Collection	Experiments
Lomentaria catenata		
Sargassum linearifolium		

Species	Seaweeds Collection	Experiments
Sargassum podacanthum		

Species	Seaweeds Collection	Experiments
S. podacanthum and *S. linearifolium* cultured oudoor	 	

Appendix 2. List of Publications

Appendix 2.1. Article in Journal of Aquaculture Research

Bui, H. T. T., Luu, T. Q., Fotedar, R., & Tantulo, U. (2017). Productivity of *Sargassum linearifolium* in potassium fortified inland saline water under laboratory conditions. *Aquaculture Research, 48*(11), 5631-5639. http://dx.doi.org/10.1111/are.13385

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ORIGINAL ARTICLE

WILEY Aquaculture Research

Productivity of *Sargassum linearifolium* in potassium fortified inland saline water under laboratory conditions

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Abstract

Growing aquatic species in inland saline water (ISW) is one way to reduce the adverse impact of ISW to agriculture farms. This 84-day laboratory-trial was conducted to study the growth of *Sargassum linearifolium* cultured in ocean water (OW), ISW, ISW fortified with potassium equivalent to 100% (ISW100), 66% (ISW66) and 33% (ISW33) of potassium in OW at 35 g/L. The biomass and cumulative specific growth rate (SGR) of *S. linearifolium* increased significantly ($p < .05$) with increased potassium in ISW until 56 days and then declined. The ISW100 and OW resulted in similar growth patterns and yielded peak biomass at day 42, proving static biomass for the next 28 days before declining. The biomass of *S. linearifolium* cultured in ISW and ISW33 significantly ($p < .05$) decreased and was lower than in ISW100 and died after day 56. The SGR of *S. linearifolium* in OW, ISW100 and ISW66 levelled off and showed no difference during the first 56 days. The *S. linearifolium* biomass and SGR negatively and significantly ($p < .05$) correlated with the concentrations of nitrate, phosphate in all waters. The increased potassium concentration in ISW similar to its concentration in SW brought the growth of *S. linearifolium* cultured to a level that was similar in OW.

KEYWORDS
inland saline water, potassium, potassium fortification, *Sargassum linearifolium*

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Appendix 2.2. Article in American Journal of Applied Sciences

Bui HTT, Luu TQ, Fotedar R., 2018. Effects of enriching nitrogen and phosphorus on the growth of *Sargassum podacanthum* cultured in potassium-fortified inland saline water. American Journal of Applied Science, 15 (3), 149-161. http://dx.doi.org/10.3844/ajassp.2018.149.161

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Original Research Paper

Effects of Enriching Nitrogen and Phosphorus on the Growth of *Sargassum Podacanthum* Cultured in Potassium-Fortified Inland Saline Water

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Abstract: Potassium-fortified inland saline water (K^+ISW) has shown potential for growing marine species, including seaweed species. The response of a brown seaweed species, *Sargassum podacanthum,* to nitrogen and phosphorus enrichments were evaluated by culturing the species for 84 days in K^+ISW and comparing it with Ocean Water (OW). The culture media were enriched weekly with ammonium chloride and sodium dihydrogen phosphate, with ammonium and phosphate ratios of 10:1 at five different concentrations 80:8, 120:12, 160:16, 200:20 and 240:24 µM. The culture medium with no enrichment was used as a control. The water quality and biomass of *S. podacanthum* were measured fortnightly. The *S. podacanthum* biomass increase significantly with different concentrations of the nutrient supplementations. The standing biomass and Specific Growth Rate (SGR) of *S. podacanthum* were similar in OW and K^+ISW in the absence of any nutrient supplementation and at the supplement concentration of ammonium and phosphate 160:16 µM. However, from day 42 onwards, at the ratios of 80:8, 120:12, 200:20 and 240:24, *S. podacanthum* cultured in OW grew significantly faster than in K^+ISW. In K^+ISW, optimal growth of *S. podacanthum* was observed at the 160:16 and the increase in biomass was significantly higher than the initial biomass until day 70, whereas at the other four nutrient supplement concentrations, the *S. podacanthum* biomass remained unchanged during the entire culture period. The nitrite, total Kjeldahl nitrogen and phosphate concentrations in water were found to be significantly ($p<0.05$) and negatively correlated ($p<0.05$) with *S. podacanthum* biomass. Therefore, the results showed that the enrichment of 160 µM ammonium and 16 µM phosphate is required in the K^+ISW for *S. podacanthum* to achieve optimal growth.

Keywords: Inland Saline Water, *Sargassum podacanthum,* Nutrient Enrichment, Potassium Fortification, Ammonium, Phosphate

Introduction

Mariculture, including seaweed culture, in Inland Saline Water (ISW) is considered as a potential expansion and diversification of aquaculture industry in Australia (Allan *et al.*, 2001). Seaweed culture can make use of salt-affected agricultural farms as it is less constrained by additional requirement for resources and changes in infrastructure than the culture of marine finfish and crustacean species. Therefore, growing *Sargassum*, in ISW can provide another source of commodity to the farmers with a lower capital investment than farming in the sea (Borowitzka, 1997) and can be an additional tool to protect the inland environment in Australia by combating the salinity problems (Ogburn, 1997).

At the same salinity, the level of potassium (K^+) concentration in ISW is lower than in Ocean Water (OW) in Australia (Allan and Fielder, 1997; Dinh, 2016) and USA (Boyd and Thunjai, 2003; Forsberg *et al.*, 1996) although other ionic profiles can be similar (Fotedar *et al.*, 2011; Prangnell and Fotedar, 2006a). Potassium is vital

Appendix 2.3. Article in American Journal of Applied Sciences

Bui HTT, Luu TQ, Fotedar R., 2018. Effects of temperature and pH on the growth of *Sargassum linearifolium* and *S. podacanthum* in potassium-fortified inland saline water. American Journal of Applied Science, 15 (3), 186-197. http://dx.doi.org/10.3844/ajassp.2018.186.197

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Original Research Paper

Effects of Temperature and pH on the Growth of *Sargassum linearifolium* and *S. podacanthum* in Potassium-Fortified Inland Saline Water

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Abstract: This study tested the effects of temperature and pH on water quality and the growth of *Sargassum linearifolium* and *S. podacanthum* in potassium-fortified Inland Saline Water (ISW) of Western Australia (WA), at two levels of pH (low pH range of 5.5-6.5 and ambient pH 7.0-8.2) and two levels of temperature (high temperature 26-27°C and ambient room temperature of 20-22°C) in triplicate for 42 days. The pH of ISW in WA varies from 3.9 to 9.1, whereas the temperature is from 6.1-28.1°C. The results showed that the high temperature initiated the mortalities of the both *Sargassum* species from the first 14 days of culture period. The high temperature also resulted in a reduction of dried weight and ash content of these two species of *Sargassum* by the end of the trial. *S. linearifolium* temperature tolerant threshold was larger than *S. podacanthum*. Since the day 14, the *S. linearifolium* biomass and specific growth rate were higher than *S. podacanthum* at both temperature levels under ambient pH. Higher crude protein in *S. linearifolium* than *S. podacanthum* was also recorded at high temperature. Ambient pH and ambient temperature resulted in higher biomass and higher specific growth rate than low pH and high temperature in both species, which is recommended for *Sargassum* spp. growth.

Keywords: pH, Temperature, *Sargassum linearifolium*, *Sargassum podacanthum*, Biomass

Introduction

Australia has a significant Inland Saline Water (ISW) resource (Nulsen, 1997; Allan *et al*., 2001; Timms, 2005). The wheat-belt area in Western Australia (WA), covering approximately 18 million hectares is the largest underground source of ISW in Australia (Doupé *et al*., 2003; Lymbery *et al*., 2006) that could provide a source of water for inland marine aquaculture (Partridge, 2008). Targeting to the farm sustainability and environmental protection, the land management of nearly 30,000 farms in Australia has changed to prevent the expansion of salinization, 470,000 hectares of land were fenced and 210,000 km of levees, banks, drains for salinity management has been built (ABS, 2002), providing an available water source for ISW aquaculture. Building onshore farm to culture seaweeds is cheaper than seaweed farms in the open sea (Borowitzka, 1997), as well as contributing to environmental protection by reducing the salinity contamination (Ogburn, 1997), considering the availability of inland water resources and farm infrastructure.

Sargassum has been cultivated in many countries, such as Korea, Japan and India, for human consumption (Bast, 2014) includes *S. naozhouense* and *S. fusiforme* (Wang *et al*., 2010a; Bast, 2014). The *Sargassum* have been used commonly in Asia as a source of alginate and medicine for human (Yende *et al*., 2014; Wiltshire *et al*., 2015). For instance, *S. naozhouense* has been used as a source of food and drugs for traditional orientation treatments (Hur *et al*., 2008; Wang *et al*., 2010b). *Sargassum* also provides a source of sargaquinoic acid, sargachromenol for neurite growth and survival (Hur *et al*., 2008). The *Sargassum* can also be used for agriculture as biochemical compounds, cattle food, fertilizer (Ara *et al*., 1997; Huisman, 2000).

Both *S. linearifolium* and *S. podacanthum* can be found in Western Australia including around Perth beaches (Womersley, 1987). In South Australia, only rope-culture trial of *S. linearifolium* in the ocean has been practiced with low specific growth rate (Wiltshire *et al*., 2015), specially under summer conditions, when the temperature is from 28-32°C

Appendix 2.4. Article in Journal of Environmental Risk Assessment and Remediation

Bui HTT, Luu TQ, Fotedar R, 2017. Growth feasibility of *Lomentaria* sp. in laboratory conditions. Environmental Risk Assessment and Remediation, 1 (2), 47-55.

Research Article *http://www.alliedacademies.org/environmental-risk-assessment-and-remediation/*

The growth feasibility of *Lomentaria* sp. in Laboratory conditions.

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Abstract

The growth feasibility of Lomentaria sp. in ocean water (OW) and inland saline water (ISW) at salinity 30‰ was tested in a series of four experiments. To grow Lomentaria sp., potassium chloride (KCl) was used to fortify ISW to approximately 100%, 66%, and 33% (ISW100, ISW66, and ISW33 respectively) of [K^+] in OW and compared to two controls of OW and ISW. The results showed that the ISW66 medium resulted in the highest ($P<0.05$) Lomentaria sp biomass from day 14-56. The Lomentaria sp. was then cultured in OW, ISW and ISW66 enriched weekly with ammonium (NH_4) 100 µmol by NH_4Cl. A significantly slower reduction of specific growth rate (SGR) of Lomentaria sp. was recorded in the NH_4 enriched waters than non-enriched waters. The effect of three temperature levels of 18-19°C, 21-22°C, and 25-26°C were also tested on the growth of Lomentaria sp. The 18-19°C resulted in highest biomass loss, whereas the higher temperatures resulted in similar SGRs of Lomentaria sp in both OW and ISW66. Four levels of NH_4:PO_4 including 0:0, 75:7.5, 150:15, and 300:30 µmol L^{-1} NH_4:PO_4 by NH_4Cl and Na_2HPO_4, were weekly added to OW and ISW66, and these combined nutrient supplementation showed no effect on the Lomentaria sp. SGR. This study identified the suitable conditions for Lomentaria sp. growth in captivity as a temperature of 21-26°C, a supply of [NH_4] no greater than 100 µmol L^{-1} in K^+ fortification ISW 33-66% of [K^+] in OW for higher biomass gain.

Keywords: Biomass, inland saline water, *Lomentaria* sp., potassium, temperatures.

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Introduction

Of 5,000 red seaweed species, Rhodophyta, 1,300 species are found in Australian waters [1]. Rhodymeniales, which contains three families and 38 genera, 17 genera have been recorded in Australia, of which three species of *Lomentaria* genus have been identified in Southern Australia, including *L. australis, L. pyramidalis, L. monochlamypdea* [2]. The *Lomentaria* thallus "erect or forming entangled clumps, much branched, with or without percurrent axes, branches terete or compressed, hollow, basally constricted with solid septa; holdfast discoid or hapteroid. Structure multiaxial, with a cluster of apical cells developing an inner cortex 2-3 cells thick and an outer cortex of small cells sometimes forming rosettes" (p. 34) with a life cycle of isomorphic gametophytes and tetrasporophytes [2]. The red seaweed can be used as a source of food, to extract agar, and producing fertilizer [1]. However, little is known about the benefit of *Lomentaria* sp. yet, and there has been no record on growing *Lomentaria* sp either in ocean water (OW) and or in inland saline water (ISW).

In Australia, ISW is available in the form of large reserves of underground [3], which could provide a source of water for inland marine aquaculture [4]. About 2.2 and 5.7 million hectares of land was salt-affected in 1996 and 2000, respectively [3,5], which is expected to increase to 17 million hectares in 2050 [5]. Agricultural land, wildlife habitats and native vegetation are adversely affected due to ISW areas rising [6]. Inland marine culture can be a way to contribute to limit the impact of ISW expansion in Australia [6].

Potassium (K^+) is crucial for algal growth [7], and it shares 1-2% of dry plant biomass [8]. K^+ is an important internal cation in algae [9], and in the red algae *Chondrus crispus* and *Porphyra tenera*, it comprises 37 and 43%, respectively, of total internal cations [10]. K^+ plays an important role in photosynthesis and respiration of the plant [11]. [K^+] of 230-350 mg L^{-1} at 35‰ is suitable for the red seaweed *Caloglossa leprieurii* (Montagne) J. Agardh growth, but another red seaweed, *Bostrychia radicans* Montagne, prefers higher [K^+] at 400-500 mg L^{-1} [12]. K^+ fortification for ISW to sustain the growth of marine species is needed [13-16] when K^+-deficient ISW is common in Australia [17-19]. Studies on the K^+ effect is important to determine the requirement of [K^+] for seaweed growth.

Ammonium (NH_4), the most common type of ammonia (NH_3) in OW [20], and phosphate (PO_4) are the preferred source of nitrogen (N) and phosphorus (P) for seaweed growth [21-24] . However, N and P in water do not always meet the algal demand [25]. For higher seaweed growth, supplying NH_4 is more efficient than nitrate (NO_3) [26]. In addition, the combination of NH_4 and PO_4 have a positive effect on the growth of *Sargassum baccularia* than either NH_4 or PO_4 alone [24]. As it is the first study on growing *Lomentaria* sp., it is necessary to identify the need of NH_4 and PO_4 for optimal *Lomentaria* sp. growth.

Temperature strongly affects the growth of algae [27]. The temperature of ISW in Western Australia (WA) is approximately 18°C, and varies around 6.3-28.1°C [28]. These temperatures are suitable for the growth of many red seaweeds. *Hypnea cervicornis* and *Gracilaria tikvahiae* prefer 20-25°C for optimal growth [29,30], when *Hypnea musciformis* and

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